The clinical features, aetiology, and economic burden of paediatric encephalitis in southern Vietnam

Nguyen Hoang Thien Huong

Linacre College

Clinical Medicine

A thesis submitted for the degree of

Doctor of Philosophy

2025

Acknowledgements

This research project would not have been possible without the help and support from many people. First and foremost, I would like to express my enduring gratitude to my supervisors Assoc. Prof. Le Van Tan (University of Oxford, UK), Prof. Sarosh R. Irani (Mayo Clinic, USA and University of Oxford, UK), and Assoc. Prof. Nguyen Thanh Hung (Children's Hospital 1, Vietnam) for providing me invaluable guidance and support. Their willingness to motivate me contributed tremendously to complete this project.

I would like to show my gratitude to Assoc. Prof. Nguyen Thanh Hung, Dr. Truong Huu Khanh, Dr. Du Tuan Quy, Dr. Le Nguyen Thanh Nhan, Dr. Ngo Ngoc Quang Minh, Dr. Le Quoc Thinh, Ms. Le Thi Ngoc Dung, and Ms. Nguyen Ngoc Viet Nga from the Department of Infectious Diseases and Neurology and the Department of Clinical Microbiology at Children's Hospital 1 in Ho Chi Minh city in Vietnam for facilitating patient recruitment and clinical data collection.

Words are inadequate in offering my thanks to Assoc. Prof. Le Van Tan, Prof. C. Louise Thwaites, Dr. Nguyen To Anh, Dr. Tran Tan Thanh, Ms. Nguyen Thi Thu Hong, and Ms. Do Duong Kim Han from the Emerging Infections Group at the Oxford University Clinical Research Unit for their significant contributions to the laboratory work.

I would like to acknowledge Dr. Cristina Tato, Dr. Gowtham Thakku, Dr. Elizabeth Fashbender, and Dr. Vida Ahyong from the Chan Zuckerberg Biohub Rapid Response Team for their invaluable and high-quality molecular microbiology training.

I also want to appreciate Dr. Hugo C. Turner from the Department of Infectious Disease Epidemiology at Imperial College London for his expertise in health economics.

The support from staff in various departments and units, particularly in administrative and logistics roles, was crucial to the success of these interdisciplinary components.

Finally, I would like to express my heartfelt thanks to my husband Nguyen Duc Toan and my son Nguyen Duc Khoi (Nghe). They have been my support system with their love, patience and encouraging me all the way. I would like to thank all other members in my family who have provided me with unwavering moral and emotional support. Their care and concern have been a big motivating force to complete this work.

Declaration

Apart from the support I received from colleagues outlined in the acknowledgements, all the work presented in this thesis is my own, and was conducted under the supervision of my supervisors Assoc. Prof. Le Van Tan, Prof. Sarosh R. Irani, and Assoc. Prof. Nguyen Thanh Hung at the Oxford University Clinical Research Unit (OUCRU) and the Children's Hospital 1 (CH1) in Vietnam. Information from this study has not been submitted for any degree or other qualification elsewhere.

The University of Oxford's policies on artificial intelligence (AI) encourage students to use AI to support their writing. Accordingly, as I revised my thesis based on the examiners' comments, I used Grok 3, developed by xAI, to refine the wording and sentence structure of my literature review. Grok 3 was not used to generate original ideas, results, or critical thinking, which remain my own. I carefully cited all relevant references to avoid plagiarism, and I critically reviewed all AI-assisted outputs for accuracy. My thesis fully complies with the University of Oxford's academic integrity and AI policies.

Publications

Publications as parts of my PhD research:

1. **Huong NHT**, Toan ND, Quy DT, Khanh TH, Thinh LQ, Nhan LNT, Minh NNQ, Turner H, Thwaites L, Irani S, Hung NT, Tan LV. **Study protocol: The clinical features, epidemiology, and causes of paediatric encephalitis in southern Vietnam**. *Wellcome Open Res*. 2021 Jun 30;6:133. doi: 10.12688/wellcomeopenres.16770.2. PMID: 36300174; PMCID: PMC9579742.
2. **Huong NHT**, Toan ND, Thien TB, Khanh TH, Tuan NM, Truc TT, Nghia NA, Thinh LQ, Thoa NTK, Nhan LNT, Minh NNQ, Turner HC, Thwaites CL, Hung NT, Tan LV, Irani SR, Quy DT. **In Children, N-Methyl-D-Aspartate Receptor Antibody Encephalitis Incidence Exceeds That of Japanese Encephalitis in Vietnam**. *Open Forum Infect Dis*. 2024 Dec 6;11(12):ofae710. doi: 10.1093/ofid/ofae710. PMID: 39691294; PMCID: PMC11651147.
3. **Huong NHT**, Toan ND, Khanh TH, Thinh LQ, Nhan LNT, Minh NNQ, Thoa NTK, Hung NT, Quy DT, Thwaites CL, Irani SR, Tan LV, Turner HC. **A Cost of Illness Analysis of Children with Encephalitis Presenting to a Major Hospital in Vietnam**. *Am J Trop Med Hyg*. 2024 Nov 19;112(2):422-430. doi: 10.4269/ajtmh.24-0409. PMID: 39561390; PMCID: PMC11803661.
4. **Huong NHT**, Hong NTT, Anh NT, Toan ND, Thien TB, Thinh LQ, Nhan LNT, Minh NNQ, Turner HC, Thwaites CL, Hung NT, Irani SR, Tan LV, Quy DT. **Viral causes of paediatric encephalitis in Southern Vietnam: results of a 6-year prospective observational study using extensive diagnostic workup**. (manuscript in preparation).

Impacts of the COVID-19 Pandemic and Emerging Infections

Although I have been occupied by my PhD research, as a clinician I have also actively participated in local outbreak responses including COVID-19 and hand foot and mouth disease (HFMD). The former had affected everybody globally, while the latter has been confined to Southeast Asia. The COVID-19 pandemic might have impacted my clinical and laboratory studies on encephalitis at Children's Hospital 1 (CH1) in Vietnam. Due to the Vietnamese government's lockdown policies, children with encephalitis were isolated in their local residences and hospitals, and travel restrictions could have prevented some with severe diseases from being transferred to CH1 for treatment. Therefore, while patient recruitment for the clinical study continued during the COVID-19 pandemic time, the isolation and lockdown measures likely reduced the number of encephalitis cases admitted to CH1 during the pandemic period. In terms of the laboratory study, the planned polymerase chain reaction (PCR) and metagenomic next-generation sequencing (mNGS) analyses, originally scheduled at CH1, were disrupted as a consequence. To overcome this, the laboratory work was relocated to the Oxford University Clinical Research Unit and the Hospital for Tropical Diseases in Vietnam, where established PCR and mNGS facilities were available to support the continuation of the research. These adaptations highlighted the challenges posed by the pandemic and the necessary adjustments to sustain the study's progress. In addition, I suspect that COVID-19 pandemic may have affected the representativeness of the patients enrolling into my study (e.g. less referrals from rural areas because of the lock down, which may have had an impact on the epidemiological findings, including the number of the JEV cases that often come from rural areas). In addition, after the COVID-19 pandemic, Vietnam experienced a very large outbreak of HFMD During January 1–June 30, 2023 and my department was fully packed with severe HFMD patients (https://wwwnc.cdc.gov/eid/article/30/2/23-1024_article).

During these two consecutive outbreaks I provided care for the patients admitted to my department.

Table of Contents

The clinical features, aetiology, and economic burden of paediatric encephalitis in southern Vietnam

Nguyen Hoang Thien Huong, Linacre College, D.Phil. Thesis, 2025

Abstract

My PhD thesis consisted a body of novel work on the epidemiology, clinical features, outcome and illness costs of encephalitis in Vietnamese children. To address my research hypotheses, I based my analysis on 164 children with encephalitis recruited into an observational study conducted at a major children's hospital in Southern Vietnam between March 2020 and December 2022. I applied a multidisciplinary approach to establish the possible infectious and non-infectious causes, and to estimate the illness costs of the disease. Accordingly, Chapter 1 and 2 provide an overview describing the background and methodologies. In Chapter 3, I demonstrate that N-Methyl-D-Aspartate Receptor (NMDAR)-antibody encephalitis has become a leading cause in Vietnamese children with encephalitis, surpassing Japanese encephalitis virus (JEV), the most common viral cause of infectious encephalitis. While there were considerable similarities in clinical presentations, in NMDAR-antibody encephalitis patients, psychiatric features, cognitive dysfunction, language changes, abnormal movements were predominantly documented. Additionally, patients with NMDAR-antibody encephalitis had a longer duration of hospital stay than JEV encephalitis patients. As a diverse range of pathogens can cause encephalitis, in Chapters 4 and 5, I applied an extensive diagnostic workup including PCR and metagenomic next generation sequencing (mNGS) to better identify infectious causes of encephalitis in 89 patients with clinically suspected infectious encephalitis. Collective findings in these two Chapters showed that the overall the diagnostic yield increased from 18/89 (20.2%) after routine diagnosis to 34/89 (38.2%) after extensive PCR testing, and to 36/89 (40.4%) after mNGS, representing a doubling

in diagnoses. However, this still left ~60% of children with suspected infectious encephalitis having no cause identified. To inform health policy makers with evidence-based decisions on resource allocation for encephalitis research and management, Chapter 6 reports my comprehensive analysis to estimate the cost of illness attributed to encephalitis in Vietnamese children. The results showed that the illness costs were considerable and higher in more severe patients, patients with sequelae, and ventilated patients. Notably, I also found that, despite high health insurance coverage, encephalitis patients and families still incur significant costs. Of note, many of the children in my study suffered from JEV, a vaccine-preventable disease, indicating the potential of preventative public health measures to impact and reduce these cost outcomes. Finally, in Chapter 7, I provide a summary of my PhD findings and offer some associated future research directions.

List of Tables

List of Figures

Abbreviations

Abbreviations	Meaning
ADEM	acute disseminated encephalomyelitis
AdV	adenovirus
AI	artificial intelligence
AK5	adenylate kinase 5
AMPAR	α-amino3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ANNA 1/2	anti-nuclear neuronal autoantibody type ½
BHQ	black hole quencher
CASPR2	contactin-associated protein-like 2
CH1	Children's Hospital 1
CMV	cytomegalovirus
CNS	central nervous system
COVID-19	coronavirus disease 2019
CSF	cerebrospinal fluid
CT	computed tomography
Ct	cycle threshold
Cy5	Cyanine 5
CZ ID	Chan Zuckerberg Infectious Diseases
DEET	N,N-diethyl-3-methylbenzamide
DENV	Dengue virus
DNA	deoxyribonucleic acid
DNER	delta/Notch-like epidermal growth factor-related receptor
DPT	diphtheria, pertussis, tetanus
ds	double-stranded
DWI	diffusion-weighted imaging
EAV	Equine arteritis virus
EBV	Epstein-Barr virus
EEG	electroencephalogram
EI	Emerging Infections
ELISA	enzyme-linked immunosorbent assay
EPI	Expanded Programme on Immunisation
EV	enterovirus
$GABA_AR$	γ-aminobutyric acid A receptor
$GABA_BR$	γ-aminobutyric acid B receptor
GAD65	glutamic acid decarboxylase (65 kDa isoform)
GCS	Glasgow coma scale
GFAP	glial fibrillary acid protein
GlyR	glycine receptor
HeV	Hendra virus
HFMD	hand, foot, and mouth disease

HHV	human herpesvirus
HIV	human immunodeficiency virus
HPV	human papillomavirus
HPyV-2	human polyomavirus 2
HRV	human rhinovirus
HSV	herpes simplex virus
HTD	Hospital for Tropical Diseases
HuGkV-2	human associated gemykibivirus 2
IAV	influenza A virus
IgG	immunoglobulin G
IgM	immunoglobulin M
IQR	interquartile range
IVIG	intravenous immunoglobulin
JE	Japanese encephalitis
JEV	Japanese encephalitis virus
KLHL11	kelch-like protein 11
LGI1	leucine-rich glioma-inactivated 1
LMICs	LMICs
LP	lumbar puncture
mGluR5	metabotropic glutamate receptor 5
MMR	measles, mumps, rubella
mNGS	metagenomic next-generation sequencing
MOG	myelin oligodendrocyte protein
MOG	myelin oligodendrocyte glycoprotein
MOGAD	myelin oligodendrocyte glycoprotein antibody-associated disease
MRI	magnetic resonance imaging
mRS	modified Rankin scale
NA	nucleic acid
NA	not available
NMDAR	N-Methyl-D-Aspartate Receptor
OUCRU	Oxford University Clinical Research Unit
OxTREC	Oxford Tropical Research Ethics Committee
P	patient
PCA	Purkinje cell cytoplasmatic autoantibodies
PCR	polymerase chain reaction
PCV	pneumococcal conjugate vaccine
PhHV	Phocid herpes virus
PI	principal investigator
PICU	paediatric intensive care unit
PIV	parainfluenza virus
RNA	ribonucleic acid

RS	rectal swab
RSV	respiratory syncytial virus
RT-PCR	real-time polymerase chain reaction
Sez6L2	seizure-related 6 homolog like 2
TS	throat swab
UK	United Kingdom
US	United States
VSBV-1	variegated squirrel bornavirus-1
VZV	varicella-zoster virus
WBC	white blood cell
WNV	West Nile virus

Chapter 1. Background and Aims

Chapter 1. Background and Aims

1.1. Infectious encephalitis

Central nervous system (CNS) infections include meningitis, meningoencephalitis and encephalitis, which can be caused by over 100 pathogens (1), of which encephalitis is the most devastating clinical condition, associated with high morbidity and mortality that affects both children and adults, particularly in low- and middle- income countries. Both infectious and autoimmune causes can be responsible for encephalitis in both children and adults. Because of the focus of my thesis, herein, I will focus my literature review on the case definition, epidemiology, causes, diagnosis, management, prevention and illness costs of paediatric encephalitis. The associated information about encephalitis in adults will be mentioned where appropriate.

1.1.1. Case definition of infectious encephalitis

Encephalitis is defined as inflammation of cerebral parenchyma associated with neurological dysfunction (2). Accordingly, a clinical diagnosis of encephalitis is made when a patient has altered mental status (decreased or altered level of consciousness, lethargy, or personality change) lasting ≥24 hours with no alternative cause and ≥2 of these following criteria: fever ≥38°C within 72 hours (before or after) presentation, generalised or partial seizures not fully attributable to a pre-existing seizure disorder, new onset focal neurological findings, cerebrospinal fluid (CSF) white blood cell count ≥5 cells/mm^3, abnormality of cerebral parenchyma on neuroimaging suggestive of encephalitis that is new or appears to have acute onset, and abnormality on electroencephalogram that is consistent with encephalitis and not attributable to any other causes (1,3–6).

1.1.2. General clinical features of infectious encephalitis

The clinical manifestations of encephalitis vary and can be dependent on the responsible pathogens, the affected regions of the brain, the patients' age, and the immune condition (1,3–6). In children, common clinical presentations of encephalitis include fever, psychiatric symptoms, emotional changes, movement disorder, seizures, decreased level of consciousness (stupor, lethargy, coma), or neurological deficit (hemiparesis, cranial nerve defect). In severe cases, status epilepticus, cerebral oedema, syndrome of inappropriate secretion of antidiuretic hormone (SIADH), and cardiorespiratory failure may occur (1,3–6).

1.1.3. Epidemiology and the global burden of infectious encephalitis in children

Encephalitis is responsible for a significant burden, especially in children, worldwide (7). The estimated incidence of hospital admission due to encephalitis in children ranged from 3 to 13 admissions per 100,000 children per year (8–16). In a recent study in the United States, of >7000 paediatric patients admitted with suspected encephalitis between 2004 and 2013, the median age of the patients was 9 years old, and 40% of children were admitted to the paediatric intensive care unit (PICU), with a median length of hospital stay of 16 days (9). In England and Wales, it was estimated that 4 to 10 children per 100,000 were hospitalised with encephalitis every year (8). Between July 2017 and July 2020, a cohort study of 202 children with encephalitis recruited from 14 hospitals of the Colombian Network of Encephalitis found that 78 patients (38.6%) required management in the paediatric intensive care unit (PICU) (17). Another 10-year retrospective cohort study published their results in 2023 found that 175 children with encephalitis were admitted to PICU requiring mechanical ventilation (74 cases, 42%); haemodynamic support (28 cases, 16%), extracorporeal membrane oxygenation (ECMO) support (3 cases, 1.7%), and 11 patients died (case fatality rate 6.3%) (18). As a consequence of the

high demand for specialised treatment and prolonged hospital stays, encephalitis is associated with the illness high cost (9,12,17–19), which will be further discussed in section 1.7.

The overall mortality of childhood encephalitis ranges from 2.8% to 31.0% (20,21). However, neurological deficits of the patients are mostly documented at hospital discharge, and may include personality change, behaviour disorder (such as attention deficit disorder), movement disorder (such as tic disorders), intellectual disability, learning disorders, blindness, paresis, ataxia, recurrent headaches, and sleeping problems (22,23). Up to 50% of children with infective encephalitis develop short- to medium-term neurological sequelae (21). In a study at National Children's Medical Centre in China from 01 January 2015 to 31 July 2024, 85.7% of survivors of paediatric infectious brainstem encephalitis suffered from sequelae such as motor disorders (24).

1.1.4. Infectious causes of encephalitis

Although bacteria, fungi, and parasites can also cause encephalitis, viruses are regarded as the most common pathogens (25). The spectrum of the infectious causes varies across the globe and is dependent on the various factors, including vaccination coverage, age and geographics. However, there are over 100 viruses can cause encephalitis, and around >=50% of patients with encephalitis have no aetiology identified despite extensive laboratory investigations, Figure 1.1 (25–27). The viral causes of encephalitis vary according to geographical region. In Asia including Vietnam, Japanese encephalitis virus (JEV) is the main cause of encephalitis in children. Equine encephalitis and West Nile viruses circulate in America whereas tick-borne encephalitis is prevalent in Europe (28).

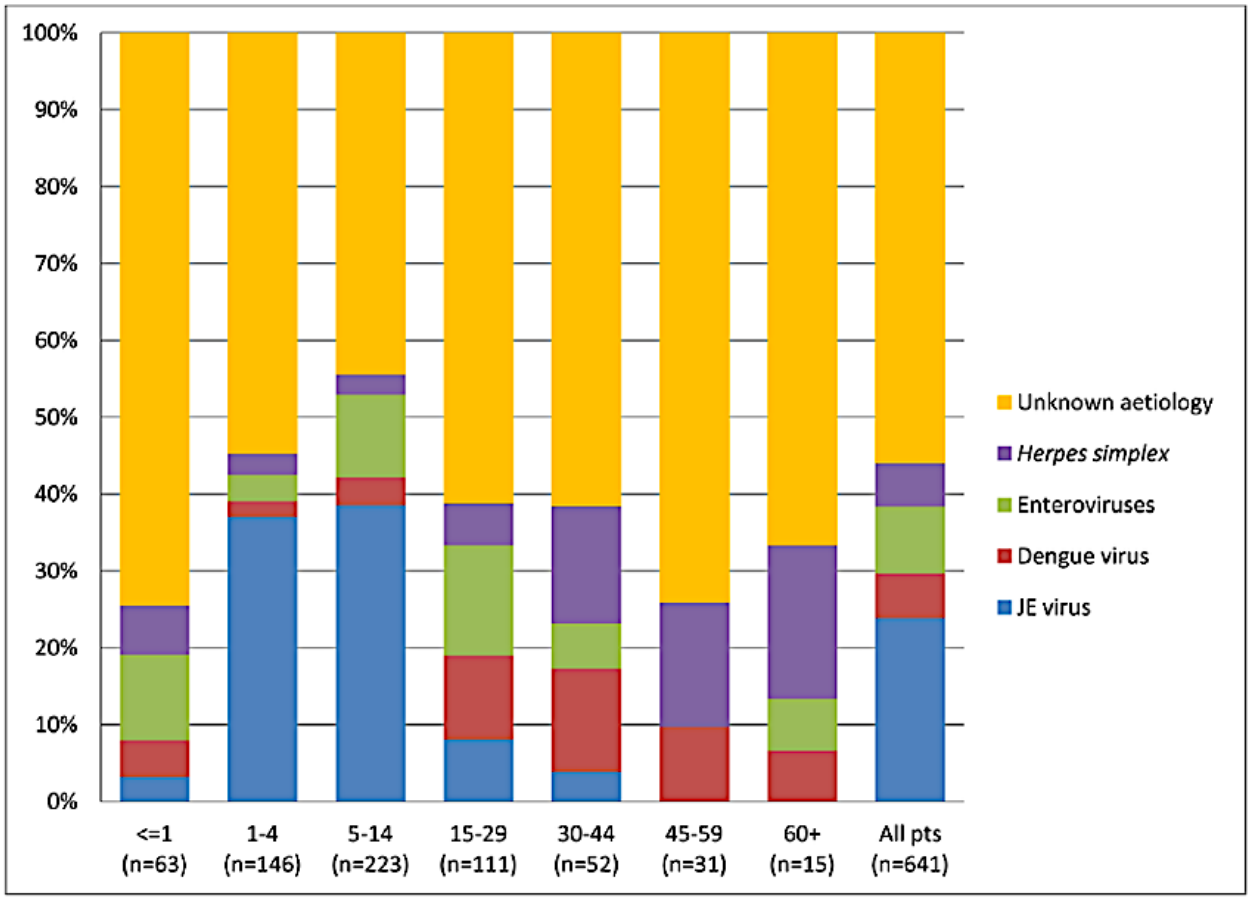


Figure 1.1. Causes of viral encephalitis/meningitis across age groups in Vietnam

Note: Adapted from "Aetiologies of central nervous system infection in Viet Nam: a prospective provincial hospital-based descriptive surveillance study", by Ho Dang Trung N et al. *PLoS One*. 2012;7(5):e37825 (29).

Laboratory confirmed aetiology per age group (bars) for patients meeting the case definition of viral meningitis/encephalitis (excluding dual infection cases).

Arboviruses

Arboviruses include viruses of the families *Togaviridae*, *Flaviviridae*, *Bunyaviridae*, and *Reoviridae* that are transmitted via mosquitos, ticks, or sand-flies (30). Encephalitis is a rare manifestation of arboviral infection (Table 1.1) but not all arboviruses can cause encephalitis. The endemic regions of arboviruses causing encephalitis can be geographically restricted and is outlined in Figure 1.2 and Table 1.1.

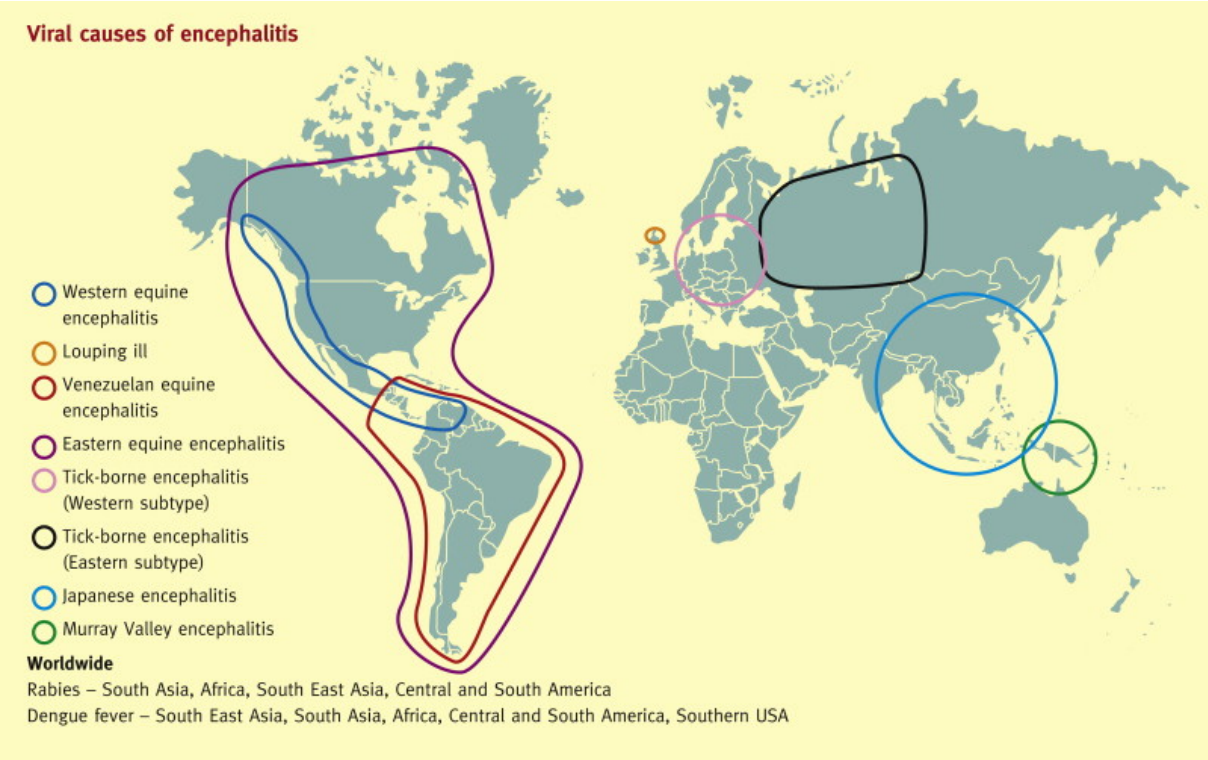


Figure 1.2. The global distribution of arboviruses causing encephalitis that are geographically restricted in certain areas of the world

Note: Adapted from "Viral meningitis and encephalitis", by Rice P. *Medicine*. 2017;45(11): 664-669 (28).

Table 1.1. Vectors and geographical distribution associated with neuroinvasive arboviruses

Family	Virus	Vector	Geographical distribution
Togaviridae	Chikungunya	Mosquitoes	Africa, Americas, Asia, Europe, Pacific Islands
	Eastern equine encephalitis	Mosquitoes	Americas
	Western equine encephalitis	Mosquitoes	Americas
	Venezuelan equine encephalitis	Mosquitoes	Central and South America
Flaviviridae	Dengue	Mosquitoes	Africa, Americas, Asia, Australia, Europe, Pacific Islands
	Japanese encephalitis	Mosquitoes	Asia
	Murray Valley encephalitis	Mosquitoes	Australia
	St. Louis encephalitis	Mosquitoes	Americas
	West Nile encephalitis	Mosquitoes	Asia, Africa, North America
	Powassan	Ticks	Canada, Europe, Northern United States
	Tick-borne encephalitis	Ticks	Asia, Europe
	Zika	Mosquitoes	Africa, Americas, Asia
Bunyaviridae	La Crosse encephalitis	Mosquitoes	United States
	California encephalitis group	Mosquitoes	Asia, Europe, North America
Reoviridae	Colorado tick fever	Ticks	Western Canada and United States

Note: Adapted from: Mangat R, Louie T. Arbovirus Encephalitis. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2025.

https://www.ncbi.nlm.nih.gov/books/NBK560866/

JEV is the main cause of paediatric encephalitis in South East and East Asia (Figure 1.2) which will be further discussed in a separated section below (28). In the United States, West Nile virus associated encephalitis has been reported across the country, especially in adults, since 1999 when the virus was first introduced into the country (31). La Crosse virus causing encephalitis most commonly affects children from five to nine years of age, and appears to be restricted to the United States. Encephalitis due to Powassan virus has been reported in Canada, Europe, Northern United States, with the mortality rate greater than 10%. Notably, the reported cases have increased from an average of 1 case/year prior to 2006 to 21–43 cases/year between 2016 and 2019 (32). This increase in the reported incidence relates to a new strain, termed Powassan virus lineage II/Deer Tick virus, spread by the aggressive *Ixodes scapularis* tick (33), which merits further research. The transmission of the virus from tick vector to human host can occur after just 15 minutes of attachment. Other arboviral causes of encephalopathy include Colorado tick virus in the western Canada and United States. Infection with Dengue virus (DENV) may also result in encephalopathy or encephalitis, albeit rare.

Japanese encephalitis virus (JEV)

An overview

JEV is transmitted by mosquitoes of the *Culex* species (Figure 1.3), especially *Culex tritaeniorhynchus*. JEV is responsible for Japanese encephalitis (JE) which is associated with high morbidity and mortality in South and Southeast Asia (Figure 1.4) (34). Currently, there is clinically proven effective antivirals to offer the hospitalised patients. Effective vaccine is available but the disease remains a public health threat across the affected countries.

Figure 1.3. Female *Culex* mosquito taking a blood meal from a person

Note: Adapted from "Life cycle of Culex mosquitoes". CDC. April 16, 2024.

https://www.cdc.gov/mosquitoes/about/life-cycle-of-culex-mosquitoes.html

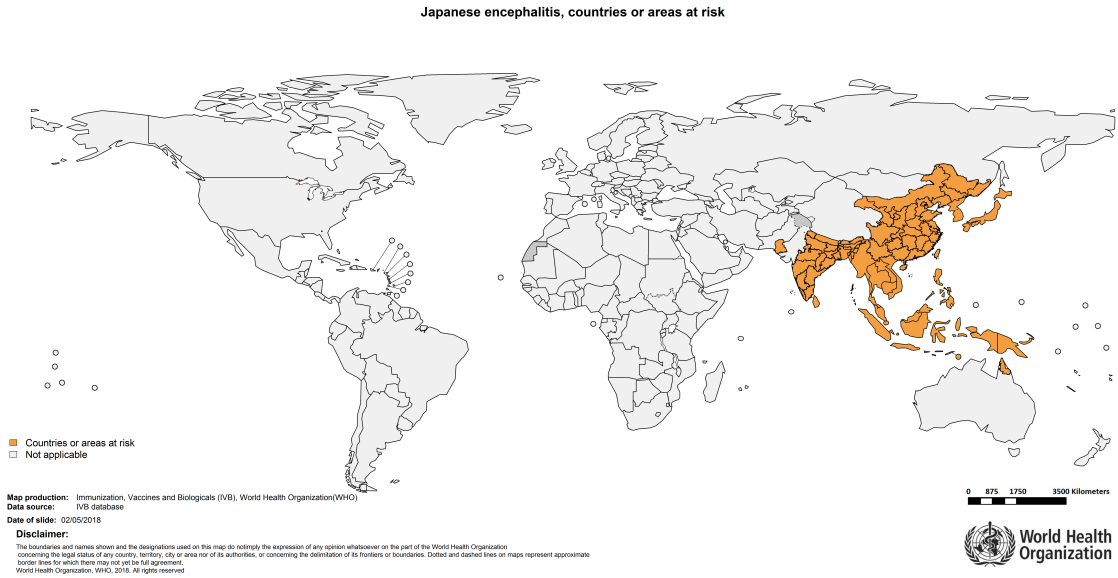


Figure 1.4. Countries or regions at risk of Japanese encephalitis

Note: Adapted from "Japanese encephalitis". WHO. May 2, 2018.

https://www.who.int/news-room/fact-sheets/detail/japanese-encephalitis

A mathematical modelling study based on age-stratified case data estimated that in 2015 around 100,308 JE cases (95% CI: 61,720–157,522) and 25,125 deaths (95% CI: 14,550–46,031) occurred worldwide, in particular in Asia (35). In a recent hospital-based study of paediatric encephalitis conducted between July 28, 2014, and Dec 31, 2017 in Cambodia, Vietnam, Laos, and Myanmar, JEV was detected in 216/664 cases (33%) (36). Although JEV is endemic in Asia, it has the potential to spread to other geographic areas of the world (34). In 2022, community transmission of JEV was reported in Australia for the first time, causing 41 human infections as of May 2022 (37). This had led to the declaration of JEV as 'Communicable Disease Incident of National Significance' in Australia (37).

Clinical features of JEV encephalitis in children

JEV is a significant cause of viral encephalitis in children, particularly in the Asia-Pacific region. JE patients present with a wide spectrum of clinical features, ranging from asymptomatic infection, to mild and severe neurological manifestations (38–40). The exact proportion of JEV patients with these respective outcomes are unknown. The initial symptoms are often non-specific, resembling a flu-like illness, and include fever, headache, and malaise, which typically develop after an incubation period of 5–15 days. In children, fever is frequently high-grade, often exceeding 38.5°C, accompanied by chills and fatigue (38–40).

The associated neurological symptoms in JE patients can include altered mental status, ranging from confusion and irritability to stupor or coma, reflecting the virus's impact on the central nervous system. Seizures are a hallmark feature in paediatric cases, occurring in up to 50–80% of children with JEV encephalitis, often presenting as generalized tonic-clonic seizures or, less commonly, focal seizures. Younger children may exhibit subtle seizure activity, and movement disorders such as lip smacking or eye deviation, which

can be mistaken for non-specific irritability. Motor deficits, including weakness or paralysis, are common, with some children developing spastic quadriparesis or hemiparesis due to involvement of the motor cortex or subcortical structures. Tremors, rigidity, and other extrapyramidal signs, such as dystonia, may also occur, reflecting basal ganglia involvement. Cranial nerve palsies, particularly affecting the facial or oculomotor nerves, can lead to symptoms like facial drooping or diplopia. In severe cases, children may develop signs of raised intracranial pressure, such as vomiting, severe headache, or papilledema, which can progress to herniation if untreated. Autonomic dysfunction, including irregular breathing patterns, tachycardia, or temperature dysregulation, is also observed in critical cases, often signalling a poor prognosis. Sensory disturbances are less common but may include hyperesthesia or paraesthesia (38–40).

The clinical course in children is often more rapid and severe than in adults, with a higher likelihood of neurological sequelae, such as cognitive impairment, motor disabilities, or epilepsy, in survivors (38–40). Notably, the severity of symptoms varies widely; some children may present with mild encephalitis, while others rapidly progress to severe, life-threatening disease (38–40).

Parkinsonian phase in JEV encephalitis in children

The parkinsonian phase of JEV-induced encephalitis is a critical clinical stage characterized by extrapyramidal symptoms resembling Parkinson's disease, typically emerging during the acute or subacute phase of the illness (38–44). This phase is marked by symptoms such as bradykinesia, rigidity, resting tremors, hypomimia, and postural instability, which result from viral invasion of specific brain area such as the basal ganglia, particularly the substantia nigra and thalamus. In children, these symptoms may be accompanied by other neurological manifestations like seizures, altered consciousness, and focal deficits, complicating diagnosis and management (38–44).

The parkinsonian features arise due to JEV's neurotropism, which leads to neuronal loss, gliosis, and inflammatory infiltrates in the basal ganglia, as observed in neuroimaging studies like MRI, which often reveal hyperintensities in these regions (38–44). The pathophysiology involves direct viral invasion and immune-mediated damage, with cytokines such as TNF-α and IL-6 contributing to neuroinflammation. In paediatric cases, the parkinsonian phase is particularly concerning because it can persist into the convalescent phase, leading to long-term motor impairments and cognitive deficits. The severity of symptoms varies, with some children showing partial recovery with supportive care, while others develop chronic neurological sequelae (38–44).

Movement disorders in JEV encephalitis in children

Movement disorders are a significant neurological manifestation in children with JEV-induced encephalitis, often contributing to long-term morbidity. Common movement disorders observed include parkinsonism, dystonia, chorea, and tremors, with parkinsonism being the most frequently reported (38–44).

Parkinsonian features, such as bradykinesia, rigidity, and tremors, often emerge in the acute or subacute phase of the illness and may persist, leading to significant functional impairment (38–44). Dystonia, characterized by sustained muscle contractions causing abnormal postures, is another prominent feature, particularly in severe cases, and can be focal, segmental, or generalized (38–44). Chorea, marked by involuntary, irregular movements, and tremors are less common but can occur, especially in the recovery phase (38–44).

These movement disorders are thought to result from JEV's neurotropism, which triggers inflammation, neuronal loss, and gliosis in affected brain regions (38–44). In children, the developing nervous system may be particularly vulnerable, leading to more pronounced and persistent motor deficits compared to adults. The severity of movement disorders

often correlates with the extent of brain involvement, as seen on neuroimaging, which frequently reveals hyperintensities in the basal ganglia and thalamus on MRI. The pathophysiology involves both direct viral damage and immune-mediated injury, with cytokines and excitotoxicity exacerbating neuronal damage (38–44). Long-term follow-up studies indicate that up to 30-50% of children with JEV encephalitis may experience residual neurological sequelae, including movement disorders, impacting their quality of life (38–44).

Clinical progression of JEV encephalitis in children

The progression of encephalitis caused by the JEV in children typically follows a distinct clinical course, characterized by an incubation period, prodromal phase, acute encephalitic phase, and recovery or chronic sequelae (38–40).

After a mosquito bite introduces JEV, the incubation period lasts 5 to 15 days, during which children are usually asymptomatic (38–40). The prodromal phase then emerges, marked by nonspecific symptoms such as fever, headache, fatigue, and malaise, lasting 1 to 6 days (38–40). Gastrointestinal symptoms, including nausea, vomiting, and abdominal pain, may also occur. These presentations make early diagnosis challenging due to their similarity to other viral infections (38–40).

As the disease progresses to the acute encephalitic phase, typically within 3 to 5 days of symptom onset, neurological symptoms become prominent (38–40). Children may develop high fever (38–40°C), altered mental status ranging from confusion to coma, seizures (particularly generalized tonic-clonic seizures, more common in children than adults), and focal neurological deficits such as weakness or paralysis. Tremors, rigidity, and abnormal movements, including dystonia, may also be observed, reflecting JEV's predilection for the basal ganglia, thalamus, and brainstem. Approximately 20–30% of children develop status epilepticus, a life-threatening complication requiring urgent

medical intervention. The acute phase typically lasts 1 to 2 weeks, with mortality rates ranging from 5–30%, highest in younger children (38–40).

Among the survivors, the recovery phase begins as fever subsides and neurological symptoms stabilize, but this phase varies widely (38–40). Some children recover fully within weeks, while others experience prolonged recovery over months, often with residual neurological deficits. Long-term sequelae, affecting 30–50% of survivors, include cognitive impairment, motor deficits, behavioural changes, and epilepsy, particularly in cases with severe initial presentations or delayed management (38–40).

Laboratory, cerebrospinal fluid (CSF), and neuroimaging findings in JEV encephalitis in children

Laboratory investigations typically reveal a leukocytosis in peripheral blood, with a predominance of neutrophils, though lymphocytosis may occur in later stages (38–41). C-reactive protein levels are often elevated, reflecting an inflammatory response, while liver function tests may show mild transaminitis due to systemic viral effects (38–41).

CSF analysis in JEV encephalitis typically shows a lymphocytic pleocytosis, with cell counts ranging from 10 to 1000 cells/mm^3, predominantly lymphocytes (38–41). The CSF protein level is moderately elevated (50-200 mg/dL), while glucose levels remain normal or slightly reduced, distinguishing JEV encephalitis from bacterial meningitis (38–41).

IgM antibodies are detectable in CSF by day 4-7 of illness in most cases (38–41). Therefore, JEV specific serological tests, such as IgM capture ELISA (enzyme-linked immunosorbent assay) for detection of JEV IgM in CSF, are highly sensitive and are recommended. Polymerase chain reaction (PCR) for JEV RNA in CSF, are clinically not sensitive due to the transient presence of viral RNA in the CSF but can be valuable in early diagnosis (38–41). JEV RNA can also be detected in urine by molecular assays including PCR and metagenomics (45). In countries where JEV and other flaviviruses

such as dengue are co-circulating, where resources are available, testing for IgM for both viruses are recommended. Repeating IgM testing can be helpful to rule out the possibility of false negative results obtained from early samples.

Magnetic resonance imaging (MRI) is a cornerstone of neuroimaging in JEV encephalitis, revealing characteristic findings in approximately 70-80% of paediatric cases (38–41). The most common MRI abnormalities include bilateral hyperintense lesions on T2-weighted and fluid-attenuated inversion recovery (FLAIR) sequences, primarily affecting the thalami, basal ganglia, and brainstem (38–41). Thalamic involvement is particularly suggestive of JEV, though not pathognomonic, as similar patterns may occur in other viral encephalitis. Lesions are typically hypointense on T1-weighted images and may show restricted diffusion on diffusion-weighted imaging (DWI) in acute stages, reflecting cytotoxic oedema. The hippocampus and temporal lobes are occasionally involved, but cortical lesions are less common. Gadolinium enhancement is variable and often minimal, indicating limited blood-brain barrier disruption. In severe cases, haemorrhagic changes in the thalami or basal ganglia may be observed, correlating with poor prognosis (38–41).

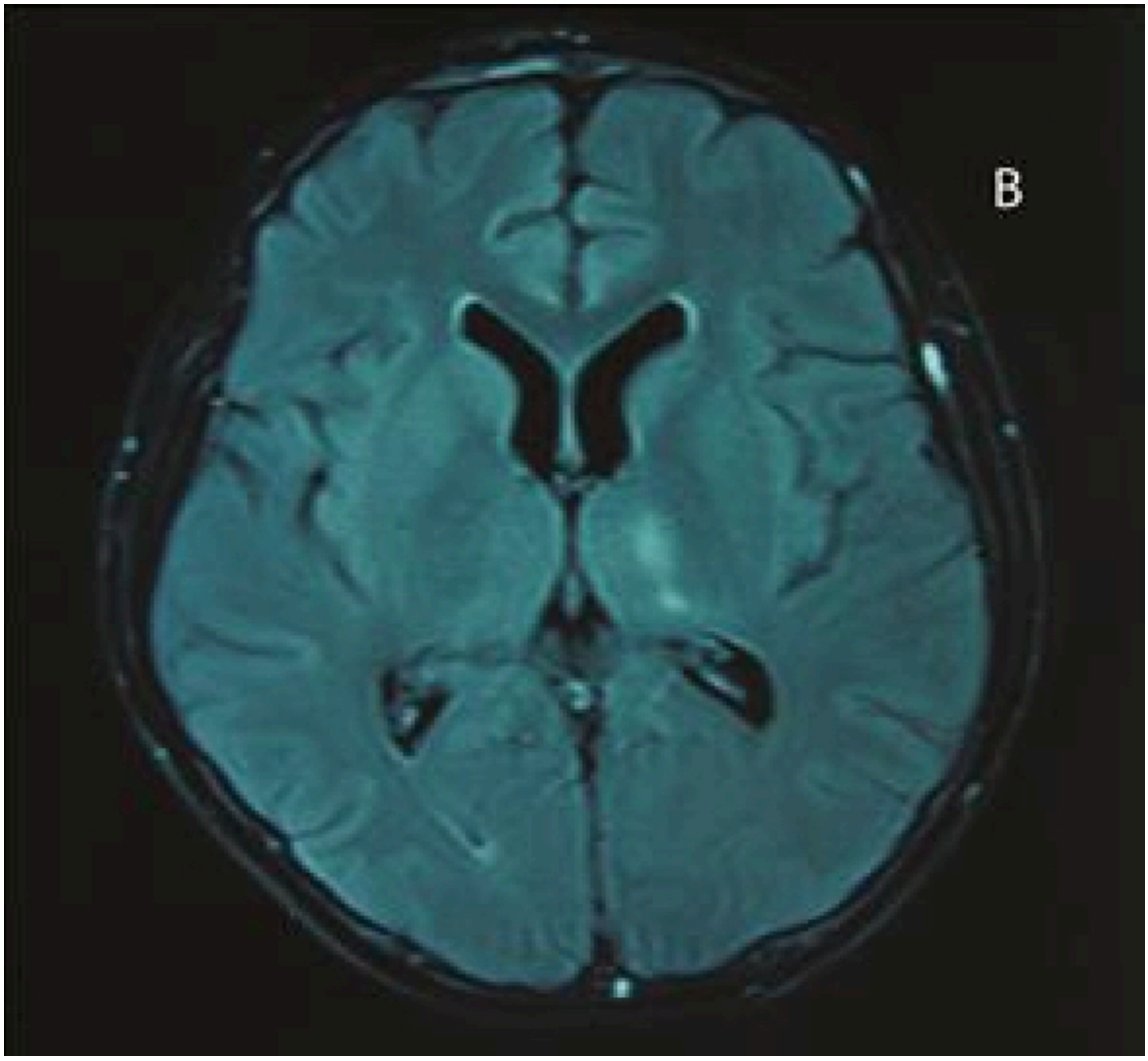


Figure 1.5. Brain magnetic resonance imaging showing a lesion of the putamen with a high signal on a fluid-attenuated inversion recovery image

Note: Adapted from "Central Nervous System Infection Diagnosis by Next-Generation Sequencing: A Glimpse into the Future?", by Nguyen Thi Hoang Mai, Nguyen Hoan Phu, et al. Open Forum Infectious Diseases. 2017;4(2):ofx046 (45).

Electroencephalography (EEG) may complement MRI findings, showing diffuse slowing or epileptiform activity, but it is not specific to JEV (38–41). These laboratory, CSF, and MRI findings collectively aid in differentiating JEV encephalitis from other causes of acute encephalitis syndrome in children, guiding timely management. However, the variability in findings underscores the importance of integrating clinical, epidemiological, and diagnostic data for accurate diagnosis (38–41).

Treatment of JEV encephalitis in children

Treatment of JEV-induced encephalitis in children is primarily supportive, as no specific antiviral therapy is currently available (38–42). The cornerstone of management involves early recognition and prompt initiation of supportive care to mitigate complications. Children presenting with symptoms such as fever, seizures, altered consciousness, or focal neurological deficits require hospitalization, often in an intensive care unit for severe cases (38–42).

Key interventions include seizure control with anticonvulsants, management of raised intracranial pressure using mannitol or hypertonic saline, and maintenance of adequate oxygenation and hydration (38–42). Mechanical ventilation may be necessary for children with respiratory failure due to brainstem involvement or severe obtundation. Corticosteroids, such as dexamethasone, have been studied for their potential to reduce inflammation and cerebral oedema, but evidence on their efficacy remains inconclusive, with some trials showing no significant benefit (38–42). Nutritional support and physiotherapy are critical during recovery to address prolonged immobility and prevent complications like contractures (38–42).

Outcomes and sequelae of JEV encephalitis in children

The outcomes and sequelae of JEV-induced encephalitis in children vary widely, ranging from complete recovery to severe neurological impairments or death (38–43).

Approximately 20–30% of children with JEV encephalitis succumb to the disease, with mortality rates higher in resource-limited settings due to delayed diagnosis and inadequate supportive care (38–43).

Among survivors, 30–50% experience long-term neurological and psychological sequelae, significantly impacting quality of life (38–43). Children who recover may face persistent neurological deficits, such as cognitive impairment, motor weakness, spasticity, or ataxia, with younger children being particularly vulnerable due to their developing brains (38–43). Behavioural and psychiatric issues, including aggression, memory deficits, and emotional lability, are also common, affecting social integration and academic performance (38–43). Seizure disorders, including epilepsy, develop in up to 20% of survivors, often requiring long-term anticonvulsant therapy (38–43). The severity of initial presentation, including prolonged seizures or deep coma, correlates with worse outcomes (38–43).

Rehabilitation, including physical and occupational therapy, can mitigate some deficits, but access to such services is limited in endemic areas (38–43). Socioeconomic factors, malnutrition, and delayed medical intervention exacerbate poor outcomes (38–43). Long-term follow-up studies indicate that some children show gradual improvement over years, but profound disabilities often persist, placing a significant burden on families and healthcare systems (38–43). Research continues to explore neuroprotective therapies and improved supportive care to reduce sequelae, but no specific antiviral treatment for JEV exists (38–44).

1.1.5. Prevention of JEV

Preventive measures, such as vaccination and vector control, remain the most effective strategies to reduce the burden of JEV-related neurological complications (38–44). Vaccination remains the most effective preventive strategy, with safe and efficacious

vaccines like the live-attenuated SA14-14-2 vaccine widely used in endemic regions (38–42). Inactivated vaccines available for Japanese encephalitis virus (JEV) include Ixiaro (JE-VC), JEEV, and inactivated mouse-brain-derived vaccines like JE-Vax, primarily used in endemic regions or for travellers (38–42). The use of mouse-brain-derived vaccine however was stopped because of its side effects.

Once infection occurs, supportive care is the mainstay, as antiviral drugs like ribavirin have shown limited efficacy in clinical trials (38–42). Experimental therapies, including interferon-alpha and monoclonal antibodies, are under investigation but not yet standard practice (38–42). Long-term management involves rehabilitation to address neurological deficits, such as cognitive impairment, motor dysfunction, or epilepsy, which may persist in survivors. Multidisciplinary care involving paediatric neurologists, physiotherapists, and psychologists is essential for optimizing outcomes (38–42). Challenges in resource-limited settings, common in JEV-endemic areas, include delayed diagnosis, limited access to intensive care, and inadequate rehabilitation services, which exacerbate morbidity and mortality. Ongoing research aims to develop targeted antivirals and improve supportive care protocols to reduce the burden of JEV in children (38–42).

Enteroviruses (EV)

Enteroviruses (EVs) are RNA viruses with single positive-strand genomic RNA structure. EVs belong to the family *Picornaviridae* which consists of more than 100 serotypes. EVs mainly infect humans through oral-faecal route, and in some instance, e.g. enterovirus D68 (EV-D68) and rhinovirus through respiratory droplets.

EV infection is associated with encephalitis with an incidence of 3%, which can result in high morbidity and mortality. Various EV serotypes, especially enterovirus A71 (EV-A71) and EV-D68, have been reported to be associated with encephalitis worldwide (46)

and have emerged as important causes of CNS infections, including encephalitis, in children in Southeast Asia and the United States of America, respectively.

In patients with EV encephalitis, the affected areas of the brain may include the motor cortex, cerebellum, thalamus, hypothalamus, midbrain, brainstem, and medulla. EV encephalitis may lead to death of neurons and cause neurological paralysis, including monoplegia, hemiplegia, paraplegia, or quadriplegia. The neurological paralysis is caused by the loss of muscle function, occurring when nerve signals between the brain and muscles are disrupted. The clinical presentation of the disease reflexes the level and progression of cellular destruction in the brain. The clinical features of EVs associated encephalitis includes fever, headache, altered level of consciousness (lethargy, drowsiness, coma), splenomegaly, hepatomegaly, muscular weakness, abnormal reflexes, meningeal signs and manifestation of brain stem dysfunction in rhombencephalitis such as ataxia, tremor, myoclonic jerks, oculomotor problems (nystagmus, strabismus, or gaze paresis), and bulbar palsy (dysphagia, dysarthria, dysphonia, and facial weakness) (46).

Enterovirus A71 (EV-A71) is an emerging pathogen causing severe hand foot and mouth disease (HFMD) in young children in Southeast Asia since 1997, which can result in encephalitis as the most severe form of complication, and can be fatal. EV-A71 associated encephalitis primarily affects the brainstem, especially the medulla oblongata and pontine tegmentum, with occasional involvement of the cerebellum, spinal cord, and cortex (46). The most severe form, rhombencephalitis, targets the brainstem's ventral, medial, and caudal regions, leading to significant neurological complications. MRI often shows hyperintense lesions on T2-weighted and FLAIR sequences in these areas, aiding diagnosis (46). Clinical presentation of EV-A71 encephalitis varies by severity. Mild cases of EV-A71 encephalitis may present with HFMD or herpangina, followed by neurological symptoms like myoclonic jerks, ataxia, tremor, or aseptic meningitis.

Severe cases of EV-A71 encephalitis manifest as brainstem encephalitis, characterized by lethargy, altered consciousness, seizures, cranial nerve palsies, nystagmus, or autonomic dysfunction (e.g., fluctuating blood pressure) (46). Critical complications of EV-A71 encephalitis include neurogenic pulmonary oedema or cardiopulmonary failure, which can be fatal, particularly in young children. Symptoms in EV-A71 encephalitis like fever, vomiting, and irritability often precede neurological signs, which emerge 1–5 days after HFMD onset (47). Long-term sequelae of EV-A71 encephalitis in survivors may include limb weakness, dysphagia, hypoventilation, cerebellar dysfunction, or neurodevelopmental delays. No specific antiviral treatment exists for EV-A71 encephalitis; management includes supportive care, intravenous immunoglobulin, and milrinone for severe cases. The high morbidity and mortality of EV-A71 encephalitis, especially in Asia-Pacific outbreaks, underscore the need for early diagnosis and intervention (46).

Herpes simplex virus (HSV)

HSV is a common cause of encephalitis worldwide, but the disease more commonly occurs in adults than in children. The prevalence of HSV encephalitis in children is around 5% (48,49). HSV-1 is the most commonly confirmed cause of death in encephalitis, with an estimated incidence of approximately 2.2 cases per million people per year (48,49). While HSV-2 predominantly cause infection in neonates (48,49) and tends to be in the clinical context of genital herpes, HSV-1 is reported to be a more common aetiology of encephalitis in children.

HSV encephalitis is associated with extremely high mortality if not timely treated with intravenous acyclovir. In untreated patients, the mortality can be up to 70%. However, its subtle and non-specific clinical manifestation represent a challenge to establish the diagnosis based on clinical assessment alone (11). Prompt and careful examination with

suitable blood and CSF testing are crucial in order to diagnose the disease and to optimise its management. A study conducted by the Division of Pediatric Infectious Diseases at the University of Alabama at Birmingham, US found that survival rate for patients treated with high-dose acyclovir (60 mg/kg/day) was significantly higher than for patients treated with standard-dose acyclovir (30 mg/kg/day) (OR=3.3 with 95% CI 1.5–7.3) (50). Patients treated with high-dose acyclovir were 6.6 times (adjusted OR; 95% CI: 0.8–113.6) to develop normally at one year of age as patients treated with standard-dose acyclovir (50). In children, the physiopathology of HSV associated encephalitis mainly focus on the inborn errors of immunity, leading to uncontrolled primary infection or reactivation and HSV-1 encephalitis may be due to a mutation in an E3 ubiquitin ligase (51). The term E3 in E3 ubiquitin ligase refers to its role as the third enzyme in a sequential cascade of enzymatic reactions involved in the ubiquitination process, which marks proteins for degradation, regulation, or localization (51). Therefore, appropriate evaluation for underlying immunodeficiency should be considered in all children following HSV encephalitis (52). At least two-thirds of children and adolescents surviving HSV encephalitis suffer from neurological disability including seizure, developmental delay, and neurological deficits (48).

Other herpes viruses

Other herpesviruses that can cause encephalitis in children, especially in immunocompromised individuals, include Epstein-Barr virus (EBV), Cytomegalovirus (CMV), Human herpesvirus 6&7 (HHV-6&7 and Varicella-zoster virus (VZV). However, the detection of those viruses, especially EBV, HHV-6&7 and CMV in the CSF may merely reflect an incidental finding of their infection of the white blood cells rather than an association with an ongoing CNS infection.

In a prospective study of 216 children with encephalitis conducted at The Hospital for Sick Children in Toronto, Canada (1994 to 2003), EBV constituted 6% of all cases (15). Neurological manifestations are common at the onset of EBV encephalitis and the disease caused by EBV did not necessarily follow an infectious mononucleosis syndrome (53). VZV was previously described as a common cause of encephalitis in children but it has decreased significantly with immunisation (54).

Influenza viruses and other respiratory viruses

Influenza A and B viruses, and respiratory viruses such as respiratory syncytial virus (RSV) A and B, rhinovirus A and B, coronavirus, parainfluenza 1, 2, 3, and metapneumovirus are common causes of respiratory diseases. However, their infections may be associated with encephalopathy/encephalitis, albeit rarely reported. In a prospective study of 311 children with encephalitis admitted to the Hospital for Sick Children in Toronto, Canada (1994 to 2005), influenza was detected in 5% (55). The majority of encephalitis cases (11/14 cases) associated with influenza viruses occur in children <5 years of age. The clinical course tends to be acute rather than post-infectious encephalitis. The pathogenesis of encephalitis caused by influenza virus is currently not well characterised.

Likewise, globally respiratory syncytial virus (RSV) and other respiratory viruses, including human metapneumovirus, adenovirus, and parainfluenza virus, have been linked with neurological infections (including encephalopathy/encephalitis) in 5% of paediatric patients presenting with encephalitis (56,57). In China, a total of 544 children were hospitalized due to adenoviral encephalitis were highlighted with data from medical records at twenty-seven children's hospitals between January 2016 and December 2018 (58).

Hendra and Nipah viruses

Both Hendra virus (HeV) and Nipah viruses are emerging zoonotic viruses and belong to the *Paramyxoviridae* family. Nipah virus was firstly described as a cause of encephalitis in pig farmers in Malaysia in 1998 with fruit bats as the primary reservoir (59). Later outbreaks of encephalitis in Bangladesh and India have been linked to palm sap contaminated by bat saliva, or to interpersonal transmission (60). Exposure to infected horses was described in an outbreak of Nipah virus encephalitis in the Philippines in 2014 (61).

Hendra virus infection causes severe and often fatal disease in both infected horses and humans (62). The natural host of the virus has been identified as fruit bats of the *Pteropodidae* Family, *Pteropus* genus. HeV was identified during the first recorded outbreak in Brisbane, Australia, in 1994 with 21 horses and 2 people affected (62). As of July 2016, 53 disease events relating to more than 70 horses have been recognised: all in the north-eastern coast of Australia (62) and related to these, were infections in 7 people (62).

Variegated squirrel bornavirus 1

Variegated squirrel bornavirus 1 (VSBV-1) is a recently discovered zoonotic virus in Germany (63,64). Its infection can result in encephalitis. It was identified as a pathogen of many deaths caused by encephalitis in exotic animal breeders in Germany and was subsequently confirmed as causes of deaths with encephalitis among zoo employees (63,64).

Measles, mumps, and rubella

Measles, mumps, and rubella can cause encephalitis. Nowadays, outbreaks of those viruses still occur in countries where vaccination coverage is low or when there is a drop in vaccination coverage (65–67). Measles can cause acute encephalitis or subacute sclerosing pan-encephalitis (SSPE) children. Although, measles was declared to be

eradicated in the United States in 2000, it recently has re-emerged as a pathogen of encephalitis in this country mainly associated with unvaccinated international travellers or under-vaccinated communities (67). The United Kingdom recently reported an increase in cases of SSPE caused by measles virus in children, with six cases identified from 2017 to 2019: the highest incidence of SSPE in the UK since 2000 (66). In Vietnam, the first four months of 2025 recorded a total of over 81,000 cases across the countries.

Non-viral pathogens

Reported bacterial pathogens of encephalitis in children (68) include *Mycoplasma pneumonia*, *Chlamydia pneumonia*, *Borrelia burgdorferi* (Lyme disease), *Bartonella henselae* (cat scratch disease), *Listeria monocytogenes*, and *Mycobacterium tuberculosis* (Table 1.2) (12). Primary amoebic meningoencephalitis (PAM) caused by *Naegleria fowleri* has been documented as a universally fatal form of encephalitis in southern states in the US, Iowa, and Nebraska (69), hypothesised to be due to temperature rise, water warming, and extreme weather promoting the growth of this thermophilic organism (70). Eosinophilic encephalitis caused by *Baylisascaris procyonis* following ingestion of soil containing raccoon faeces has been found to be severe, often fatal, with neuropsychiatric conditions, and mostly occur in young children or adults with pica or occupational exposure with poor hand hygiene (71).

Table 1.2. Non-viral pathogens of encephalitis in children

Non-viral pathogens of encephalitis		
Bacterial pathogens	**Fungal pathogens**	**Parasitic pathogens**
• *Mycobacterium tuberculosis* • *Mycoplasma pneumoniae* • *Listeria monocytogenes* • *Borrelia burgdorferi* • *Brucella* species • *Leptospira* species • *Legionella* species • *Tropheryma whipplei* (Whipple's disease) • *Nocardia actinomyces* • *Treponema pallidum* • *Salmonella typhi* • Rickettsiae causing: Rocky Mountain spotted fever Endemic and epidemic typhus Q fever Ehrlichiosis	• *Cryptococcus* • Aspergillosis • Candidiasis	• Human African trypanosomiasis • Cerebral malaria • *Toxoplasma gondii* • Schistosomiasis • *Naegleria fowleri* • *Balamuthia mandrillaris* • *Acanthamoeba* species • *Baylisascaris procyonis*

Note: Adapted from "Encephalitis in US Children", by Messacar K, Fischer M, Dominguez SR, Tyler KL, Abzug MJ. Infect Dis Clin North Am. 2018 Mar;32(1):145-162 (12).

1.2. Autoimmune encephalitis

1.2.1. Case definition of autoimmune encephalitis

Autoimmune encephalitis is characterised by prominent neuropsychiatric manifestations and is caused by autoantibodies against neuronal cell surface proteins, ion channels, or receptors (72). Clinically, there are considerable overlap in presentations in patients with autoimmune encephalitis and infectious encephalitis. Currently, there are sixteen disorders where immunoglobulin G (IgG) autoantibodies against cell surface or synaptic proteins have been recognised (73). Of these, 12 manifest as autoimmune encephalitis including N-methyl-D-aspartate receptor (NMDAR), leucine-rich glioma-inactivated 1 (LGI1), contactin-associated protein-like 2 (CASPR2), gamma aminobutyric acid-A ($GABA_AR$), gamma aminobutyric acid-B ($GABA_BR$), dipeptidyl-peptidase-like protein 6 (DPPX), glutamic-acid-decarboxylase 65 (GAD65), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR), immunoglobulin-like cell adhesion molecule 5 (lgLON5), glycine receptor (GLyR), metabotropic glutamate receptor 1 (mGluR1), and neurexin-3-alpha (73). Of these, NMDAR-antibody encephalitis is the most frequent form reported worldwide (74–77).

The recognition of autoimmune encephalitis in 2005 has changed clinical practice. Accordingly, clinicians will now consider the diagnosis and treatment strategies in patients neurological and psychiatric syndromes that were previously considered to be idiopathic or not even suspected to be immune-mediated disorders (73). In support of this changes in clinical practice, in 2023, Sarosh R. Irani and colleagues also published a review updating clinical and biological advances of autoantibody-associated neurological diseases, detailing the associated clinical phenotypes, recent autoantibody discoveries, and a further knowledge of immunological, neurobiological, and pathophysiological mechanisms of these diseases (78). Additionally, the review also comprehensively

outlined the frequency syndromes associated with specific forms autoimmune encephalitis and the associated syndromes as shown in Figure 1.4 (78).

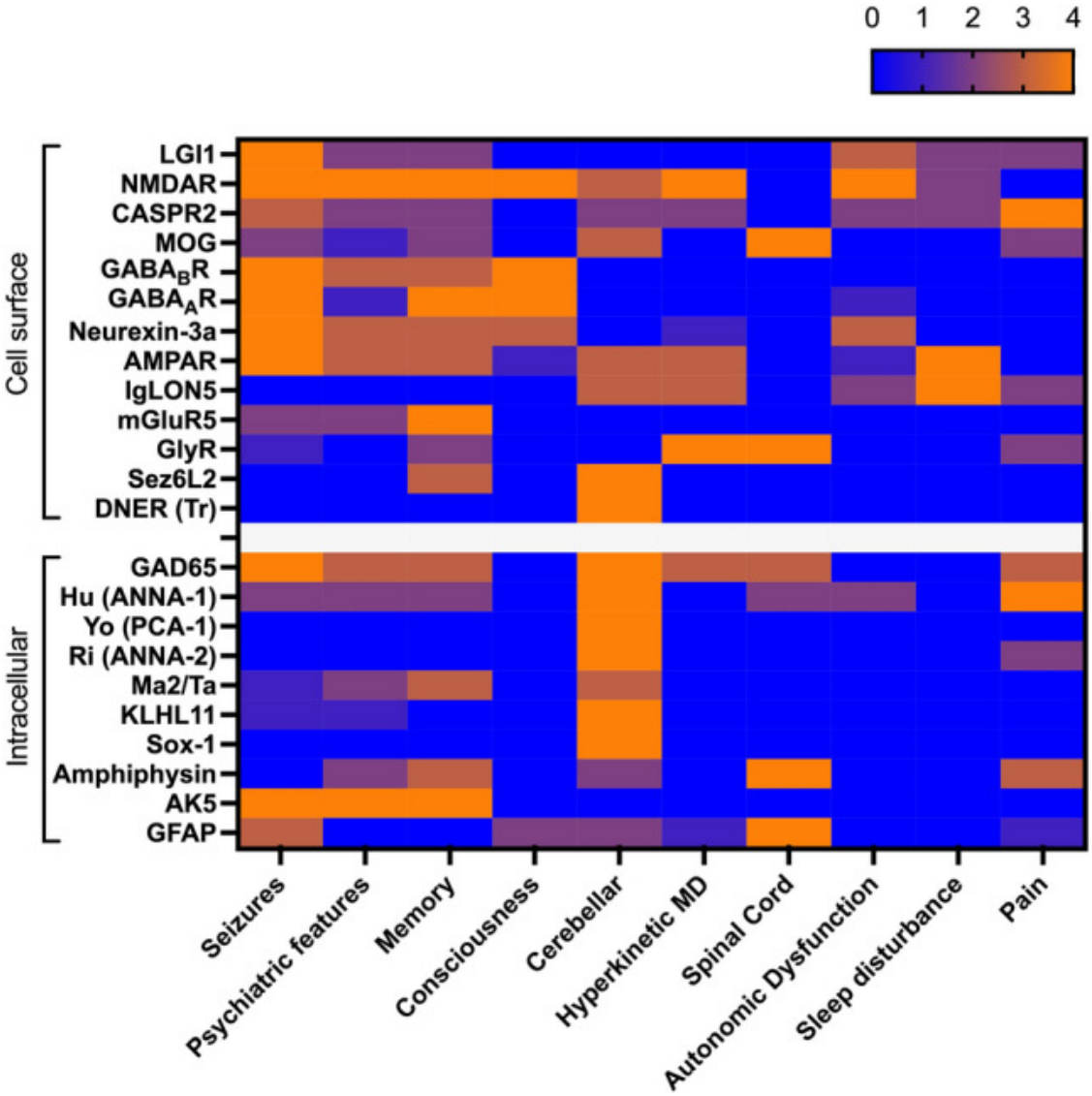


Figure 1.6. Heatmap illustrating the frequency of clinical features associated with autoantibodies in autoimmune encephalitis syndromes

Note: Adapted from "Autoimmune encephalitis: recent clinical and biological advances", by Varley JA, Strippel C, Handel A, Irani SR. *J Neurol*. 2023 Aug;270(8):4118-4131 (78).
Frequencies of features from rare or unknown (0=teal) to common (4=red).
LGI1 (leucine-rich glioma-inactivated 1), NMDAR (N-methyl-d-aspartate receptor), CASPR2 (contactin-associated protein-like 2), MOG (myelin oligodendrocyte protein), GABA$_B$R (γ-aminobutyric acid B receptor), GABA$_A$R (γ-aminobutyric acid A receptor), AMPAR (α-amino3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor), mGluR5 (metabotropic glutamate receptor 5), GlyR (glycine receptor)r, Sez6L2 (seizure-related 6 homolog like 2), DNER (delta/Notch-like epidermal growth factor-related receptor), GAD65 (glutamic acid decarboxylase (65 kDa isoform)), ANNA 1/2 (anti-nuclear neuronal autoantibody type ½), PCA (Purkinje cell cytoplasmatic autoantibodies), KLHL11 (kelch-like protein 11), AK5 (adenylate kinase 5), GFAP (Glial Fibrillary acid protein).

In 2017, the Autoimmune Encephalitis Alliance (USA), the Encephalitis Society (UK), the Anti-NMDA Receptor Encephalitis Foundation Inc (Canada), and the Anti-NMDA Receptor Encephalitis Patient Initiative (Germany) have proposed the guidelines entitled "A clinical approach to diagnosis of autoimmune encephalitis" in 2017 (1,3–5). Accordingly, autoimmune encephalitis is diagnosed when all three of the following criteria are met: 1. Subacute onset (rapid progression of less than 3 months) of working memory deficits (short-term memory loss), altered mental status, or psychiatric symptoms; 2. At least one of the following: new focal CNS findings, seizures not explained by a previously known seizure disorder, CSF pleocytosis (white blood cell count of more than five cells/mm^3), MRI features suggestive of encephalitis, and 3. Reasonable exclusion of alternative causes (1,3–5). It was also recommended that the diagnosis of NMDAR-antibody encephalitis can be made when all three of the following criteria have been met: 1. Rapid onset (<3 months) of at least four of the six major groups of symptoms including abnormal (psychiatric) behaviour or cognitive dysfunction, speech dysfunction (pressured speech, verbal reduction, mutism), seizures, movement disorder, dyskinesias, or rigidity/abnormal postures, decreased level of consciousness, autonomic dysfunction or central hypoventilation; 2. At least one of the following laboratory study results: abnormal EEG (focal or diffuse slow or disorganised activity, epileptic activity, or extreme delta brush), CSF with pleocytosis or oligoclonal bands, and 3. Reasonable exclusion of other disorders. Diagnosis can also be made in the presence of three of the above groups of symptoms accompanied by a systemic teratoma. A definite diagnosis of autoimmune encephalitis can be when evidence of the corresponding autoantibodies is detected in CSF of the patients (1,3–5).

1.2.2. Clinical features

The clinical course of autoimmune encephalitis including NMDAR-antibody encephalitis consists of four phases: 1-4, (4,79–84). The prodromal phase (phase 1) is characterised by fever, headache, nausea, vomiting, upper respiratory tract infection, and flu-like syndrome. In the illness phase (phase 2), cerebral MRI abnormalities or CSF pleocytosis can be seen, which decreases over several weeks without any obvious changes in the symptoms. The illness phase includes psychiatric phase and neurological phase. The psychiatric phase lasts for 1 to 2 weeks and the patient may present with behavioural changes, irritability, tantrums, coma, manic symptoms, behavioural outbursts, sleep dysfunction, and hyperactivity. The neurological phase lasts for weeks to months during which the patient may present with seizures (focal, motor, complex partial), dystonia, or status epilepticus). Speech and language changes will be recognised in weeks such as mutism and decreased responsiveness. In severe cases, cardiorespiratory dysfunction and failure in addition to autonomic instability can be seen which requires intensive treatment in the PICU. Speech dysfunction is more common than autonomic dysfunction and ventilation abnormality in children. Motor dysfunction or movement disorders, particularly orofacial dyskinesia, in addition to seizures can develop in this phase. Ataxia, difficulty walking and even lose the ability to walk can be seen in younger children. The recovery phase (phase 3) is characterised by the reversal of the signs and symptoms outlined above. Cognitive and psychiatric dysfunctions may last longer and these can be the last functions to improve. It may take a few months of treatment with immunotherapy and multidisciplinary care for patients to recover. During this phase, the evidence of inflammation on MRI and CSF is minimal and antibodies can even persist a few weeks after the complete recovery. In the late phase (phase 4), most patients make a full recovery in term of cognitive and behavioural function documented at discharge.

1.2.3. Epidemiology

In children, the first reported cases with NMDAR-antibody encephalitis came from China and dated back in 2010. NMDAR-antibody encephalitis is now recognised more frequently and commonly in the paediatric population and to some extent in some regions globally, this aetiology may surpass the viral causes for encephalitis (85). The reported incidence of NMDAR-antibody encephalitis in children is around 1.5 per million population per year, with the predominance of female patients, accounting for 75% of the reported cases (3,86–89). However, scarce data exists regarding the prevalence NMDAR-antibody encephalitis in children in Southeast Asia, including Vietnam.

1.2.4. Pathophysiology of NMDAR-antibody encephalitis

When NMDARs are activated, sodium and calcium ions are allowed to pass through these channels. The activation occurs because the magnesium block is removed, allowing glutamate and glycine to bind to their respective sites (82,90,91). These antibodies are produced in the CNS by the cells that can cross the blood-brain barrier. When antibodies bind to NMDARs, these receptors are internalised from the cell surface and lead to receptor dysfunction. It is believed that the dysfunctional receptor mechanisms in which the hypoactive receptors prevent normal tonic inhibition on the dopaminergic mesolimbic pathway lead to psychotic symptoms (90). It is also hypothesised that the expression in high density of NMDA receptors in the frontotemporal area specifically in the dorsolateral prefrontal cortex, hippocampus and ventral tegmental area leads to the characteristic abnormalities in behaviour, learning and memory of patients (92).

Figure 1.5 shows immunological facts and hypotheses for the pathophysiology of NMDAR-antibody encephalitis are showed in Figure 1.5 (73). Approximately 50% of young women with NMDAR-antibody encephalitis have an ovarian teratoma that contains nervous tissue. However, in children and men, the frequency of tumours is lower

and the histology is different when older men and women have carcinomas instead of teratomas. It is hypothesised that antigen released by apoptotic tumour cells is taken up by antigen-presenting cells, and then processed and presented to the immunologic system at the regional lymph nodes where memory B cells are generated and the antibody production by plasma cells is initiated (73). After crossing the blood-brain barrier through or the choroid plexus to reach the brain, the memory B cells would experience re-stimulation, antigen-driven affinity maturation, clonal expansion, and maturation into antibody-producing plasma cells, the presence of which has been demonstrated in studies of brain tissue from patients (73). Plasma cells are long-lived (for several months to years), refractory to the most frequently used immunotherapies (plasma exchange, IV immunoglobulin, rituximab), and protected by the blood-brain barrier from systemically administered drugs. These plasma cells lead to a prolonged synthesis of antibodies within the CNS, demonstrated by a relatively high concentration of antibodies in CSF compared to serum (intrathecal synthesis). CSF antibodies remain detectable for at least as long as the patients have active disease or substantial neurologic deficits. In some patients, the CSF and serum antibodies are detectable several months or years after clinical recovery (73).

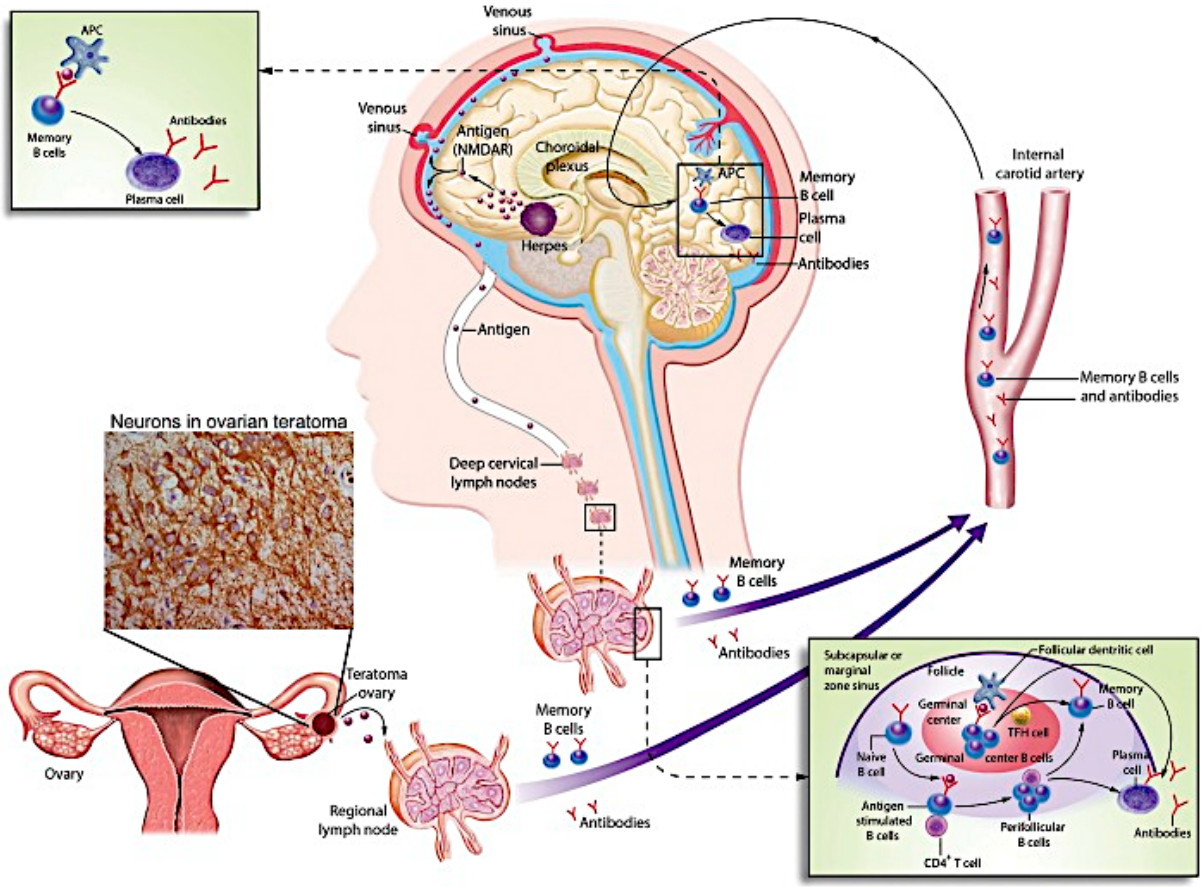


Figure 1.7. Immunologic triggers in NMDAR-antibody encephalitis

Note: Adapted from "NMDA receptor encephalitis and other antibody-mediated disorders of the synapse: The 2016 Cotzias Lecture", by Dalmau J. *Neurology*. 2016;87(23):2471-2482 (73).

1.2.5. Differential diagnosis

Autoimmune encephalitis including NMDAR-antibody encephalitis must be differentiated from other encephalitic conditions in children including drugs, post-infectious syndromes, autoimmune neuropsychiatric disorders associated with Streptococcal infections, autistic syndrome in acute encephalopathy, immunological chorea encephalopathy syndrome, acute diffuse lymphocytic meningoencephalitis, limbic encephalitis, acute juvenile non-herpetic encephalitis, acute disseminated encephalomyelitis (ADEM), inborn errors of metabolism, environmental toxins and medication overdose, rheumatological conditions such as neuropsychiatric lupus and primary psychiatric conditions such as schizophrenia. Due to the extensive differential diagnosis, the patient's diagnostic workup should be individualised (4,82–84,93).

1.2.6. Complications

Early diagnosis and treatment of autoimmune encephalitis, specifically NMDAR-antibody encephalitis have been proven to result in less damage to the hippocampus, however, the optimal time window between disease onset and treatment initiation is still not identified. It has been emphasised that recovery develops as a multi-stage process that occurs as the reversal of signs and symptoms (86,94).

1.3. Approach to patients with encephalitis

1.3.1. Medical history

The medical history may provide clues for a particular cause. When a patient presents with clinical features compatible with encephalitis, it is important to ask for specific information about travel and exposures to specific animals, insects, toxins, etc, particularly within the two to three weeks before the onset of clinical signs and symptoms. Vaccination history and immune status of the patient should also be reviewed (1,3,84,95).

This may be helpful in guiding the subsequent diagnostic pathway targeting the most likely causes.

1.3.2. Physical examination

The physical examination should include careful neurological investigations for specific signs or symptoms. Neurological evaluation should include an assessment of mental status, motor, sensory, cranial nerve, cerebellar, and reflex function. The paediatric Glasgow coma scale (GCS) score is helpful in quantifying the level of consciousness and monitoring neurological progression (1,3,84,95).

When combined with information about travel history, examination findings may suggest a possible aetiology but are not highly specific. For example, in individuals recently returning from a JEV endemic country, JE is possible. The vesicular rash in neonates with encephalitis may be suggestive of HSV, while maculopapular rash can happen those with West Nile virus (WNV) disease. Lesions compatible with hand, foot, and mouth disease in young children are suggestive of an EV, in particular EV-A71, infection (1,3,84,95).

1.3.3. Laboratory investigations

a. Routine blood tests

Laboratory blood tests that should be routinely performed in all patients with suspected encephalitis include a complete blood count, plasma electrolytes, glucose, blood urea nitrogen, creatinine, plasma aminotransferases (alanine aminotransferase, aspartate aminotransferase), and coagulation studies.

b. CSF analysis

Lumbar puncture (LP) must be performed in all patients with suspected encephalitis unless there are contraindications such as coagulopathy and intracranial hypertension. The contraindications of LP including mass lesion and midline shift would require the performance of neuroimaging such as brain ultrasound, MRI, or CT scan. Samples of CSF

should be sent for cell count and measurements of glucose, protein and lactate levels (1,3,84,95). The CSF indices in viral encephalitis are similar to those in viral meningitis and meningoencephalitis but it can also be confused with those of bacterial meningitis. CSF Protein is usually moderately elevated but generally <150 mg/dL. The CSF: plasma glucose ratio is usually normal range. Moderate reduction in CSF glucose can be seen with HSV and mumps; marked depression, often <10 mg/dL, occurs with tuberculous meningitis. Notably, CSF findings are completely normal in 3 – 5% encephalitis patients both adults and children (1,3,84,95).

1.3.4. Laboratory testing for infectious causes of encephalitis

a. Serological testing

The serological diagnosis is helpful for pathogens such as JEV and other arboviruses as they are often absent in CSF at the time hospital admission. (25,95,96). Albeit not specific, the choice of specific serological assays can be guided by clinical features, travel history, initial laboratory findings of the CSF, and the epidemiological factors (time of year, geographic locale, exposures). A four-fold change in antibody titers between two samples, especially plasma, collected 1-2 weeks apart is required to establish a confirmed diagnosis. However, in the absence of other causes, the detection of pathogen specific antibodies, e.g. JEV antibodies, in a single CSF samples can be considered as a confirmed diagnosis. A challenge in interpreting the results of serological diagnosis is the potential cross-reactivities between closely related species (e.g. JEV and DENV) in localities where they are co-circulating. In this circumstance, testing for multiple targets (e.g. JEV and DENV) are required and the obtained results should be interpreted based on the readouts, with a higher value favouring the infection caused by the corresponding virus.

b. Molecular testing of CSF and non-CSF samples

CSF PCR is the most commonly used molecular approach to identify a causative agent in patients with central nervous system infections, including encephalitis (97). Recent advancements in multiplex PCR technologies such as Biofire (98) and Seegene (99,100) have enabled at least 14 pathogens to be captured in a single assay, with an overall 94.2% sensitivity and 99.8% specificity.

Compared with CSF samples, other samples, including urine and plasma, and in particular sample from non-sterile sites (throat swabs and rectal swabs) might offer a higher detection rate. However, the detection of an infectious cause, in particular in cases of respiratory viruses, in those samples may not reflect a direct link with the pathology of encephalitis.

For EVs identification, although CSF PCR is the method of choice, PCR testing of throat and rectal swab might be useful. However, CSF PCR might be negative in EV encephalitis, and in case of EV-A71 associated encephalitis in patients with hand foot and mouth disease, the virus is rarely detected in CSF by PCR (96,101).

For HSV, CSF PCR is considered the gold standard and is the priority in patients with encephalitis. Additionally, culture and/or direct immunofluorescence assay (DFA) of skin lesions if present will also be helpful in detecting HSV, but those assays have limited clinical implication owing the long turnaround time and/or the specificity (98,103), and virus culture expertise is hardly maintained in clinical laboratories around the world nowadays.

For other herpesviruses (HHV-6, CMV, and EBV), as outlined above, CSF PCR results should be interpreted with caution because of latency and/or chromosomal integration (11,49,96,103). Further information about the diagnostic approaches to representatives of encephalitis pathogens are provided in Table 1.3 (6).

Extensive PCR testing has helped increase the diagnostic yields in patients with encephalitis. However, the number of cases with unknown causes remains higher than the number of cases with identified causes (104,105). Potential reasons might include the emergence of novel or rare pathogens, the miss-inclusion of cases with autoimmune, and the sensitivities of the PCR methods.

Table 1.3. Geographical location, risk factors and diagnostic evaluation of various pathogens causing encephalitis

Pathogen	Geographical location	Specific risk factor(s)	Ancillary investigation
Bartonella bacilliformis	South America	Sand-fly bite	- Antibody testing in blood and CSF - CSF and blood culture - CSF PCR
B. henselae	Worldwide	Contact with cats	- Antibody testing in blood and CSF - CSF culture - CSF and lymph node biopsy PCR
Borrelia burgdorferi	Europe, North America	Tick bite	- Antibody testing in blood and CSF - CSF PCR (low sensitivity)
Brucella spp	Africa, Middle East, southern Europe	Consumption of unpasteurized milk	- Antibody testing in blood and CSF - Blood PCR - Serum agglutination (titre >1:160)
Chikungunya virus	South America, Southeast Asia, China	Mosquito bite	- Antibody testing in blood and CSF
Coxiella burnetii	Worldwide	Contact with sheep and goats, consumption of raw meat	- Antibody testing in blood
Cryptococcus neoformans	Worldwide	Immunocompromised (HIV infection)	- CSF fungal culture - Cryptococcal antigen testing (CrAg) in CSF and blood - CSF India Ink staining - CSF PCR
Cytomegalovirus	Worldwide	Immunocompromised (HIV infection, transplant recipients), neonates	- CSF PCR
Dengue virus	(Central) America, South America, Southeast Asia, China, Australia	Mosquito bite	- Antibody testing in blood and CSF - Antigen (NS1) detection in blood or CSF
Eastern equine encephalitis virus	North, Central and South America	Mosquito bite	- Antibody testing in blood and CSF
Enteroviruses	Worldwide	Children, immunocompromised, unvaccinated (in case of poliovirus)	- CSF PCR - Throat or rectal swab PCR
Epstein–Barr virus	Worldwide	Immunocompromised (HIV infection, transplant recipients), primary infection in adolescents	- CSF PCR - Antibody testing in blood and CSF
Herpes simplex virus type 1 and 2	Worldwide	—	- CSF PCR - Antibody testing in blood and CSF (incl. ratios)
Human herpes virus 6 and 7	Worldwide	Immunocompromised (haematopoietic cell transplant recipients)	- CSF PCR
HIV	Worldwide	Risk factors for HIV infection	- CSF and blood PCR
Human polyomavirus 2 (JC virus)	Worldwide	Immunocompromised (HIV infection, T-cell impaired immunity)	- CSF PCR - MRI for confluent white matter lesions
Japanese encephalitis virus	Southeast Asia, India, Nepal, China	Mosquito bite	- Antibody testing in blood and CSF

Leptospira spp	Worldwide	Freshwater exposure, contact with rats	- CSF PCR - Antibody testing in blood and CSF - Urine PCR
Listeria monocytogenes	Worldwide	Immunocompromised (pregnancy, glucocorticoid therapy, haematological malignancies), consumption of raw meat and unpasteurized dairy	- CSF and blood culture - CSF PCR
Measles virus	Worldwide	Unvaccinated	- Antibody testing in blood and CSF - CSF PCR - Nasopharynx and urine PCR
Mumps virus	Worldwide	Unvaccinated	- Antibody testing in blood and CSF - CSF or saliva PCR
Murray Valley encephalitis virus	Southeast Asia, China, Australia	Mosquito bite	- Antibody testing in blood and CSF
Mycobacterium tuberculosis	Worldwide, more common in developing countries	Immunocompromised (HIV infection)	- CSF culture - Ziehl-Neelsen staining CSF - CSF PCR
Nipah virus	Southeast Asia, China	Contact with pigs and bats	- Antibody testing in blood and CSF
Rabies virus	Central and South America, Africa, India, Nepal	Bite or scratches from infected mammals (e.g. dogs, bats, monkeys, foxes, and raccoons)	- Antibody testing in blood and CSF - CSF or saliva PCR
Rickettsia rickettsii	North, Central and South America	Tick bite	- Antibody testing in blood and CSF - PCR on skin biopsy in case of skin lesions
Rift Valley fever virus	Africa	Mosquito bite, contact with infected animal (can occur in cows, sheep, goats, camels)	- Antibody testing in blood-Blood PCR
Rubella virus	Worldwide	Unvaccinated	- Antibody testing in blood and CSF
St. Louis encephalitis virus	North, Central and South America	Mosquito bite	- Antibody testing in blood and CSF
Tick-borne encephalitis virus	Europe, Russia, Southeast Asia, China	Tick bite	- Antibody testing in blood and CSF
Toscana phlebovirus	Southern Europe	Sand-fly bite	- Antibody testing in blood and CSF
Toxoplasma gondii	Worldwide	Immunocompromised (HIV infection), contact with cats, unpasteurized milk	- Antibody testing in blood and CSF - CSF PCR - MRI brain for typical lesions
Trypanosoma brucei spp.	Africa	Tsetse fly bite	- Direct microscopy - Antibody testing in blood and CSF
Usustu virus	Europe, Africa	Mosquito bite	- Antibody testing in blood and CSF
Varicella zoster virus	Worldwide	Immunocompromised (transplant recipients, immunosuppressive therapy)	- CSF PCR - Antibody testing in blood and CSF (incl. CSF to blood ratios) - PCR skin lesion
Venezuelan equine encephalitis virus	North, Central and South America	Mosquito bite	- Antibody testing in blood and CSF

West Nile virus	North and Central America, Europe, Africa, Middle East	Mosquito bite	- Antibody testing in blood and CSF
Western equine encephalitis virus	(Central) America, South America	Mosquito bite	- Antibody testing in blood and CSF
Zika virus	Southern hemisphere	Mosquito bite	- CSF PCR - Antibody testing in blood and CSF - Urine PCR

Note: Adapted from "Diagnosing infectious encephalitis: a narrative review", by Olie SE, Staal SL, van de Beek D, and Brouwer MC. *Clinical Microbiology and Infection*. 2025 Apr;31(4):522–8 (6).

CSF: cerebrospinal fluid; MRI: magnetic resonance imaging.

Central nervous system lymphoma and post-transplant lymphoproliferative disease should be considered if Epstein–Barr virus DNA is found in CSF.

c. Metagenomic next-generation sequencing

Metagenomic next-generation sequencing (mNGS) is an assay that can be used for the detection of known/unknown pathogens, with a pooled sensitivity of 77% (95% CI: 70-82%) and a pooled specificity of 96% (95% CI: 93-98%) for all pathogens (106). mNGS overcomes the limitations of conventional diagnostic methods such as PCR and culture because it does not require any prior knowledge/assumptions about the targeted causative agents present in the tested samples. Therefore, in a single assay, mNGS could potentially detect all viral agents (including known and previously unknown viruses) in various clinical sample types. This offers a new opportunity for virus detection and discovery, critical to pandemic preparedness and response.

A negative mNGS finding should be interpreted with caution owing to the possibility of false negative results as the sensitivity of mNGS has not been well verified for all possible causes (107–109). However, when mNGS is performed in combination with conventional testing, it may potentially be useful in ruling out the infectious causes.

Where viral loads are sufficiently high, mNGS can generate enough sequence date for pathogen evolutionary analysis. After the COVID-19 pandemic, genomic surveillance and monitoring of novel pathogens causing diseases are high priorities for the World Health Organisation. Genomics can provide key insights into pathogen evolutionary trajectories and the associated clinical and public health consequences. This is critical to the development of intervention strategies and health policies, as demonstrated during the COVID-19 pandemic, and equally relevant to endemic and emerging infections. In this context, mNGS has the potential to transform my current diagnostic approach in patients with infectious diseases. Yet, analysing the massive datasets generated by mNGS remains a major challenge, requiring sophisticated computational tools and expertise. Additional challenges include high cost and long turnaround time. Those collective challenges

represent major barriers in bringing mNGS into clinical laboratories, and its use has been restricted to research purpose only.

1.3.5. Laboratory testing for the causes of autoimmune encephalitis

The CSF and/or plasma samples of patients can be immunologically tested for common auto-antibodies against glutamate receptor types NMDAR (N-methyl-D-aspartate) and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid), GABA (γ-amino butyric acid) receptors, components of the voltage-gated potassium channels (VGKC, DPPX: dipeptidyl aminopeptidase-like protein 6) or VGKC-associated proteins (LGI1: leucine-rich glioma-inactivated protein 1, CASPR2: contactin-associated protein 2). CSF is more sensitive than plasma/serum in diagnosis autoimmune encephalitis.

1.3.6. Neuroimaging studies

Patients with suspected encephalitis should undergo neuroimaging with magnetic resonance imaging (MRI) or computed tomography (CT) (110). Neuroimaging findings in paediatric encephalitis are different from adults (111,112) ranging from normal MRI to severe conditions including haemorrhagic lesions, necrotising lesions and brain oedema (113). The association between abnormal brain MRI images with severity of encephalitis and short-term neurological outcomes has been reported when MRI findings has been found to directly correlate with length of hospital stay and the severity of encephalitis (113,114). Poor long-term outcome is significantly associated with abnormal brain MRI and parenchymal involvement, areas with diffusion restriction, and focal cortical findings on brain MRI (115). Abnormalities recognised by MRI may include cerebral oedema and inflammation of the cerebral cortex, gray-white matter junction, thalamus, or basal ganglia (116). Haemorrhagic findings on MRI may be recognised in HSV encephalitis (117).

MRI is the first of choice for neuroimaging in paediatric encephalitis because it is more sensitive to detect lesions for encephalitis than CT (113). In a study of The California Encephalitis Project between 2005 and 2012 at Rady Children's Hospital San Diego in California, US, abnormal findings were recognised on 23% (22/94) of CT and 50% (67/134) of MRI studies (113). Notably, 20 paediatric encephalitis cases with normal CT at admission had abnormal findings on MRI performed within 2 days (113). Abnormal MRI findings are more common than abnormal CT findings in paediatric encephalitis (113). Increasing complexity of MRI findings correlated with disease severity as evidenced by longer length of stay, but were not specific for an identifiable pathogen (113).

In NMDAR-antibody encephalitis, MRI images are often subtle and nonspecific, with common abnormal findings such as T2/FLAIR hyperintensity in the limbic system (including temporal lobes and hippocampus) (Figure 1.6) (118), cerebral cortex, and less commonly, the basal ganglia, brainstem, and cerebellum (119).

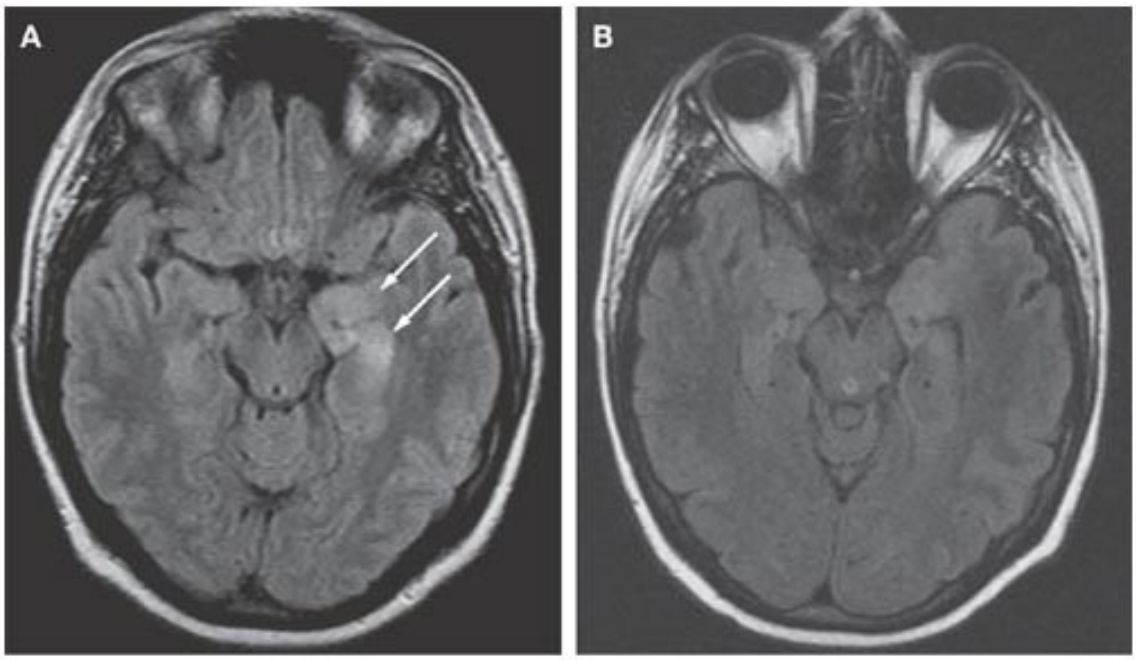


Figure 1.8. MRI study of a patient with NMDAR-antibody encephalitis

Note: Adapted from "A patient with encephalitis associated with NMDA receptor antibodies", by Sansing L., Tüzün E., et al. *Nat Rev Neurol*. 2007 (3):291–296 (118).

(A) MRI fluid-attenuated inversion recovery (FLAIR) obtained at symptom presentation demonstrates bilateral medial temporal lobe hyperintense signal, predominantly involving the left hippocampus (arrows).

(B) Follow-up MRI obtained during recovery, 4 months after the initial MRI, shows considerable improvement of the FLAIR hyperintensity.

If MRI is unavailable or is not feasible, CT with and without contrast enhancement is an acceptable alternative particularly in the initial evaluation (1,95). In clinical practice, because CT is often more readily available than MRI is, and because it is faster and generally does not require sedation/anaesthesia, many children undergo initial evaluation with CT before LP to exclude mass lesion, midline shift haemorrhage and then subsequently underwent MRI. In addition to the standard T1- and T2-weighted images, diffusion-weighted imaging (DWI) increase the sensitivity of MRI, particularly in the early course of encephalitis. In some circumstances, findings on MRI may lead to a presumptive diagnosis of HSV encephalitis despite an initial negative CSF HSV PCR (120). MRI findings depend on the specific viral aetiology. However, most findings are not highly sensitive or specific for a particular pathogen. Typical findings include (116,117,120):

HSV typical appearances include temporal lobe enhancement, however, temporal localisation also may occur with other herpes viruses and syphilis. Flavivirus, Eastern equine encephalitis virus may have lesions in the thalamus, basal ganglia, and midbrain that are of mixed intensity or hypodense on T1 and hyperdense on T2 and fluid-attenuated inversion recovery (FLAIR) images. EV 71 encephalitis often show hyperintense T2 and FLAIR lesions in the midbrain, pons, and medulla. Respiratory virus encephalitis (e.g., influenza, parainfluenza, adenovirus, respiratory syncytial virus) is characterised by abnormalities in the thalamus or basal ganglia.

Neuroimaging also may detect other conditions that are in the differential diagnosis (116,117,120) such as post-infectious encephalitis or acute disseminated encephalomyelitis (ADEM) with deep and subcortical white matter lesions are typically multiple and bilateral but may be asymmetric. Brainstem and spinal cord abnormalities are common. Gray matter lesions may be observed in the thalami and basal ganglia.

However, particularly for head trauma or intracranial haemorrhage, in most cases, CT is the preferred imaging modality for the initial evaluation of suspected head trauma or intracranial haemorrhage. CT is also more helpful than MRI for the detection of CNS tumours, congenital infection, space-occupying lesions or unifocal or multifocal ring-enhancing lesions.

Cerebral MRI in NMDAR-antibody encephalitis demonstrates bilateral T2 or FLAIR signal hyperintensities in hippocampi, frontal cortex, medial temporal lobe, cerebellar cortex, spinal cord, and medulla oblongata. However, these findings were evident in only 30% of the patients in a multi-institutional observational study conducted by Titulaer and colleagues between Jan 1, 2007, and Jan 1, 2012 with enrolled 577 patients both adults and children (median age 21 years, range 8 months to 85 years), 211 of whom were children (<18 years) at the Hospitals of the Universities of Pennsylvania in the US and Barcelona in Spain (121). Most commonly, lesions present in the hippocampus, and therefore, they are the main predictor of poor prognosis in patients (121).

1.3.7. Electroencephalogram

Electroencephalogram (EEG) may help to differentiate encephalitis from seizure activity caused by other neurological diseases (i.e. partial complex seizures, absence seizures) including epilepsy, head injuries, brain tumours, genetic conditions, etc. For practical reasons, the EEG is typically performed after the initial evaluation in the emergency department or other acute care settings (1,95,122). Continuous EEG may be helpful in documenting or excluding seizures and seizure-mimic conditions if routine EEG is negative or not conclusive (1,95,122). EEG is abnormal in 87 to 96% of children with encephalitis and most findings are nonspecific. Delta brush was found to be commonly associated with NMDAR-antibody encephalitis (Figure 1.7) (123). EEG findings range from generalised slowing to patterns characteristic of specific aetiologies such as the

temporal focus of HSV encephalitis (1,95,122). EEG in NMDAR-antibody encephalitis shows a slow, continuous rhythmic and disorganised activity in delta and theta range superimposed seizures, as 90% of patients have a slowing of EEG at some point during the illness (89).

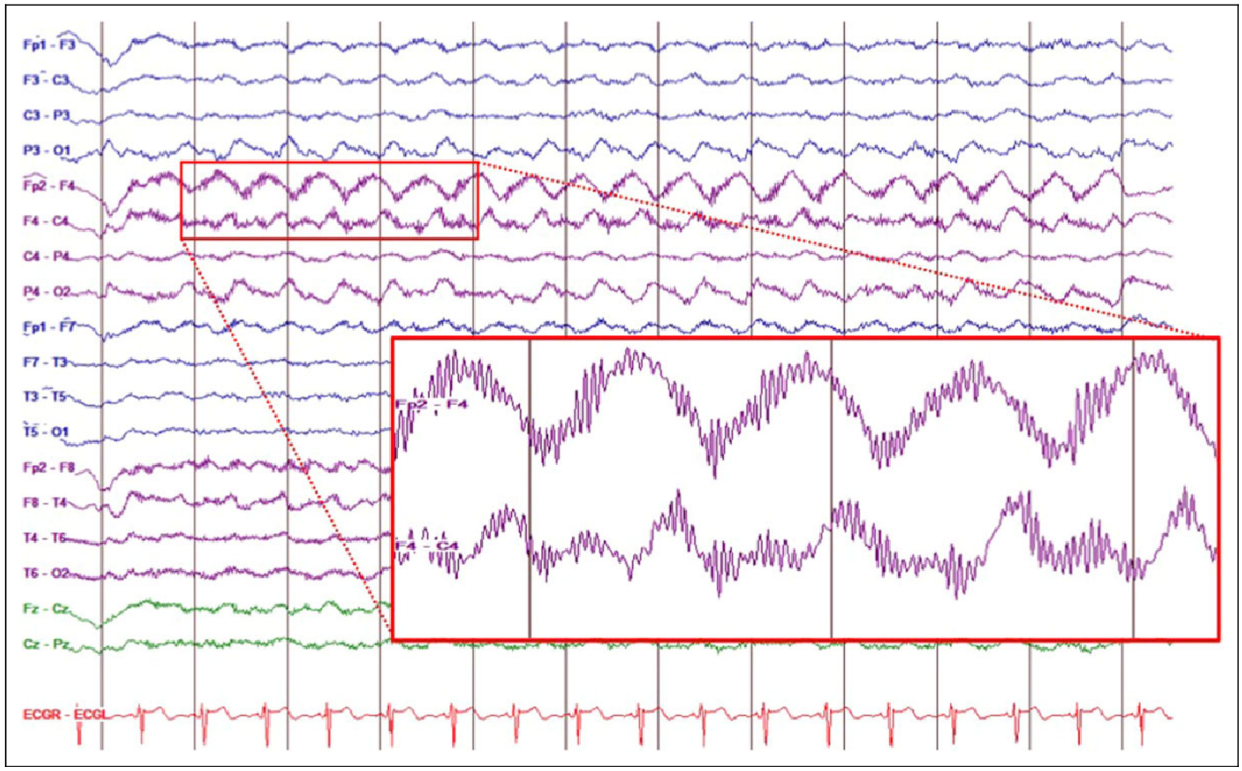


Figure 1.9. Extreme Delta Brush from EEG reading of a patient with NMDAR-antibody encephalitis

Note: Adapted from "Extreme Delta Brush in NMDA Receptor Encephalitis", by Castellano J, Glover R, Robinson J. *Neurohospitalist*. 2017 Jul 14;7(3):NP3–4 (123).

1.3.8. Evaluation for other causes of encephalopathy

Additional testing is performed to exclude other causes of encephalopathy including bacterial sepsis or meningitis, metabolic disorders, drugs and toxins. These investigations include CSF Gram stain, acid fast stain, and culture, urine and serum toxicology screening, and metabolic studies (plasma ammonia, lactate, and blood gases) if they are clinically indicated (1,3–5). Cerebral biopsy is the last resort, rarely performed because of the ethical issue, and it may be indicated if a patient with an unknown aetiology of encephalitis continues to deteriorate neurologically despite acyclovir therapy and all other investigations (25,95).

1.4. Treatment

1.4.1. Principles of treatment

Encephalitis is an acute, life-threatening emergency clinical situation, requiring prompt intervention. Assessment and management are often performed simultaneously. Provision of empiric antimicrobial therapy and supportive care are the cornerstones of therapy for viral encephalitis. Empiric therapy can be changed if and when a specific pathogen or alternative diagnosis is identified (1,5,95,102,124).

1.4.2. Infectious encephalitis treatment

a. Empiric acyclovir

For infants and children beyond the neonatal period who present with suspected encephalitis, it is recommended that intravenous (IV) acyclovir should be promptly initiated pending viral investigation (1,5,95,102,124). HSV encephalitis is a devastating infection with a mortality of up to 70% if untreated but the survivors still have neurological deficits even when appropriately treated (48).

The dose of acyclovir varies depending on age (1,5,95,102,124) including >28 days to <3 months (20 mg/kg per dose IV every eight hours); ≥3 months to <12 years (10 to 15 mg/kg per dose IV every eight hours); an increased dose (20 mg/kg per dose every eight hours) is approved by the US Food and Drug Administration for the treatment of HSV encephalitis in this age group, but the risk of nephrotoxicity may be increased; consultation with an infectious disease or pharmacology specialist may be warranted if administered with other nephrotoxic drugs or weight-based dosing exceeds 800 mg per dose; ≥12 years (10 mg/kg per dose IV every eight hours).

The duration of empiric acyclovir therapy depends upon laboratory results (1,5,95,102,124). In cases with confirmed or probable HSV, if HSV polymerase chain reaction (PCR) from CSF or other sites is positive, acyclovir should be continued for 21 days (1,5,95,102,124). LP should be performed near the end of acyclovir treatment to ensure that HSV PCR is negative; acyclovir therapy should be continued if CSF HSV PCR remains positive (1,5,95,102,124). In cases with HSV negative PCR, the decision to continue acyclovir therapy must be individualised. Repeat LP may be warranted in some cases because CSF HSV PCR testing can be negative during the first few days of the illness. Likewise, if there are strong clinical indicators of HSV encephalitis (e.g., temporal spikes on electroencephalogram (EEG) or temporal lobe involvement on imaging), repeat LP may be warranted to exclude the possibility of a false-negative result on initial testing. Repeat LP may also be warranted in patients with severe neurological dysfunction even in the absence of clinical indicators of HSV, particularly if no specific alternative aetiology has been identified.

b. Empiric antibiotics treatment

The clinical manifestations and CSF values of bacterial meningitis and viral encephalitis may overlap. Given the poor outcomes of delayed use of antibiotics for bacterial

meningitis, the threshold for the initiation of empiric antimicrobial therapy in patients with suspected viral encephalitis and not yet excluded bacterial meningitis should be relatively low (1,5,95,102,124,125).

c. Other treatments

Levodopa or dopaminergic agents have been trialled in some cases to alleviate parkinsonian symptoms, but their efficacy in JEV-related parkinsonism remains limited and poorly studied (38–44). The parkinsonian phase, while not exclusive to JEV, is a hallmark of its neurological impact in children, emphasizing the importance of early diagnosis and intervention to mitigate long-term disability (38–44).

Management of movement disorders in JEV encephalitis is challenging, as no specific antiviral therapy exists, and supportive care remains the cornerstone (38–44). Symptomatic treatment with levodopa or anticholinergics may provide partial relief for parkinsonism, while dystonia may respond to benzodiazepines or baclofen, though outcomes vary (38–44). Rehabilitation and physiotherapy are critical to improve functional outcomes, particularly in children with persistent deficits.

1.4.3. Autoimmune encephalitis treatment

a. Immunotherapy for paediatric encephalitis

The International Consensus Recommendations for the treatment of first event of paediatric NMDAR-antibody encephalitis has been published in 2021 by a panel of 27 experts with representation from all continents (Figure 1.8) (125). Accordingly, first-line immunotherapy for paediatric NMDAR-antibody encephalitis includes intravenous high-dose steroids (methylprednisolone), intravenous immunoglobulin (IVIG), and/or plasmapheresis (4,81,84,126). Second-line immunotherapy includes targeted B-cell therapy with rituximab and cyclophosphamide (4,81,84,126).

Although first-line immunotherapy (corticosteroids, IVIG, plasmapheresis) is widely used in the treatment of NMDAR-antibody encephalitis, evidence regarding the best efficacy and safety of different first-line immunotherapy combinations is still limited and contrasting. A meta-analysis of use and safety of immunotherapy in NMDAR-antibody encephalitis with data from 1550 patients from 652 articles (127) found that plasmapheresis alone (increased odds of good outcome = 5.6); combination of corticosteroids and IVIG (increased odds of good outcome = 2.7); combination of corticosteroids, IVIG, and plasmapheresis (increased odds of good outcome = 2.8) were effective in the treatment of NMDAR-antibody encephalitis.

Importantly, lack of immunotherapy within 30 days of disease onset (363/728 patients (49.9%) in the total literature review cohort) was associated with 2.7-fold increased odds of poor outcome. Also, with this review and meta-analysis (127), regarding second-line immunotherapies, rituximab administration was associated with 5.9-fold reduced odds of relapse after 24 months or more follow-up. There has been an emergence in the use of second-line therapies, such as intravenous or intrathecal methotrexate, subcutaneous or intravenous bortezomib, and intravenous tocilizumab; however, their efficacy and safety in NMDAR-antibody encephalitis need further research (127).

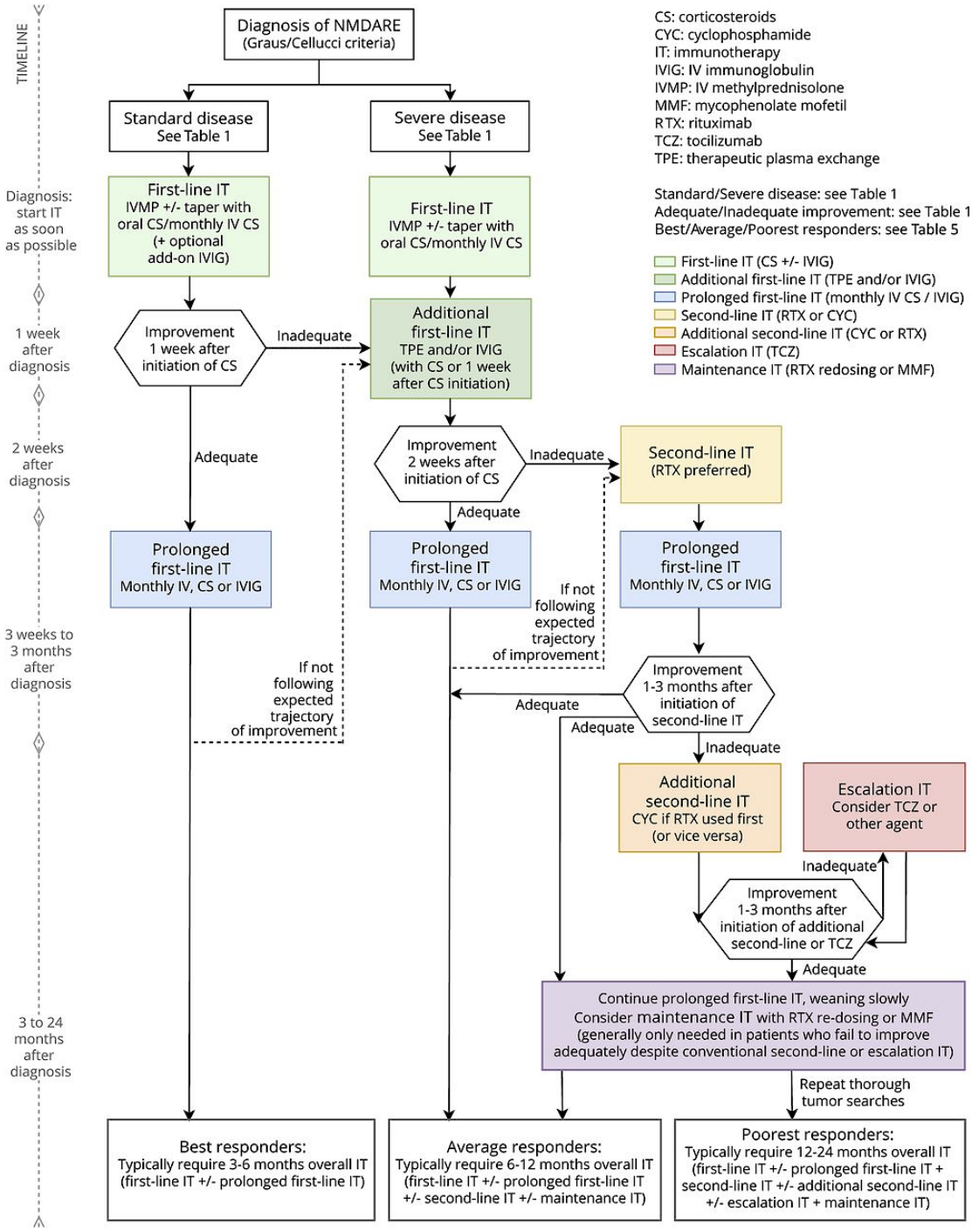


Figure 1.10. International Consensus Recommendations for the Treatment of First Event of Pediatric NMDAR-antibody Encephalitis

Note: Adapted from "International Consensus Recommendations for the Treatment of Pediatric NMDAR Antibody Encephalitis", by Nosadini M et al. *Neurol Neuroimmunol Neuroinflamm*. 2021 Jul 22;8(5):e1052 (125).

b. Symptomatic treatment in autoimmune encephalitis

Melatonin (<5 years: 1-2 mg/dose, 6-12 years: 1-3 mg/dose, >12 years: 1-5 mg/dose) can help to reverse sleep disturbances in children. Valproate (10-15 mg/kg/day divided into 2 doses, maximum 60 mg/kg/day); levetiracetam (10-20 mg/kg/day divided into 2 doses, maximum 40-60 mg/kg/day); carbamazepine (10-30 mg/kg/day divided into 2 doses); oxcarbazepine (10-60 mg/kg/day divided into 2 doses), midazolam (0.1-0.2 mg/kg/dose, maximum 3 doses); and phenobarbital (intravenous dose: 10-20 mg/kg/dose, can be repeated after 8-12 hours (maximum 30 mg/kg/day), oral dose: 3-8 mg/kg/day) are commonly used medications for treating seizures or epilepsy in paediatric encephalitis (4,81,84,126). Movement disorders can be treated with clonazepam (oral dose: 0.02-0.2 mg/kg/day, maximum 1 mg/day or slow intravenous dose: 0.1-0.2 mg/kg/dose, maximum 3 doses); diazepam (oral dose: 0.4-1 mg/kg/day, divided into 2-3 doses/day, maximum 5 mg/dose); trihexyphenidyl (oral dose: 0.2-2.5 mg/kg/day, divided into 2 doses), baclofen (oral dose: 0.75-2 mg/kg/day, divided into 2 doses), and risperidone (oral dose: 0.01-0.06 mg/kg/day, divided into 2 doses) (80,82–84,93,128).

c. Intensive care treatment of autoimmune encephalitis

Autonomic dysfunction, abnormal ventilation, cardiac arrhythmia, or hyperkinetic crisis can occur in children presenting with encephalitis clinical features. These children should be managed in the neurocritical or intensive care unit. Teratoma resection must be guaranteed if the tumour is present. Cardiac arrhythmia is one of the most common autonomic and hemodynamic instabilities seen in the paediatric population suffering from NMDAR-antibody encephalitis. Bradycardia is associated with seizures in the paediatric age group; hence, round-the-clock ECG is essential. Medications such as glycopyrrolate or theophylline have been shown to prevent severe bradycardia. Autonomic dysfunction can be recognised as fluctuating temperature and abnormal blood pressure. Movement

disorders exacerbate during a febrile state and it is essential to maintain temperature control using antipyretics (84). Mechanical ventilation with sedation including morphine or midazolam or fentanyl infusion can be considered in cases of cardiorespiratory failure. Noise control can help to avoid agitation. Muscle relaxants concomitant to corticosteroid therapy should be avoided in children in order to prevent myopathies (84,129,130). Tracheostomy and gastrostomy have been shown to be effective for facilitating respiratory improvement and nutritional support. A comprehensive rehabilitation program is essential and should include physical therapy, occupational therapy, and speech therapy (131).

d. Adjunctive therapies

Based on the currently available evidence, it is recommended not to routinely treat children with infectious encephalitis with adjunctive therapies including glucocorticoids, intravenous immune globulin, interferon-alpha, and therapeutic hypothermia (132–135). The adjunctive therapy in autoimmune encephalitis include complementary therapies like yoga, acupuncture, massage, nutrition, and mindfulness, as well as potential medications like tocilizumab or bortezomib for refractory cases (136). Although observational studies have described beneficial effects in some limited settings, evidence from controlled trials is lacking. Pending results of an ongoing multicentre randomised trial evaluating adjunctive dexamethasone for herpes simplex virus (HSV) encephalitis, glucocorticoids are not recommended in the routine management of children with HSV encephalitis (137).

e. Monitoring

Paediatric patients with severe encephalitis with clinical features including seizures, cardiorespiratory compromise, coma, or severe neurologic compromise) should closely monitored. The paediatric GCS can be helpful in quantifying the level of consciousness and monitoring neurological progression. Acute deterioration in the neurological status such as new focal findings, loss of pupillary reactivity, and rapid decline in paediatric

GCS score should prompt neuroimaging study to evaluate for cerebral oedema, haemorrhage, or other acute changes. Repeat neuroimaging may also be needed in patients who do not improve as expected over the initial treatment. CT is typically performed to evaluate acute changes and MRI is performed when more details for diagnosis and prognosis is required (137).

f. Fluid balance and electrolytes

Fluid and electrolyte disturbances are commonly associated with severe encephalitis in critically ill children and it is important to monitor input-output balance and daily weight, as well as plasma electrolytes through medical biochemistry testing. Appropriate volume expansion with 20 mL/kg of normal saline bolus may be of benefit in hypovolemia. Subsequent fluid management generally consists of intravenous fluids to maintain euvolaemia. Enteral feeding tubes are used to provide adequate nutrition in patients who are not able to feed by mouth. Parenteral nutrition is generally reserved for patients who are unable to resume enteral feeding after one week.

g. Management of complications

Potential complications recognised in encephalitis include status epilepticus, cerebral oedema, fluid and electrolyte disturbances caused by the syndrome of inappropriate antidiuretic hormone secretion (SIADH), and sudden cardiac and respiratory collapse. Status epilepticus should be treated intensively. There is insufficient evidence for the routine use of antiepileptic drugs for the prevention of seizure caused by high body temperature in viral encephalitis (1,5,95,102,124). Fluid restriction is not typically necessary except that for cerebral oedema which is treated with mannitol or sodium chloride 3%.

1.4.4. Sequelae

In self-limiting cases, lethargy and coma gradually improve over days to weeks. In severe cases, the long-term outcome varies with devastating conditions and neurological deficits may last longer. JEV may suffer from long-term sequelae including paralysis, hemiplegia, and reduced muscle strength. In a case series of 93 children with acute encephalitis in 2000–2004 at Neuropediatric Unit, Department of Women's and Children's Health, Karolinska University Hospital, Stockholm, Sweden, 71 were eligible for follow-up evaluations, and only 24/71 children (33.8%) had complete recovery within 6 to 12 months (22). Neurological features including personality change, behaviour disorder (including attention deficit disorder), movement disorder (including tic disorders), intellectual disability, learning disorders, blindness, paresis, ataxia, recurrent headaches, and sleeping problems may persist over a longer time (23). In a study at Rady Children's Hospital San Diego from the California Encephalitis Project in the US of 99 children with encephalitis at a mean duration of follow-up of 35.6 months sequalae occurred in half of the cases: learning problems (23%), developmental delay (19%), behavioural problems (10%), motor deficit (2%), visual defects (1%), hearing impairment (1%), and bladder spasticity (1%) (138).

1.5. Prevention

1.5.1. General preventive measurements

Because treatment options for encephalitis are limited, prevention is paramount (139,140). Primary prevention measures include hand washing; appropriate identification and treatment of genital herpes simplex virus (HSV) infection during pregnancy to prevent neonatal HSV; routine immunisation of infants, children, and adolescents (for measles, mumps, rubella, influenza) and appropriate immunisation of travellers

depending upon their destination(s) (e.g., Japanese encephalitis vaccine, tick-borne encephalitis virus vaccine); insect control and avoidance of mosquito and tick exposure (e.g., draining stagnant water, appropriate dress, use of mosquito and tick repellents, such as DEET [N,N-diethyl-3-methylbenzamide], mosquito netting, looking for ticks after hiking, etc); and appropriate viral screening of blood products (e.g., West Nile virus).

1.5.2. Vietnam immunisation programme and its coverage of viral pathogens of encephalitis

The Expanded Programme on Immunisation (EPI) was introduced as the Vietnamese national immunisation programme in 1981, and currently provides vaccines including: *Mycobacterium tuberculosis*, DPT (diphtheria, pertussis, tetanus), MMR (measles, mumps, rubella), varicella-zoster virus (VZV), poliovirus, hepatitis B virus (HBV), *Haemophilus influenzae* type b, and JEV (141). The rabies vaccine is currently not given to children in Vietnam as part of the EPI. The rabies vaccine is not given routinely to all children in Vietnam. Pre-exposure prophylaxis is optional and recommended for high-risk groups, while post-exposure prophylaxis is given only after suspected rabies exposure. Likewise, pneumococcal vaccines including PCV13 (pneumococcal conjugate vaccine 13-valent) and PCV23 (pneumococcal conjugate vaccine 23-valent) have not been included in the EPI. Vietnam has planned to introduce 4 vaccines including Dengue virus, pneumococcal vaccines, human papillomavirus (HPV), and seasonal influenza vaccine into the EPI from 2025 onward. All viral pathogens listed above have been reported to be causative agents of encephalitis in human including children (142). Therefore, several infectious causes of encephalitis are preventable by the current EPI.

1.6. Cost of illness of encephalitis

The total cost of illness consists of direct medical costs, direct nonmedical costs, and productivity costs, which encompassed the period before hospital admission, during hospitalisation and post hospital discharge. Yet few studies, especially from LMICs, have been conducted to comprehensively assess the illness costs of paediatric encephalitis, in particular in the context of the emergence of autoimmune encephalitis. Based on a nationally representative database of hospitalisation the total cost of encephalitis-associated hospitalisation in the USA was estimated to be US$2.0 billion in 2010 (143). A longitudinal follow-up study in Nepal of acute encephalitis syndrome in children from two hospitals in Nepal (144) where the median out-of-pocket cost to families, including medical bills, medication and lost earnings, was US$1,151 for children with severe/moderate impairment and US$524 for those with mild/no impairment (144). In a Cambodian study that interviewed affected families, it was found that costs related to acute hospital admission were the major cost drivers (145). Medication was identified to be the predominant acute medical cost associated with paediatric encephalitis in Indonesia (146). The average economic burden due to hospitalisation of a child with encephalitis in the US is estimated to be between US$64,000 and US$260,000, depending on the level of healthcare and rehabilitation (9,12,19). However, detailed data on their economic burden remains highly limited in the literature. In addition to medical costs associated with hospitalisation, paediatric encephalitis causes an additional burden for the affected families and the societies of the endemic countries. In Vietnam, there has been only one cross-sectional retrospective study using a micro-costing approach from the health system and household perspectives that was conducted to estimate the cost of acute care, initial rehabilitation and sequelae care with 242 patients in Vietnam and 65 patients in Laos, with laboratory-confirmed Japanese encephalitis. The study found that in Vietnam, the

mean total cost was $3,371 per acute Japanese encephalitis episode (median $2,071, standard error $464) while annual costs were $404 for initial sequelae care (median $0, SE $220) and $320 for long-term sequelae care (median $0, standard error $108); while in Laos, the mean hospitalisation costs in acute stage were $2,005 (median $1,698, SE $279) and the mean annual costs were $2,317 (median $0, standard error $2,233) for initial sequelae care and $89 (median $0, standard error $57) for long-term sequelae care. Japanese encephalitis patients and their families in Vietnam and Laos suffer extreme medical, economic, and social hardship (147).

1.7. Knowledge gaps and hypotheses

In summary, encephalitis is a devastating clinical problem worldwide, but especially in low- and middle-income countries. The emergence of new pathogens that can cause encephalitis (including Nipah virus, enterovirus A71 and Hendra virus), the wide spectrum of known viruses responsible for this devastating condition, the low sensitivity of current diagnostic methods and the high mortality and morbidity associated with encephalitis represent major challenges for routine diagnostics. However, this in turn makes encephalitis an attractive candidate for molecular diagnostic assays, especially multiplex real-time PCR and in particular sequence-independent assays such as metagenomics. However, few studies exploring the potential of multiplex real-time PCR and metagenomics in patients with encephalitis have been conducted in Southeast Asia. Additionally, over the last decades, autoimmune encephalitis has emerged as an important differential diagnosis in patients presenting with encephalitis. Of these, NMDAR-antibody encephalitis is a predominant cause. Yet, there are no existing data detailing the epidemiology of autoimmune encephalitis, particularly NMDAR-antibody encephalitis in Vietnamese children. In addition, data on illness costs of paediatric encephalitis is

currently lacking, but such knowledge is crucial to inform local policymakers in prioritising resources for improved diagnosis and treatment and public health interventions.

1.8. Aims, objectives, and result chapter outline

Collectively, in order to inform future intervention studies, my PhD research aims to improve current knowledge about the causes of paediatric encephalitis in southern Vietnam and the associated epidemiology, clinical characteristics, outcomes, and illness costs. I hypothesise that i) NMDAR-antibody encephalitis is an important differential diagnosis in Vietnamese children presenting with encephalitis, and is associated with high illness costs, and ii) molecular methods (PCR and in particular mNGS) can improve the diagnostic yield in Vietnamese children with clinically suspected infectious encephalitis. Accordingly, to address those research hypotheses, I set out to conduct an observational study of encephalitis in children admitted to the Children's Hospital 1 in Ho Chi Minh City, Vietnam, a tertiary, referral hospital for children from southern provinces in Vietnam. My specific objectives are:

1. To unravel the epidemiology, clinical profiles, and in-hospital outcomes of NMDAR-antibody encephalitis in children in Southern Vietnam;
2. To comprehensively identify the infectious causes of paediatric encephalitis in Southern Vietnam;
3. To estimate the illness costs attributed to NMDAR-antibody encephalitis and infectious encephalitis in children in Southern Vietnam.

Accordingly, my PhD thesis consists of four results chapters:

- In children, NMDAR-antibody encephalitis incidence exceeds that of Japanese encephalitis in Vietnam.

- Viral aetiology of encephalitis in Vietnamese children: results of extensive real-time polymerase chain reaction (PCR) analysis of biological samples.
- Metagenomics next-generation sequencing (mNGS) analysis of biological samples from children presenting with encephalitis of unknown origin after routine diagnosis.
- A cost of illness analysis of children with encephalitis presenting to a major hospital in Vietnam.

Chapter 2. Materials and methods

Chapter 2. Materials and Methods

This chapter outlines the clinical study collecting patient data (demographic, clinical, outcome and illness costs) and samples underpinning the analyses described in the result chapters (3, 4, 5 and 6). Detailed information about laboratory methods used will be described under the corresponding result chapters.

My contributions: As a principal investigator (PI), I was responsible for funding acquisition from the Gates Foundation to fund the clinical study, including proposal writing, budgeting, submission and liaising with the funder. I also led the study design, coordination and execution of the study. My key activities included protocol development, patients' recruitment, data collection, entry and cleaning up, and statistical analyses (result Chapters). Specifically, I designed the patient recruitment protocol, obtained ethical approvals, managed the collection of clinical data through clinical examination of the study participants, and ensured data accuracy.

2.1. Study design and setting

The clinical study was prospectively conducted at Children's Hospital 1 (CH1) and the Oxford University Clinical Research Unit (OUCRU) in Ho Chi Minh City, Vietnam during March 2020 to December 2022 (154). CH1 is a 1,600-bed hospital, and is the largest tertiary hospital for children coming southern provinces of Vietnam with a population of over 40 million (Figure 2.1). Annually, CH1 (Figure 2.2) has approximately 90,000 admissions with 150-200 cases presented with encephalitis (155). The age limit that defines an individual as a child and may be managed at or admitted to CH1 is 16 years of age or younger.

OUCRU is hosted by the Hospital for Tropical Diseases (HTD) in HCMC, a tertiary referral hospital for infectious diseases of southern Vietnam. OUCRU has a large clinical and scientific research programme which focuses on the most significant infectious diseases in Vietnam (Figure 2.3). The OUCRU laboratory capacity covers multidisciplinary subspecialties including emerging infections, Dengue haemorrhagic fever, central nervous system (CNS) infections, zoonotic infections, statistics, mathematical modelling, health economics, epidemiology, clinical trials.

Figure 2.1. Location of Children's Hospital 1, Ho Chi Minh City and Vietnam

Figure 2.2. Children's Hospital 1 in Ho Chi Minh City in Vietnam (Panel A) and the Infectious Diseases Department where recruitment was conducted (Panel B)

Figure 2.3. The Hospital for Tropical Diseases (Panel A) and the Oxford University Clinical Research Unit (OUCRU, Panel B)

2.2. Participants

2.2.1. Inclusion criteria

Any children (16 years of age or younger) admitted to CH1 and fulfilling the case definition of encephalitis were eligible to be invited to participate in the study.

2.2.2. Exclusion criteria

Patients were excluded if they refused to consent to participate in the study.

2.3. Case definitions

2.3.1. Case definition of encephalitis

A case of encephalitis was defined as a patient presenting with altered mental status (i.e., decreased or altered level of consciousness, lethargy, or personality change) lasting ≥24 hours with no alternative cause identified and ≥2 of these following criteria: documented fever ≥38°C (100.4°F) within 72 hours (before or after) presentation, generalized or partial seizures not fully attributable to pre-existing seizure disorder, new onset focal neurologic findings, CSF white blood cell count ≥5 cells/mm^3, abnormality of brain parenchyma on neuroimaging suggestive of encephalitis that is new or appears to have acute onset, and abnormality on electroencephalogram that is consistent with encephalitis and not attributable to any other causes (1,3–5).

2.3.2. Case definition of autoimmune encephalitis

A case of autoimmune encephalitis was defined as a patient presenting with all three of the following criteria: 1. Subacute onset (rapid progression of less than 3 months) of working memory deficits (short-term memory loss), altered mental status, or psychiatric symptoms; 2. At least one of the following: new focal CNS findings, seizures not explained by a previously known seizure disorder, CSF pleocytosis (white blood cell

count of more than five cells per mm^3), MRI features suggestive of encephalitis; and 3. Reasonable exclusion of alternative causes (1,3–5).

2.3.3. Case definition of NMDAR-antibody encephalitis

A case of probable NMDAR-antibody encephalitis was defined as a patient presenting with all three of the following criteria: 1. Rapid onset (less than 3 months) of at least four of the six following major groups of symptoms: abnormal (psychiatric) behaviour or cognitive dysfunction; speech dysfunction (pressured speech, verbal reduction, mutism); seizures; movement disorder (dyskinesias, or rigidity/abnormal postures); decreased level of consciousness; and autonomic dysfunction or central hypoventilation; 2. At least one of the following laboratory study results: abnormal EEG (focal or diffuse slow or disorganised activity, epileptic activity, or extreme delta brush), CSF with pleocytosis or oligoclonal bands; and 3. Reasonable exclusion of other disorders. Diagnosis of probable NMDAR-antibody encephalitis can also be made in the presence of three of the above groups of symptoms accompanied by a systemic teratoma (1,3–5). A case of confirmed NMDAR-antibody encephalitis was defined as a patient presenting with one or more of the six major groups of symptoms and positive anti-NMDAR antibody testing (1,3–5).

2.4. Recruitment and assessment methods

The flow chart for the identification of paediatric encephalitis in this study is showed in Figure 2.4.

Study design

My clinical study was conducted as a prospective observational study to systematically investigate the clinical features of children with encephalitis admitted to the Department of Infectious Diseases and Neurology at CH1. The study systematically enrolled all eligible children with encephalitis, defined by standardised case definitions, to

comprehensively capture the clinical features, regardless of potential benefits to participants.

All patients were enrolled as soon as possible upon hospital admission, ensuring that data collection began concurrently with their clinical presentation. This prospective approach allowed us to capture real-time clinical information, minimising recall bias, and ensuring data accuracy. Upon admission, patients meeting the inclusion criteria (age ≤16 years and fulfilling the case definition of encephalitis) were enrolled. After obtaining informed consent, clinical data, including medical history, signs and symptoms, laboratory and imaging results, and treatment details, were collected directly by the study team during the admission process, rather than relying solely on the retrospective review of medical records.

Patient recruitment

Recruitment was conducted by a dedicated study team comprising paediatricians and research nurses, all of whom were well-trained to conduct observational study like the present study. As a principal investigator, I am primarily in charge of patient recruitment, ensuring consistent and thorough enrolment of participants. When I was unavailable in the department, recruitment was based on consultations and discussions between myself and other paediatricians. This systematic recruitment process ensured that no eligible cases were missed and allowed for timely enrolment, enhancing the study's representativeness. Potential participants were screened and identified at the point of admission to the Department of Infectious Diseases and Neurology at CH1. Screening and identification were conducted daily during admission hours to maximize the recruitment of eligible cases.

Clinical assessments

The study team maintained close collaboration with the clinical care team to collect all clinical data in real time. As part of routine case, clinical assessments were conducted daily during hospitalization to comprehensively monitor the progression or resolution of signs and symptoms, alongside changes in laboratory, CSF, imaging results, and treatment details. These assessments were performed by a consistent study team of paediatricians, in consultation with the treating paediatricians, to ensure reliability and continuity. Additional unscheduled assessments were conducted when significant clinical changes were reported, reflecting the study's adaptability to patients' evolving conditions. My study however, was not designed to capture the development of clinical presentation during the course of hospitalisation. The collected clinical data merely reflected the observed events documented anytime during the course of illness. All data were recorded in real time using standardised a case report form (CRF) (Appendix 3.2), and the collected data were subsequently entered into a locally developed clinical database systems namely CliRes, widely used system for data storage of clinical studies at OUCRU. Clinical assessments were conducted during the study period, including at hospital admission, during treatment, and at discharge, depending on the patient's clinical course.

Assessment of paediatric GCS and mRS

The paediatric GCS and mRS (Appendix 3.1) were assessed by members of my study team. The mRS was incorporated into the CRF following the publication of my study protocol. To ensure the consistency, all assessors were trained on the standardised application of the paediatric GCS and mRS with clear guidelines (Appendix 3.1).

During the COVID-19 pandemic

The recruitment was maintained during the study period, including the COVID-19 pandemic time. This was possible because as a tertiary referral hospital, CH1 was still in function during the COVID-19 pandemic time.

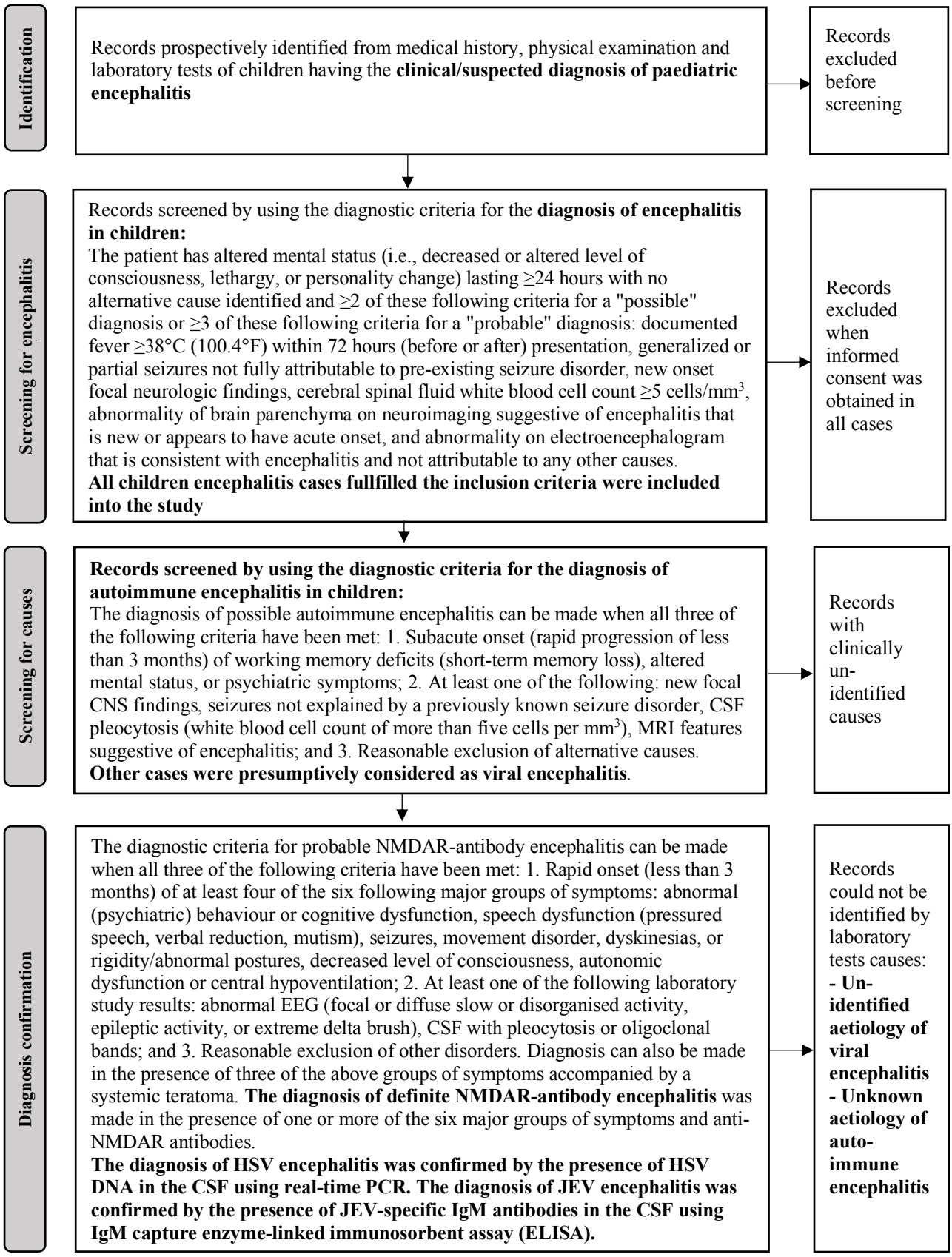


Figure 2.4. Flow chart for the identification of encephalitis in children

2.5. Investigation scheme

The “core test panel”, applied to every case as part of routine diagnosis, included a complete blood count, blood glucose, electrolyte panel, C-reactive protein (CRP), liver and kidney function tests, cerebrospinal fluid (CSF) analysis (cell count, protein, glucose, lactate), CSF autoimmune testing for autoantibodies (primarily anti-NMDA receptor, and (where resources are available) anti-AMPA R1/R2, anti-LGI1, anti-CASPR2, anti-GABA, and anti-DPPX antibodies), CSF PCR testing for HSV-1 and HSV-2, CSF ELISA testing and distinguishing between JEV- and DENV-specific IgM antibodies, brain magnetic resonance imaging (MRI), and electroencephalogram (EEG). For JEV and DENV testing, the CSF samples were submitted to the Pasteur Institute in Ho Chi Minh City, where an in-house IgM capture enzyme-linked immunosorbent assay (ELISA) was used to test for both JEV and DENV in the CSF.

Additional tests that were performed only in selected cases (e.g., when clinical symptoms suggest a tumour), included abdominal, pelvic, and chest ultrasound to screen for tumours (e.g., ovarian teratoma, thymoma, lymphoma, Hodgkin’s disease). Testicular ultrasound was used to screen for testicular tumours. If the disease progressed severely or the cause remained unclear, whole-body MRI was considered.

2.6. Sample size

Given the descriptive nature of the present study, samples size calculation was not sought. I aimed to carry out patient recruitment from March 2020 to December 2022. Based on my admission data, I anticipate that 150-200 patients were enrolled over the study period.

2.7. Data collection

2.7.1. Clinical data collection

The study participants had acute CSF samples collected alongside meta clinical data, including dates of birth, admission, and discharge; demographic data (gender and place of residence); clinical signs, symptoms, and syndromes; laboratory and imaging results (CSF, urine, and plasma testing, EEG, MRI, CT scans) obtained as part of routine care; diagnosis and differential diagnoses; associated conditions and comorbidities; treatments (antibiotics, methylprednisolone, intravenous immunoglobulin, etc.); and discharge outcomes.

Obtaining brain biopsy was not allowed as part of routine care in my setting. As per my routine practice, clinical samples are obtained within 12 hours from patients fulfilling the diagnostic criteria of possible or probable encephalitis. To maximise the chance of enrolling patients into the study during the acute illness phase, I conducted the recruitment as soon as a patient with presumed encephalitis is identified.

Paediatric Glasgow coma scale (GCS) and modified Rankin scale (mRS) for children were used to measure the hospital outcomes of patients in this study. The mRS was previously used in a UK-based surveillance study of encephalitis in children, and in another study using data from Evelina Children's Hospital, Great Ormond Street Children's Hospital, St George's Hospital, King's College Hospital in London and Birmingham Children's Hospital in Birmingham between 2007 and 2010 (74,156).

2.7.2. Economic data collection

Indirect- and direct costs of illness (including disease associated costs 7 days after hospital discharge) were evaluated. The study assessed the economic burden of encephalitis in children by collecting information regarding direct cost of illness (total cost (VND or USD) of medical health care or treatment) and indirect cost of illness (related information

of care giver(s): relationship with patient, daily work, job, time consuming for taking care of the patient before, during and after hospitalization; health care seeking activities of care giver(s) before and after hospitalization of patient (name of activities, cost of treatment, cost of traveling, hour(s) consumed)). The cost-of-illness data were collected by the same study team responsible for the clinical assessments, comprising general paediatricians and paediatricians specialising in neurology.

2.7.3. Microbiological sample collection and storage

Admission samples collected for microbiology investigation included:

a. CSF
b. Plasma
c. Urine
d. Throat swabs
e. Rectal swabs

Samples were collected at the time of hospital admission or shortly thereafter (typically within 24–48 hours) for all enrolled patients with clinically relevant encephalitis. This timing was chosen to optimize the sensitivity of diagnostic assays, particularly for HSV-PCR, as early sampling is critical for detecting acute infections. The results were interpreted in the context of clinical presentation and sample timing to minimise false negatives due to delayed antibody responses. Due to the low availability of the resources, follow-up samples were not collected.

As part of this study all clinical samples from encephalitis patients were stored and archived at –80^{o}C in deep freezer. All samples were shipped to the laboratory of Emerging Infections Group (EI Group) of OUCRU for further metagenomic characterisation and PCR testing.

Details of samples and molecular processing were stored in electronic database housed at the EI Group at OUCRU and at the department of clinical microbiology of Children's Hospital 1. All specimens, reports, study data collection, process, and administrative forms were identified by a coded number. Study databases were secured with password-protected access systems. No patient identifying information was included in publications or presentations resulting from this work.

Nucleic acid extractions were stored until all analyses for this study have been performed. Clinical samples were stored beyond the duration of the project.

The microbiology laboratory of CH1 and OUCRU held and stored the samples for this study. Nucleic acid extractions were stored until all analyses for this study have been performed.

2.8. Diagnostic methods

2.8.1. Routine diagnostics

As part of routine care at CH1, CSF samples of patients presenting with brain infections were subject to cultured and/or examined by microscopy for detection of bacterial, viral and fungal infections with the use of standard methods implemented at CHI when appropriate. The sample type used for diagnosing HSV and JEV as causes of encephalitis in this study was cerebrospinal fluid (CSF). The diagnosis of HSV encephalitis was confirmed by the presence of HSV DNA in the CSF using real-time PCR (6).

The diagnosis of JEV encephalitis was confirmed by the presence of JEV-specific IgM antibodies in the CSF using IgM capture enzyme-linked immunosorbent assay (ELISA) (6). In acknowledgement of the co-circulation of JEV and DENV in southern Vietnam and the potential for serological cross-reactivity between these two flaviviruses, the ELISA test incorporated both JEV and DENV antigens, and was carried out at the Pasteur

Institute in Ho Chi Minh City, Vietnam, as a service. The reported specificity of the CSF ELISA for JEV-IgM detection was around 99.8% (151).

Regarding dengue serology, the same diagnostic testing strategy as outlined for JEV. However, in my local practice testing for dengue virus was requested only in cases where patients presented with clinical features suggestive of dengue haemorrhagic fever, such as fever, thrombocytopenia, or haemorrhagic manifestations. This targeted approach ensured that dengue serology was conducted selectively to avoid unnecessary testing. In cases where dengue testing was performed, results were interpreted in the context of clinical presentation and other laboratory findings to distinguish between dengue-related illness and encephalitis caused by JEV or other etiologies. By focusing on CSF-based JEV-specific IgM testing and restricting dengue serology to clinically relevant cases, I aimed to enhance diagnostic specificity and reduce the impact of cross-reactivity.

For HSV PCR, as part of routine testing, CSF samples were sent to Medic-Lab, a local diagnostic medical centre in Ho Chi Minh City, Vietnam, where qualitative real-time PCR was used to detect and differentiate the specific DNA sequences of HSV-1 and HSV-2 in the CSF. Because HSV PCR was carried out as a service, the diagnostic reagents (including primer and probe sequences) were not made available.

The PCR diagnostic assays for other causes of CNS infections conducted at OUCRU are detailed in Chapter 4, and the mNGS analysis conducted at OUCRU is described in Chapter 5 (Figure 2.5).

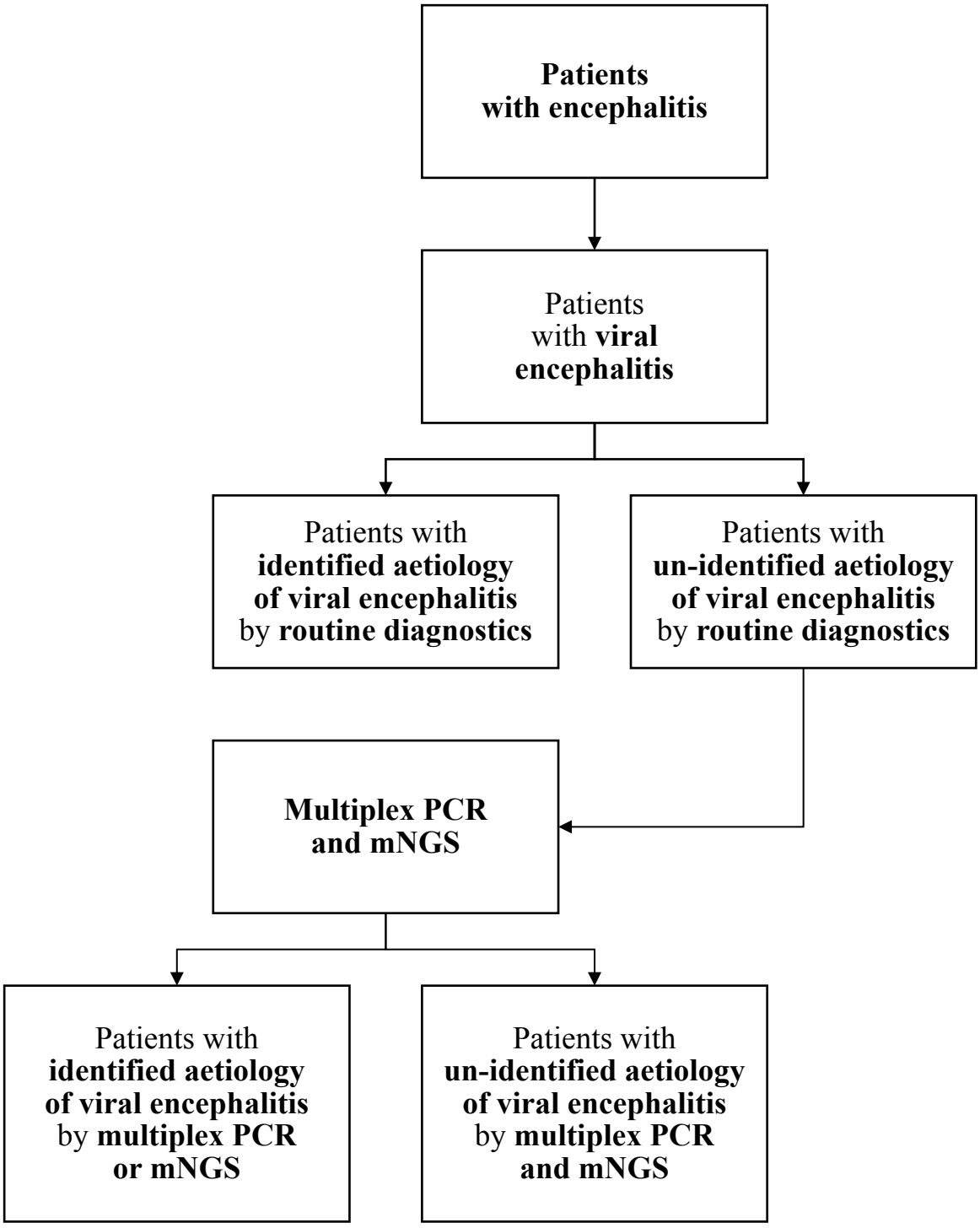


Figure 2.5 Flowchart of routine diagnostics, multiplex real-time PCR, and mNGS for the detection of viruses causing encephalitis

2.8.2. Immunological assays

The CSF of patients with clinical presentations compatible with probable autoimmune encephalitis (1,4,5) were tested for antibodies against NMDAR, and (where available) antibodies against other receptors (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA-R1/R2), gamma aminobutyric acid-A (GABAA-RB1/B2), leucine-rich glioma-inactivated 1 (LGI1), contactin-associated protein-like 2 (CASPR2), dipeptidyl-peptidase-like protein 6 (DPPX). As part of routine testing, CSF samples were sent to the laboratories of the University of Medicine and Pharmacy Medical Centre at Ho Chi Minh City, or Medic-Lab during the study period, where a commercial fixed cells-based assays (EUROIMMUN, Lübeck, Germany) was used, following manufacturer's instruction (152–154). Occasionally, testing for NMDAR antibodies were carried out the laboratory of OUCRU.

2.8.3. Metagenomic next-generation sequencing

Regardless of the results of investigation using classical assays outlined above, collected clinical samples (CSF, urine, plasma, and rectal and throat swabs) will be analysed using my in-house metagenomic next-generation sequencing (mNGS) workflow (107,109,161). CSF samples from patients with laboratory-confirmed anti-NMDAR encephalitis and PCR-grade water were used as negative controls. The obtained sequence data were analysed to define the pathogen contents (including novel viruses) in the tested specimens using CZID (czid.net) or in-house pipeline (Chapter 6). Any virus detected by metagenomics were confirmed using specific PCR of the corresponding viruses or the detected viral sequences. Only those confirmed by PCR will be considered as a true positive. The obtained mNGS results were compared against that of current (routine) diagnostic assays (especially PCRs).

2.9. Data management

All available data were entered into an electronic database (CliRes) developed by the IT department of the OUCRU. Only the named investigators or their designee(s) had access to this information. All other investigators were regularly updated and granted access to data when requested. Patients were identified by their names. The investigators were responsible for maintaining all study records. The investigators were responsible for the timeliness, completeness and accuracy of the information in the original dataset and the clinical data management system. Laboratory staff recorded specimens, their aliquots, and their storage location. All necessary tools, instruction, and training were provided to all site staff involved in data entry to ensure the correct and consistent completion database prior to the study starting.

The investigators were responsible for retaining all essential data for at least five years after the completion of the study. Original paper documents will be maintained for a minimum of five years and electronic documents retained thereafter. All stored records are to be kept secure and confidential.

The investigators were responsible for retaining all essential data for at least 5 years after the completion of the study. Original paper documents will be maintained for a minimum of 5 years and electronic documents retained thereafter. All stored records are to be kept secure and confidential.

2.10. Data analysis

Descriptive statistics were employed to compare between the epidemiology, clinical presentation, laboratory findings, patient treatment, and outcomes of patients with NMDAR-antibody and viral encephalitis. Significant differences between features of groups of patients was determined by statistical comparisons using Pearson's chi-squared

test, Fisher's exact test, and Wilcoxon rank-sum test. All statistical analyses were performed using Stata version 18 (StataCorp LP, College Station, TX, USA) and R 4.1.0 (R Core Team, 2021). The p-values of ≤0.05 were considered significant.

2.11. Quality control

The study was conducted in compliance with the current approved protocol, ICH GCP (International Conference on Harmonisation Good Clinical Practice), relevant regulations and standard operating procedures. Regular monitoring was performed according to ICH GCP. Data, samples and procedures were evaluated for compliance with the protocol, standard operating procedures, regulatory requirements and terms of ethical approval. Records were verified for accuracy against source documents and physical inventory of samples. The diagnostic laboratory at Children's Hospital 1 conducts regular internal and external quality control procedures.

2.12. Ethical issues

2.12.1. Ethical and Regulatory Guidelines

The Investigators ensured that this study was conducted in accordance with the principles of the Declaration of Helsinki (Seoul 2008) and the terms of approval of the appropriate ethical committees. The Investigators ensured that this study is conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice July 1996.

This protocol and the relevant supporting document were submitted to the EC/IRB of Children's Hospital 1 (CH1-EC/IRB) and the Oxford Tropical Research Ethics Committee (OxTREC) for review and were not be initiated at that site until after approval.

The Investigators submitted and, where necessary, obtained approval from the above parties for all substantial amendments to the original approved documents.

This protocol and the relevant supporting documents were approved by the ethical committee of Children's Hospital 1 (1503/QD-BVND1) and the Oxford Tropical Research Ethics Committee (OxTREC 7-20). The collection of all biological samples was performed as part of routine clinical assessments and are consistent with the local standard of care and good clinical practice.

2.12.2. Informed Consent

Written informed consent obtained from all study participants or their representatives before any data from patients is collected for the study. The study staff discussed the study with the parent/representative of potential participants. Study staff described the purpose of the study, the study procedures, possible risks/benefits, the rights and responsibilities of patient, and alternatives to enrolment.

The parents/representatives were invited to ask questions which were answered by study staff, and they were provided with appropriate numbers to contact if they have any questions subsequently. If the parent/representative agreed for their child to participate, they were asked to sign and date two copies of an informed consent form.

A copy of the form was given to them to keep. If required, the parent/representative was given up to 48 hours to consider for their children to take part in the study. In addition to the procedures above, illiterate signatories had the informed consent form read to them in the presence of a witness who signed to confirm that the form was read accurately and that the participant or representative agrees to participation. All informed consent forms were written in the local language and used terms that are easily understandable.

2.12.3. Risks and benefits

This was a minimal risk study because it does not involve any investigational new drugs or interventions. The collection of all biological samples was performed as part of routine clinical assessments and are consistent with the local standard of care and good clinical practice.

2.12.4. Expenses

Patients did not have to pay any expenses to participate in this study. Patients did not receive reimbursement of expenses.

2.12.5. Confidentiality

The study staff ensured that all data was stored securely at the study site in locked file cabinets or password protected devices in areas with access limited to study staff. All specimens, reports, study data collection, process, and administrative forms were identified by a coded number. Study databases were secured with password-protected access systems and controlled distribution web-based security certificates. No patient identifying information was included in publications or presentations resulting from this work.

Chapter 3. In children, NMDAR-antibody encephalitis incidence exceeds that of Japanese encephalitis in Vietnam

Chapter 3. In children, NMDAR-antibody encephalitis incidence exceeds that of Japanese encephalitis in Vietnam

3.1. Introduction

Over the last decade, autoimmune encephalitis has been increasingly recognized as an important cause of encephalitis in children, with antibodies against N-methyl-D-aspartate receptor (NMDAR)-antibody the leading cause in this age group. Studies from high-income counties, including the United States, Denmark and Germany, have shown that the frequency of autoimmune encephalitis has now exceeded infectious aetiologies (84,163–166).

This shift in encephalitis epidemiology has challenged routine diagnosis and clinical management of these patients (84,133,163–167). Yet, as existing literature is currently dominated by studies from developed countries, it remains unclear whether this shift is observed in low- and middle-income countries (LMICs) (168–170). Here, I aim to contrast the frequencies, and the associated clinical presentations and outcomes of autoimmune and infectious encephalitis in patients enrolled in the clinical study outlined in Chapter 2. Accordingly, I hypothesised that there were significant differences in clinical presentations and outcomes between NMDAR-antibody encephalitis and JE patients.

My contributions: In this chapter, I was responsible cleaning up the clinical dataset and performed statistical analyses to address the research question about the clinical features and their association with different patient groups. JEV and NMDAR-antibody testing was sent to local Institutes for analysis as a service as outlined in Chapter 2. Findings of this Chapter was published in the Open Forum Infectious Diseases Journal (PMID: 39691294).

3.2. Methods

3.2.1. Study design and setting of the clinical study

The prospective study was conducted at Children's Hospital 1 (CH1) in Ho Chi Minh City in Vietnam between March 2020 and December 2022. CH1 is a 1,600-bed hospital, and is the largest tertiary referral hospital for children coming from southern provinces of Vietnam with a catchment population of over 40 million with an estimation of 11 million children.

3.2.2. Inclusion and exclusion criteria

Inclusion criteria were any child (aged less than or equal to 16 years) who was admitted to the Department of Infectious Diseases and Neurology of CH1 during the study period, and fulfilled the clinical diagnostic criteria of encephalitis. Patients were excluded if no informed consent was obtained.

3.2.3. Data collection

At enrolment, the study participants had acute CSF samples collected alongside meta clinical data, including dates of birth, admission and discharge, demographic data, clinical features, laboratory and imaging results, diagnoses, and treatment.

Patient consciousness level was evaluated using the Paediatric Glasgow coma scale (GCS) (166). The modified Rankin scale (mRS) for children was used to assess the degree of disability or dependence in daily activities (167).

The neurological sequelae (paralysis, developmental delay, altered mental status, speech disorder, and motor disturbance) of patients with encephalitis were evaluated immediately on hospital discharge and by two qualified paediatric neurologists. If there was disagreement, then the diagnosis of neurological sequelae was discussed until a consensus decision was reached.

3.2.4. Routine laboratory diagnostic approach

As part of routine care at CH1, CSF of patients presenting with brain infections are subject to cultured and/or examined by microscopy for detection of bacterial, viral and fungal infections with the use of standard methods when appropriate. Additionally, the CSF of patients with clinically suspected encephalitis were tested for the presence of HSV DNA using real-time PCR and JEV-specific IgM using IgM capture ELISA assay (6).

The CSF of patients with clinical presentations compatible with probable autoimmune encephalitis (1,4,5) were tested for antibodies against NMDAR, and (where available) antibodies against other antigens (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA-R1/R2), gamma aminobutyric acid-A (GABAA-RB1/B2), leucine-rich glioma-inactivated 1 (LGI1), contactin-associated protein-like 2 (CASPR2), dipeptidyl-peptidase-like protein 6 (DPPX). For autoimmune encephalitis diagnosis, I used commercial fixed cells-based assays (EUROIMMUN, Lübeck, Germany) (168), following manufacturer's instruction.

3.2.5. Data analysis

Statistical analysis was employed to find the significant difference of epidemiology, clinical presentation, laboratory findings, treatment, and outcomes of patients between NMDAR-antibody encephalitis and JEV encephalitis. For these analyses, where appropriate, I applied Pearson's chi-squared test, Fisher's exact test, and Wilcoxon rank-sum tests. All statistical analyses were performed using Stata version 18 (StataCorp LP, College Station, TX, USA) and R 4.1.0 (R Core Team, 2021).

The selection of variables for statistical comparison was based on background knowledge and my clinical observations. P-values were adjusted using Bonferroni correction to control for the increased risk of false positives in multiple hypothesis tests. An alpha level of 0.05 was chosen, indicating a 5% chance of incorrectly rejecting the null hypothesis

when it is actually true. This level of significance is the most commonly used because it balances the risk of making a Type I error (false positive) and a Type II error (false negative). The adjusted P-value was calculated as: Adjusted P-value = P-value * Number of tests. If the adjusted P-value was less than or equal to my chosen alpha level, the result was considered statistically significant.

3.3. Results

3.3.1. Identification, screening, and diagnosis of encephalitis

During the study period, 458 patients were screened and subsequently 164 (35.8%) fulfilled the predefined diagnostic criteria, and were enrolled in the study (Figure 3.1).

The remaining 294 patients were excluded for several reasons (Figure 3.1). Of the 458 patients screened, those presenting with symptoms mimicking encephalitis (e.g., fever, seizures, or altered consciousness) but diagnosed with alternative conditions based on clinical features, CSF analysis, and imaging results were excluded. These conditions included bacterial meningitis (n=124), febrile seizures (n=159), and metabolic disorders (n=7). Additionally, some cases (n=4) had transient symptoms resolving within 24 hours, failing to meet the duration criterion for encephalitis (Figure 3.1).

A total of 164 children with a clinical diagnosis of encephalitis were enrolled in the study between March 2020 and December 2022. Using the criteria outlined in Chapter 2, 75 cases (45.7%) had signs and symptoms meeting the predefined criteria of probable autoimmune encephalitis. The remaining 89 (54.3%) were classified as clinically suspected infectious encephalitis (Figure 3.1).

There were no indeterminate cases (neither infectious nor autoimmune based on clinical assessment and initial laboratory tests) in this cohort.

Of the 75 patients with probable autoimmune encephalitis, all CSFs were examined for NMDAR antibodies, while 22 were also tested for antibodies against AMPA-R1/R2, $GABA_{A/B1/B2}R$, LGI1, CASPR2 or DPPX. CSF antibodies against NMDAR were detected in 23/75 (30.7%), and none had antibodies against the remaining antigens.

Of the 89 patients with a clinical diagnosis of infectious encephalitis, JEV and HSV-1 were detected in 14 (15.7%) and 4 (4.5%), respectively. Collectively, of the 164 enrolled patients, NMDAR-antibody encephalitis was detected in 23 (14.0%), while JEV or HSV-

1 were detected in 18 (11.0%; JEV in 14/164 (8.5%) and HSV-1 in 4/164 (2.4%)) (Figure 3.1).

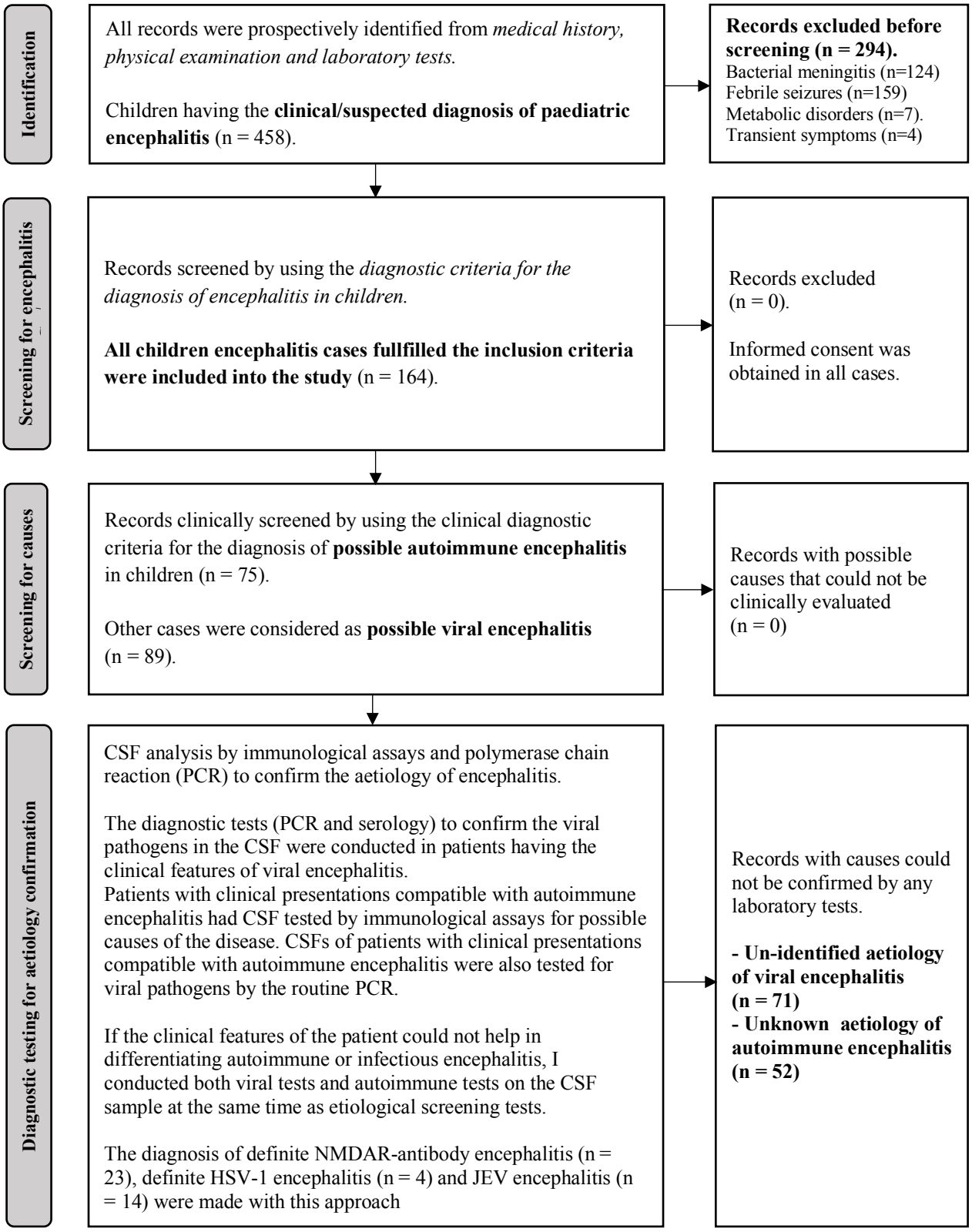


Figure 3.1. Flow chart for the identification, screening, and diagnosis of encephalitis

3.3.2. Baseline characteristics of all encephalitis patients

Among 164 children with a clinical diagnosis of encephalitis, 85 (51.8%) were males and 79 (48.2%) were females (Table 3.1). The median age was 9 years (IQR; 5 – 12 years). A higher proportion (121/164, 73.8%) were from other provinces in southern Vietnam rather than from Ho Chi Minh city (43/164, 26.2%). The clinical features of 164 cases with encephalitis were highly variable with remarkable manifestations including fever (70.1%), seizure (62.8%), neurological deficits (23.2%), and abnormal muscular tone (27.4%). In term of fever, the highest temperature during hospitalization had a median of 39.0^{o}C (IQR; 38.5^{o}C – 39.5^{o}C) and the median (IQR) of duration of fever was 5 days (3 days – 7 days). However, the proportion of children with fever was significantly higher in infectious encephalitis with 79.8% compared to 58.7% of autoimmune encephalitis (p=0.003). Other clinical features of encephalitis, particularly the six major groups of symptoms commonly observed in autoimmune encephalitis are shown in Table 3.1. MRI findings showed that cerebral cortex (37.8%), limbic system (25.0), thalamus (16.5%), and cerebellum (12.8%) were the most commonly affected regions in encephalitis. Slow waves were the main EEG abnormality with 42/164 patients (25.6%). On the whole, antiviral treatment with acyclovir was found in 125/164 patients (76.2%) while corticosteroid was used in 83/164 patients (50.6%). The in-hospital death was recognised in 3 cases of encephalitis (1.8%). The median (IQR) of length of hospital stay was 15 days (11 days – 24 days). 64/164 patients had sequelae (39.0) with a median (IQR) of modified Rankin scale of 0 (0 – 2) (Table 3.1).

Among 75 children with autoimmune encephalitis, female was significantly (p=0.002) more common in NMDAR-antibody encephalitis (defined autoimmune encephalitis) (19/23, 82.6%) than in undefined autoimmune encephalitis (23/52, 44.2%). In addition,

dyskinesia was significantly more common in NMDAR-antibody encephalitis than in undefined autoimmune encephalitis (15/23, 65.2% vs. 21/52, 40.4%, p=0.047) (Table 3.5). CSF WBC count in NMDAR-antibody encephalitis with a median (IQR) of 11 (3 – 28) cells/mm^3 was significantly higher (p=0.001) than in undefined autoimmune encephalitis with 2 (2 – 7) cells/mm^3.

Table 3.1. Baseline characteristics of all encephalitis patients

	All encephalitis patients (n = 164)
Demographic features	
Female	79 (48.2)
Age (years)	9 (5 – 12)
Ho Chi Minh city	43 (26.2)
Other provinces	121 (73.8)
Clinical features	
Illness days before admission	4 (3 – 7)
Fever	115 (70.1)
Highest temperature during hospitalization (^oC)	39.0 (38.5 – 39.5)
Duration of fever (days)	5 (3 – 7)
Abnormal psychiatric behaviour	61 (37.2)
Cognitive dysfunction	55 (33.5)
Memory deficits	5 (3.0)
Delirium	35 (21.3)
Language changes	70 (42.7)
Speech dysfunction	37 (22.6)
Mutism	10 (6.1)
Seizures	103 (62.8)
Abnormal movements	65 (39.6)
Cerebellar syndrome	21 (12.8)
Dyskinesias	47 (28.7)
Abnormal muscular tone	45 (27.4)
Paediatric Glasgow coma scale (GCS) score	11 (10 – 13)
Level of consciousness Alert (Paediatric GCS score = 14 – 15) Drowsy (Paediatric GCS score = 9 – 13) Stupor (Paediatric GCS score = 6 – 8) Coma (Paediatric GCS score = 3 – 5)	 29 (17.7) 109 (66.4) 18 (11.0) 8 (4.9)
Autonomic dysfunction	39 (23.8)
Neurological deficits	38 (23.2)
CSF investigation	
White blood cell count (cells/mm^3)	4 (2 – 30)
Neutrophils (%)	69.0 (60.0 – 75.0)
Monocytes (%)	69.0 (60.0 – 73.0)
Protein (g/L)	0.3 (0.2 – 0.5)
Glucose (mmol/L)	3.8 (3.4 – 4.4)

CSF glucose / Plasma glucose	0.7 (0.6 – 0.8)
Lactate (mmol/L)	1.6 (1.4 – 2.0)
MRI findings (performed in all cases)	
Normal	74 (45.1)
Abnormal	90 (54.9)
Cerebral cortex	62 (37.8)
Limbic system	41 (25.0)
Thalamus	27 (16.5)
Mid-brain	16 (9.8)
Cerebellum	21 (12.8)
Brain stem	13 (7.9)
EEG abnormality (performed in all cases)	
Normal	80 (48.8)
Abnormal	84 (51.2)
Delta brush	4 (2.4)
Slow waves	42 (25.6)
Spike waves	10 (6.1)
Beta waves	1 (0.6)
Seizure	2 (1.2)
Fast activity	5 (3.0)
Slow baseline activity	13 (7.9)
Low voltage	3 (1.8)
Treatment	
Corticosteroid	83 (50.6)
Immunoglobulin	27 (16.5)
Cyclophosphamide	9 (5.5)
Rituximab	6 (3.7)
Acyclovir	125 (76.2)
Mannitol	87 (53.0)
Sodium chloride 3%	23 (14.0)
Outcomes	
Length of hospital stay (days)	15 (11 – 24)
Mortality	3 (1.8)
Sequelae	64 (39.0)
Modified Rankin scale	0 (0 – 2)

Note: Data are presented as n (%) or median (IQR).

3.3.3. Clinical features of NMDAR-antibody encephalitis, JEV encephalitis, and HSV-1 encephalitis

Clinical features of NMDAR-antibody encephalitis and JEV encephalitis were compared, and four HSV-1 patients were excluded from statistical analyses (Table 3.2). The selection of variables for statistical comparison was based on background knowledge and my clinical observations. The proportion of children fever was significantly higher in infectious encephalitis with 79.8% compared to 58.7% of autoimmune encephalitis (p=0.003). Although clinical presentations of NMDAR-antibody encephalitis and undefined autoimmune encephalitis showed significant similarities, the features of JEV encephalitis differed from those of confirmed NMDAR-antibody encephalitis (Table 3.2). Of the 23 patients with NMDAR-antibody encephalitis, females were predominant (82.6%; 19/23), and males were all < 12 years. Patients with NMDAR-antibody encephalitis were older than those with JEV encephalitis and 16/23 (69.6%) came from outside of Ho Chi Minh City than JEV encephalitis patients (Table 3.2). In contrast, 12/14 (85.7%) JEV encephalitis patients were male and all were from outside Ho Chi Minh City (p=0.031). Fever recorded at enrolment was documented in all 14 JEV encephalitis patients, but in just over half (56.5%, 13/23) of NMDAR-antibody encephalitis patients, with a longer fever duration recorded in the former (median days (IQR): 7 (5 – 10) vs. 3 (3 – 7), p=0.014). Likewise, JEV encephalitis patients were associated a lower level of consciousness than those with NMDAR-antibody encephalitis; median paediatric GCS (IQR): 8 (8 – 11) vs. 11 (9 – 12) (Table 3.2). In terms of neurological features, signs and symptoms such as psychiatric disorder, delirium, and cognitive dysfunction were exclusively found in NMDAR-antibody encephalitis patients, whereas language changes, abnormal movement, and dyskinesias were less frequently recorded in JE patients (Figure 3.2).

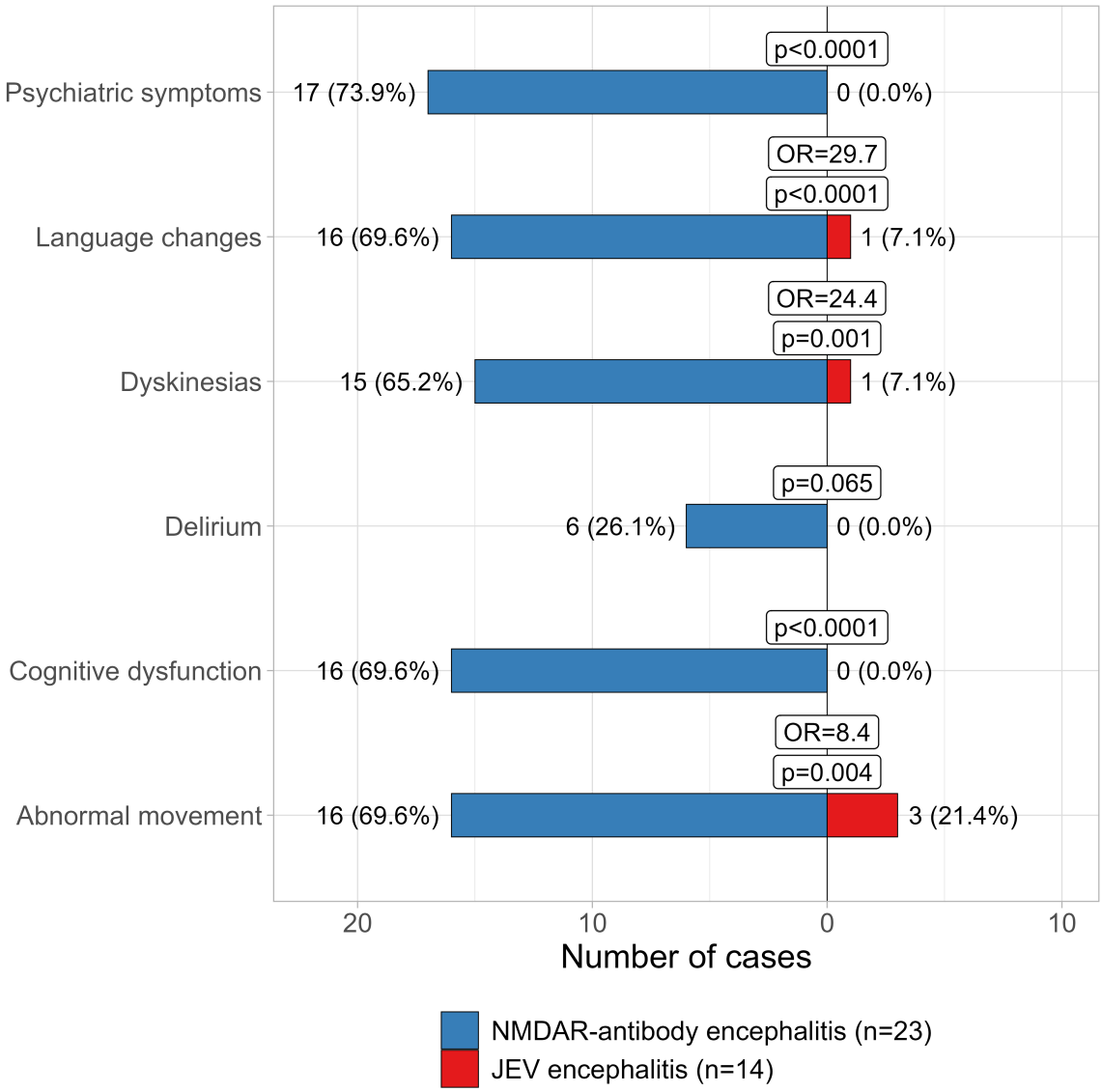


Figure 3.2. Frequency of psychological disorders and other features predominantly recorded in patients with NMDAR-antibody encephalitis as compared to that of Japanese encephalitis patients

Note: Psychiatric symptoms were described in terms of agitation, hallucinations, sleep disorders, and mood changes. Abnormal movement included dyskinesias, dystonia, stereotypical movement disorder, chorea, catatonia, bradykinesia, and tremor. Dyskinesias were described as lingual, orofacial or limb dyskinesias.

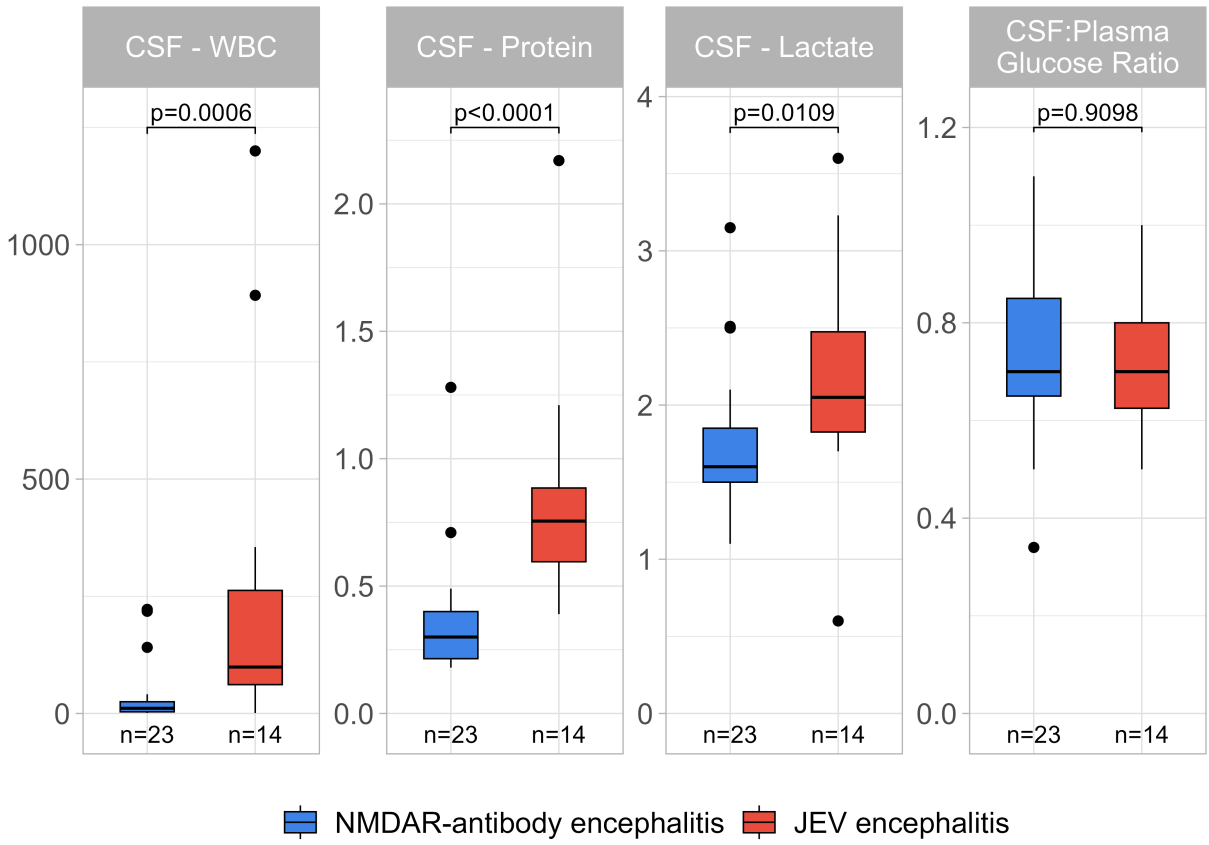


Figure 3.3. CSF laboratory data of NMDAR-antibody encephalitis in comparison with that of Japanese encephalitis patients

Note: The units of measurement for CSF WBC count, CSF protein, and CSF lactate are cells/mm^3, g/L, and mmol/L; respectively.

Compared with JEV encephalitis patients, NMDAR-antibody encephalitis patients had lower CSF white blood cell (WBC) counts (median (IQR): 11 cells/mm^3 (3 cells/mm^3 – 28 cells/mm^3) vs. 99 cells/mm^3 (59 cells/mm^3 – 300 cells/mm^3), p=0.0006; Figure 3.3), lower CSF protein and lactate levels (median (IQR): 0.3 g/L (0.2 g/L – 0.4 g/L) vs. 0.8 g/L (0.6 g/L – 0.9 g/L), p<0.0001), and 1.6 mmol/L (1.5 mmol/L – 1.9 mmol/L) vs. 2.1 mmol/L (1.8 mmol/L – 2.6 mmol/L), p=0.0109). Both patient groups had similar CSF/plasma glucose ratio (Figure 3.3).

CSFs of patients with clinical presentations compatible with autoimmune encephalitis were also tested for viral pathogens by routine PCR: only one showed positivity, with CSF PCR HSV-1 detected and the subsequent development of CSF NMDAR antibodies two months later. No association was observed between JEV and NMDAR-antibody encephalitis.

No patients in my study exhibited signs, symptoms, or were suspected of having teratomas. The abdominal ultrasound examination was performed in 2 cases of NMDAR-antibody encephalitis and 2 cases of undefined autoimmune encephalitis with upper gastrointestinal bleeding. However, no gastric teratomas were found, and the upper gastrointestinal bleeding was assumed to be a complication of corticosteroid use. In my practice, the abdominal ultrasound examination was not routinely conducted in autoimmune encephalitis for the screening of teratomas unless the patients had clinical features that are relevant to teratomas.

3.3.4. MRI and EEG findings

On magnetic resonance imaging (MRI), in patients with NMDAR-antibody encephalitis, abnormalities were frequently detected in the limbic system (15/23, 65.2%) and cerebral cortex (13/23, 56.5%), whereas thalami were affected in all JEV encephalitis patients. From the 23 NMDAR-antibody encephalitis patients, electroencephalogram showed slow

waves and delta brush were detected in 12 (52.2%) and in 3 (13.0%), respectively. No remarkable signals of electroencephalogram were documented in JEV encephalitis patients (Table 3.2).

3.3.5. Treatment

In my local practice, immunotherapy was initiated when autoimmune encephalitis was clinically suspected without waiting for confirmatory test results, and followed recent International Consensus Recommendations (81). First-line immunotherapies included methylprednisone (21 cases, 91.3%) and immunoglobulin (9 cases, 39.1%); with cyclophosphamide (n=7; 30.4%) (available at my centre since May 2020) and rituximab in 4 cases (17.4%) (available at my centre since June 2022) as second-line immunotherapies (Table 3.2).

3.3.6. Outcomes

There was a significant association between illness days before admission and sequelae (p=0.039) but not mortality (p=0.068). Patients with NMDAR-antibody encephalitis had a longer duration of hospital stay than JEV encephalitis patients; median in days (IQR): 38 (15 – 53 days) vs. 15 days (11 – 20 days, p=0.021). At discharge, fatal outcome was documented in one NMDAR-antibody encephalitis patient, but in none of JEV encephalitis patients. In addition, there was no statistically significant difference of mRS and neurological sequelae between NMDAR-antibody encephalitis and JEV encephalitis patients (Table 3.2).

Table 3.2. Clinical features of NMDAR-antibody encephalitis, JEV encephalitis, and HSV-1 encephalitis

	NMDAR-antibody encephalitis (n = 23)	JEV encephalitis (n = 14)	HSV-1 encephalitis (n = 4)	Adjusted P-values*
Demographic features				
Female	19 (82.6)	2 (14.3)	3 (75.0)	**0.000**
Age (years)	10.0 (9.0 – 13.0)	4.5 (1.0 – 9.0)	9.0 (3.5 – 13.5)	0.322
Ho Chi Minh city	7 (30.4)	0 (0.0)	0 (0.0)	1.000
Other provinces	16 (69.6)	14 (100.0)	4 (100.0)	
Clinical features				
Illness days before admission	4.0 (3.0 – 10.0)	5.0 (3.0 – 6.0)	4.5 (2.0 – 15.0)	1.000
Fever	13 (56.5)	14 (100.0)	4 (100.0)	0.276
Highest temperature during hospitalization (^oC)	38.8 (38.5 – 39.0)	39.0 (39.0 – 40.0)	39.4 (38.6 – 40.0)	0.322
Duration of fever (days)	3.0 (3.0 – 7.0)	7.0 (5.0 – 10.0)	8.0 (6.0 – 10.0)	0.644
Glasgow coma scale	11 (9 – 12)	8 (8 – 11)	7 (4 – 10)	1.000
Seizure	17 (73.9)	6 (42.9)	4 (100.0)	1.000
Neurological deficits	6 (26.1)	7 (50.0)	2 (50.0)	1.000
Abnormal muscular tone	8 (34.8)	4 (28.6)	2 (50.0)	1.000
Psychiatric symptoms	17 (73.9)	0 (0.0)	0 (0.0)	**0.000**
Language changes	16 (69.6)	1 (7.1)	1 (25.0)	**0.000**
Dyskinesias	15 (65.2)	1 (7.1)	2 (50.0)	**0.046**
Delirium	6 (26.1)	0 (0.0)	0 (0.0)	1.000
Cognitive dysfunction	16 (69.6)	0 (0.0)	0 (0.0)	**0.000**
Abnormal movement	16 (69.6)	3 (21.4)	2 (50.0)	0.184
CSF investigation				
WBC count (cells/mm^3)	11 (3 – 28)	99 (59 – 300)	8 (3 – 99)	**0.046**
Protein (g/L)	0.3 (0.2 – 0.4)	0.8 (0.6 – 0.9)	0.3 (0.3 – 0.5)	**0.000**
Lactate (mmol/L)	1.6 (1.5 – 1.9)	2.1 (1.8 – 2.6)	2.3 (1.7 – 3.4)	0.506
CSF glucose / Plasma glucose	0.7 (0.6 – 0.9)	0.7 (0.6 – 0.8)	0.7 (0.6 – 1.2)	1.000

MRI findings				
Cerebral cortex	13 (56.5)	2 (14.3)	4 (100.0)	0.506
Limbic system	15 (65.2)	2 (14.3)	0 (0.0)	0.138
Thalamus	9 (39.1)	14 (100.0)	0 (0.0)	**0.000**
Mid-brain	9 (39.1)	3 (21.4)	0 (0.0)	1.000
Cerebellum	7 (30.4)	3 (21.4)	0 (0.0)	1.000
Brain stem	4 (17.4)	1 (7.1)	0 (0.0)	1.000
EEG abnormality				
Delta brush	3 (13.0)	0 (0.0)	0 (0.0)	1.000
Slow waves	12 (52.2)	0 (0.0)	3 (75.0)	**0.046**
Spike waves	2 (8.7)	0 (0.0)	0 (0.0)	1.000
Beta waves	1 (4.3)	0 (0.0)	0 (0.0)	1.000
Seizure	1 (4.3)	0 (0.0)	0 (0.0)	1.000
Fast activity	2 (8.7)	0 (0.0)	0 (0.0)	1.000
Slow baseline activity	1 (4.3)	0 (0.0)	1 (25.0)	1.000
Low voltage	1 (4.3)	0 (0.0)	1 (25.0)	1.000
Treatment				
Corticosteroid	21 (91.3)	1 (7.1)	1 (25.0)	**0.000**
Immunoglobulin	9 (39.1)	0 (0.0)	0 (0.0)	0.322
Cyclophosphamide	7 (30.4)	0 (0.0)	0 (0.0)	1.000
Rituximab	4 (17.4)	0 (0.0)	0 (0.0)	1.000
Acyclovir	18 (78.3)	10 (71.4)	4 (100.0)	1.000
Mannitol	14 (60.9)	12 (85.7)	2 (50.0)	1.000
Sodium chloride 3%	3 (13.0)	3 (21.4)	2 (50.0)	1.000
Outcomes				
Length of hospital stay (days)	38.0 (15.0 – 53.0)	15.0 (11.0 – 20.0)	22.0 (16.0 – 47.5)	0.966
Mortality	1 (4.3)	0 (0.0)	0 (0.0)	1.000
Sequelae	13 (56.5)	9 (64.3)	3 (75.0)	1.000
Modified Rankin scale	2 (0 – 3)	1 (0 – 2)	2 (1 – 3)	1.000

Note: Data are presented as n (%) or median (IQR).

*Comparisons are made between NMDAR-antibody encephalitis and JEV encephalitis using Pearson's chi-squared test, Fisher's exact test, Wilcoxon rank-sum test. P-values were adjusted using Bonferroni correction. An adjusted p-value less than 0.05 is statistically significant.

In my study, one patient presented on day 7 of illness with clinical features suggestive of autoimmune encephalitis, including dyskinesia and abnormal behaviour. Additionally, EEG revealed slow waves, and MRI showed limbic system involvement. The patient tested positive for HSV-1 by CSF PCR during the initial evaluation at CH1. A follow-up CSF sample, collected two months later during the same hospitalization episode, tested positive for NMDAR antibodies. This case was classified as NMDAR-antibody encephalitis for two reasons. Firstly, the clinical presentation, including psychiatric symptoms, cognitive dysfunction, and movement disorders, was consistent with autoimmune encephalitis rather than typical HSV encephalitis. Secondly, NMDAR-antibody encephalitis is a recognized sequela of HSV encephalitis, as supported by prior literature (169). This patient was admitted to CH1 once and recruited into the study only once. During hospitalisation, the initial suspicion of autoimmune encephalitis prompted repeat CSF testing, which confirmed NMDAR antibodies, leading to the final diagnosis. For statistical analysis, this case was tabulated as a single case of NMDAR-antibody encephalitis in all relevant tables, reflecting its primary diagnosis based on clinical and immunological findings, and representing a single case.

3.4. Discussion

Here I report the first description of NMDAR-antibody encephalitis in children admitted to a tertiary referral hospital for children in Southern Vietnam. I identify NMDAR-antibody at a higher frequency than that of JEV encephalitis, the most common cause of viral encephalitis in Asia, including Vietnam (173–175). Additionally, I show that compared with JEV encephalitis, NMDAR-antibody encephalitis was associated with a different set of clinical features and longer hospital stays. My data revealed that fever, reduced consciousness, elevated CSF WBC count, increased CSF lactate, and elevated CSF protein were significantly more prevalent in JEV encephalitis, consistent with findings from previous studies (10,176,177). In NMDAR-antibody encephalitis, psychiatric features, cognitive dysfunction, language changes, abnormal movements have been well described, and attributed to multiple brain regions including fronto-striatal, cortico-limbic, hypothalamus and brainstem dysfunctions (176,178). My findings are consistent with those from higher income countries such as the US, which also show the frequency of autoimmune causes, especially NMDAR-antibody, now exceed infectious etiologies (84,163).

The JEV vaccine was introduced into the Vietnam's national expanded vaccination program in 2015, and could have reduced the incidence of JEV encephalitis. However, in my study, I could not assess this question. This is because, in my study, most patients lacked vaccination records. Some had records with limited details, while others relied only on their memory. Therefore, data on JEV vaccination could not be comprehensively collected, hampering informative assessment of vaccine effectiveness.

When comparing patients with NMDAR-antibody encephalitis and those with probable autoimmune encephalitis but seronegative for antibodies against NMDAR, I found considerable similarities in clinical presentations and neuroinflammation features

between the two groups. However, I did not find evidence of antibodies against AMPA-R1/R2, $GABA_A/_{B1}/_{B2}R$, LGI1, CASPR2 or DPPX antigens in a subset of 22 patients with probable autoimmune encephalitis. And the causes remained undefined in 52/75 (69.3%) patients with probable autoimmune encephalitis. While the data further emphasizes that NMDAR-antibody is the most common cause of autoimmune encephalitis in children (179–182), future research should comprehensively look at all the 23 possible autoantibodies currently recognized as potential causes of autoimmune encephalitis (88). Currently treatment pathways of NMDAR-antibody encephalitis include first-line immunotherapy (intravenous steroids, intravenous immunoglobulins, and plasma exchange), and if no improvement, second-line therapy (rituximab and cyclophosphamide) (81,181). Early treatment has been shown to be associated with better clinical outcome in patients with autoimmune encephalitis. The identification of features commonly found in patients with NMDAR-antibody encephalitis such as genders, neuroinflammation and especially clinical manifestations (psychological and movement disorder and language change) are consistent with findings from previous studies (76,81,85,132,133,165). These features therefore should be useful for the clinicians in approaching children with encephalitis, especially in resource limited setting where diagnostic assays are not readily available.

In my study, only one patient exhibited a clinical course involving initial HSV-1 positivity followed by NMDAR-antibody detection, representing a single case of NMDAR-antibody encephalitis, supporting previous reports (169,180–182). Among the 164 enrolled patients, each experienced only one episode of encephalitis during hospitalisation, resulting in a total of 164 individuals and 164 encephalitis episodes.

I acknowledge that NMDAR-antibody or other autoimmune encephalitis, along with antibodies to neuronal surface proteins, can occur secondary to JEV and are commonly

reported in children following JE, as described in the medical literature (183). However, no association between JEV and NMDAR-antibody encephalitis was observed in my study.

I appreciate that there are a lot more pathogens (other than JEV and HSV-1 as well as bacteria) of infectious encephalitis. However, unfortunately, I could only find 14 JEV and 4 HSV-1 encephalitis using routine diagnostic testing approach applied in my setting. Acute disseminated encephalomyelitis (ADEM) is a common presentation of myelin oligodendrocyte glycoprotein (MOG) antibody-associated disease (MOGAD), a group of central nervous system (CNS) demyelinating diseases. However, the detection of MOG antibodies in plasma and CSF by immunofluorescence assays to confirm the diagnosis is currently not available at my centre. In my study, the MRI and EEG studies did not find any images associated with ADEM and MOGAD.

I acknowledge that ELISA tests for JEV IgM in CSF can show cross-reactivity with dengue virus due to shared flavivirus antigens. This could reduce specificity and potentially lead to false-positive results. This complicates diagnosis in regions where both viruses are endemic, requiring confirmatory tests like PCR to distinguish between JEV and DENV infections. Therefore, my data may have overestimated the number of JEV cases. This in turn further emphasizes the burden of NMDAR-antibody encephalitis.

In this study, I did not routinely test second CSF samples in patients with clinically suspected JEV encephalitis whose admission CSF was IgM-negative. This limitation, due to constrained resources (e.g., limited laboratory capacity and challenges in obtaining follow-up samples), may have led to the under-diagnosis of JEV cases, as IgM seroconversion can occur later in infection. To mitigate this, I relied on clinical criteria and initial ELISA results, but future studies should prioritize repeat CSF testing to improve diagnostic sensitivity.

The absence of indeterminate cases in this cohort can be attributed to the stringent case definitions and the comprehensive diagnostic workup employed. Each case was evaluated against clear clinical and laboratory criteria, which minimised diagnostic ambiguity. All cases were reviewed by a multidisciplinary team of paediatricians specialising in neurology and infectious diseases, which enhanced the reliability of my classifications. Additionally, the use of advanced diagnostic tools, such as targeted autoimmune antibody testing and sensitive PCR assays, likely reduced the likelihood of unclassified cases. My study's focused inclusion criteria and thorough investigative approach likely contributed to the clear delineation of all cases into either infectious or autoimmune categories.

3.4.1. Strengths and limitations

The strength of my study is that it was conducted at a tertiary referral hospital for children in Vietnam, a low- and middle-income country, where limited data exist regarding the epidemiology of autoimmune encephalitis (154). Therefore, the results have added to the growing body of knowledge about the burden associated NMDAR-antibody encephalitis worldwide. However, owing to the nature of a single hospital-based study, the obtained results from a study with limited number of cases might not be generalizable for a wider community in Vietnam, and may mis-estimate overall aetiologies of encephalitis. In the SouthEast Asia Encephalitis Project (46), centred around encephalitis in the Greater Mekong region over around 3 years, 4 hospitals in Cambodia, Vietnam, Laos, and Myanmar recruited 664 children with encephalitis. Given different likely catchments, this rate of ~50 cases/year/hospital was equivalent to my findings of 164 children in 2.8 years from 1 hospital (~58 cases/year/hospital). Additionally, the COVID-19 lockdown during the study period could be a potential bias toward the admission of more patients from outside of HCMC, resulting an overestimation of disease burden. Finally, my study was

limited by the intrinsic difficulties in defining undiagnosed encephalitis: in the absence of microbiological and immunological confirmations, encephalitis cases in my study have been defined based on compatible manifestations of autoimmune encephalitis or viral encephalitis suggested by guidelines (1,5,61,92,183).

3.5. Conclusion

In summary, my study has revealed that NDMAR-antibody encephalitis is an important differential diagnosis in Vietnamese children presenting with clinically suspected encephalitis. The disease is associated with long hospitalisation and poor outcome. My findings could change paediatric diagnostics and treatment interventions to move toward more appropriate approaches in Vietnam.

Chapter 4. Viral aetiology of encephalitis in Vietnamese children: results of extensive multiplex real-time polymerase chain reaction analysis of biological samples

Chapter 4. Viral aetiology of encephalitis in Vietnamese children: results of extensive multiplex real-time polymerase chain reaction analysis of biological samples

4.1. Introduction

The use of sensitive multiplex real-time polymerase chain reaction (RT-PCR) has greatly improved the diagnostic yield in patients with central nervous system infection (46,184). In this chapter, I aimed to unravel the causes of inf3ectious encephalitis in children enrolled in the clinical study described in Chapter 2 and 3. Accordingly, I performed multiplex RT-PCR assays targeting at a broad range of viral and bacterial pathogens to analyse biological samples collected from children who had no cause identified after routine diagnosis.

My contributions: as a PI, I developed the analysis plans, oversaw the project and interpreted the diagnostic findings in the clinical context. The laboratory experiments were carried with technical support from the Emerging Infections Group at Oxford University Clinical Research Unit, in Ho Chi Minh City, Vietnam. Their expertise was critical in ensuring the accuracy and reliability of the molecular biology results.

4.2. Methods

4.2.1. Patients and samples

As outlined in Chapter 3, between March 2020 and December 2022, 164 children with clinical features of encephalitis were enrolled in this study. Of these, 75/164 cases (45.7%) had signs and symptoms meeting the predefined criteria of autoimmune encephalitis, of which 23/164 patients (14.0%) were confirmed as NMDAR-antibody encephalitis (Chapter 3). The remaining 89/164 cases (54.3%) were classified as clinically suspected

infectious encephalitis, and were the focus of this Chapter. Of these, 71 (79.8%) had no cause identified after routine diagnosis (Figure 4.1). Their available CSF samples and other biological samples (including plasma, urine, throat swabs and rectal swabs) were further analysed using multiplex RT-PCR, using a step-wise approach. Accordingly, available CSF and throat swabs were first tested, followed by further testing of blood, urine and rectal swab samples of the corresponding patients who had a throat swab PCR positive (Figure 4.2).

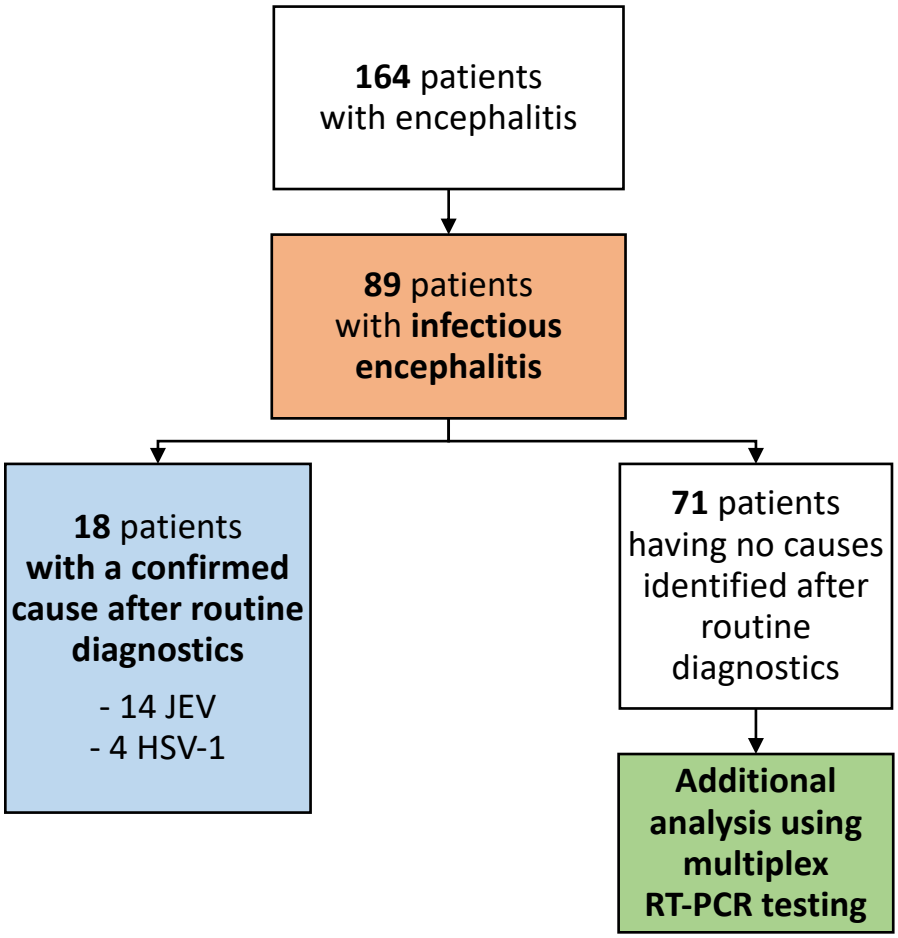


Figure 4.1. Routine diagnostics of infectious encephalitis in children

Note: HSV: herpes simplex virus, JEV: Japanese encephalitis virus

4.2.2. Nucleic acid extraction

Nucleic acids were extracted from 200ul of patient samples after the additional of internal controls provided with the PCR kits (see below), using MagNA Pure 96 DNA and viral NA Small Volume Kit (Roche Diagnostics, Mannheim, Germany) on an automatic MagNA pure 96 system, according to the manufacturer's instructions. The extracted nucleic acids were then eluted in 50ul elution buffer provided with the kit (185).

4.2.3. PCR detection of viral and bacterial causes

In addition to routine diagnosis outlined in Chapter 3, (multiplex) real-time (RT)-PCR assays targeting a wide range of viruses and bacteria were applied to analyse the CSF and non-CSF samples. For CSF samples, three Seegene multiplex PCR panels (Seoul, South Korea), including Allplex™ Meningitis-V1 Assay (Cat. No. MG9700X, MG10209Z); Allplex™ Meningitis-V2 Assay (Cat. No. MG9500X, MG10210Z), and Allplex™ Meningitis-B Assay (Cat. No. MG9600X, MG10211Z) were used (Table 4.1). These three panels cover 12 viruses and 6 bacteria that are known to cause central nervous system infections.

For throat swab and other non-CSF samples, two multiplex PCR panels including Allplex™ RV (Respiratory Virus) Essential Assay (Cat. No. RV9750X) and Allplex™ Respiratory Panel 3 (HBoV / HRV / CoV) (Cat. No. RP9601X, RP10181Z) and an established enterovirus PCR were used (Table 4.1). These PCR platforms combined cover a total 13 viruses (Table 4.1).

Table 4.1. Multiplex RT-PCR assays and targeted pathogens

Samples	Target pathogens	Technology of PCR assays	Multiplex real-time PCR assays
Cerebrospinal fluid	Herpes simplex virus 1 (HSV-1) Herpes simplex virus 2 (HSV-2) Varicella zoster virus (VZV) Epstein-Barr virus (EBV) Cytomegalovirus (CMV) Human herpes virus 6 (HHV-6) Human herpes virus 7 (HHV-7)	Seegene's proprietary MuDT™ technology	Allplex™ Meningitis-V1 Assay (Cat. No. MG9700X, MG10209Z)
	Adenovirus (AdV) Human parechovirus (HPeV) Enterovirus (EV) Mumps virus (MV) Parvovirus B19 (B19V)	Seegene's proprietary MuDT™ technology	Allplex™Meningitis-V2 Assay (Cat. No. MG9500X, MG10210Z)
	Neisseria meningitidis (NM) *Listeria monocytogenes* (LM) *Haemophilus influenzae* (HI) *Streptococcus agalactiae* (GBS) *Streptococcus pneumoniae* (SP) *Escherichia coli K1* (EC K1)	Seegene's proprietary MuDT™ technology	Allplex™ Meningitis-B Assay (Cat. No. MG9600X, MG10211Z)
Throat swab	Influenza A virus (Flu A) Influenza B virus (Flu B) Respiratory syncytial virus (RSV) Metapneumovirus (MPV) Adenovirus (AdV) Rhinovirus (HRV) Parainfluenza virus (PIV)	Seegene's proprietary MuDT™ technology	Allplex™ RV (Respiratory Virus) Essential Assay (Cat. No. RV9750X)
	Human bocavirus (HBoV) Human rhinovirus (HRV) Coronavirus 229E (229E) Coronavirus NL63 (NL63) Coronavirus OC43 (OC43)	Seegene's proprietary MuDT™ technology	Allplex™ Respiratory Panel 3 (HBoV / HRV / CoV) (Cat. No. RP9601X, RP10181Z)
Throat swab	Enterovirus (EV)	In-house PCR assay adapted from previous studies (186–189)	SuperScript® III Platinum®One-Step qRT-PCR (Invitrogen, Carlsbad, CA, USA) and LightCycler 480 II machine (Roche Diagnostics GmbH, Mannheim, Germany)

4.2.4. Seegene PCR description

The Seegene PCRs utilise the proprietary MuDT™ technology, which could accommodate multi-Ct (threshold cycle) values in a single fluorescence channel without the need of melting curve analysis on a RT-PCR instrument. The PCR reaction consisted of 17 µl of master mix provided with the kit and 8 µl of template (respiratory panels); 15 µl of master mix provided with the kit and 5 µl of template (meningitis panels) (187,190,191). The PCR reactions were then carried out on a Bio-Rad CFX96TM Dx System (Bio-Rad, California, USA) and the PCR results were analysed using the Seegene Viewer for Real-time Instruments V3 (Seegene, South Korea). The presence of specific gene sequence in the reaction is reported as a Ct value through Seegene Viewer analysis software. An exogenous gene is used as Internal Control (IC) to monitor the whole process of sample collection, nucleic acid extraction and to check for any possible PCR inhibition. The optimal dilution of IC is the dilution at which Cy5 Ct value or Quasar670 Ct value ranges from 32 to 35. Failure to detect IC may indicate improper extraction of nucleic acid from clinical samples, presence of RT-PCR inhibitor, and reagent or equipment malfunction (187,190,191).

To prevent amplification product acting as potential contaminants, Uracil-DNA glycosylase (UDG)-dUTP system is employed in all Seegene PCR reactions. The UDG-dUTP system is commonly used when performing PCR to eliminate amplicon carry-over using UDG excises uracil residues from DNA by cleaving the N-glycosylic bond.

4.2.5. PCR analysis of enterovirus (EVs) using in-house assay

The primers and probes and conditions of the in-house assay used for the detection of human enteroviruses are showed in Table 4.2. Real-time RT-PCR reactions were carried out using the SuperScript® III Platinum®One-Step qRT-PCR (Invitrogen, Carlsbad, CA, USA) and was performed on a LightCycler 480 II system (Roche Diagnostics GmbH, Mannheim, Germany). The reaction was performed in a final volume of 25 µl containing 12.5 µl 2X RT-PCR reaction Mix, primers and probes at 10nM, 0.5 µl Enzyme Mix, and 5 µl nucleic acid. The cycling conditions included one cycle of 60°C for 3 min, followed by 15 min at 53ºC and 2 min at 95ºC, and 45 cycles of 15 s at 95ºC, 1 min at 53ºC (including fluorescence acquisition) and 15 s at 72ºC.

A sample was considered positive if negative controls were negative and the tested sample was positive with a Ct value equal to or less than 40. A sample was considered negative if negative controls were negative, positive controls were positive with Ct values of about 30 (192).

Table 4.2. Primer and probe sequences and concentration used in single reaction in the PCR analysis of enterovirus

Name	Sequence (5'→3')	Final concentration	Note
ENT-F	CCCTGAATGCGGCTAAT	400 nM	Enterovirus specific primers and probe (188)
ENT-R	ATTGTCACCATAAGCAGCC	400 nM	
ENTr-probe	Cy5-ACCCAAAGTAGTCGGTTCCG-BHQ3	200 nM	

Note: Cy5 = Cyanine 5; BHQ = black hole quencher

4.2.6. Confirmed, probable, and possible causes of viral encephalitis

A confirmed cause of encephalitis was established when the pathogen was detected by PCR in the CSF sample. A probable cause of encephalitis was established when the pathogen was detected by PCR in plasma or urine or in at least both throat swab and rectal swab. A possible cause of encephalitis was established when the organism was detected by PCR in only either throat or rectal swab (Table 4.3).

Table 4.3. Confirmed, probable, and possible causes of viral encephalitis

	Detected by PCR in cerebrospinal fluid	Detected by PCR in plasma	Detected by PCR in urine	Detected by PCR in throat swab	Detected by PCR in rectal swab
Confirmed causes of encephalitis	+	–	–	–	–
	+	+	–	–	–
	+	+	+	–	–
	+	+	+	+	–
	+	+	+	+	+
Probable causes of encephalitis	–	+	–	–	–
	–	+	+	–	–
	–	+	+	+	–
	–	+	+	+	+
	–	–	+	–	–
	–	–	+	+	–
	–	–	+	+	+
	–	–	–	+	+
	–	+	–	+	+
	–	–	+	+	+
Possible causes of encephalitis	–	–	–	+	–
	–	–	–	–	+

Note:
"+": detection of a virus in the sample. There could be only one pathogen or different pathogens detected in the samples.
"–": no virus detected in the sample.

4.2.7. Data analysis

Descriptive statistics were employed to compare the epidemiology, clinical presentations, laboratory findings, patient treatment, and outcomes of patients with encephalitis. Data were presented as number of cases and percentage for qualitative variables. For quantitative variables, data were presented as median and interquartile range as my database were highly skew.

Results were illustrated in the form of tables and charts for descriptive variables. Heat map is used to illustrate the results of PCR testing including patient count, pathogen count, co-infection, and Ct values.

Statistical comparisons were made using Pearson's chi-squared test, Fisher's exact test, and Wilcoxon rank-sum test. All statistical analyses were performed using Stata version 18 (StataCorp LP, College Station, TX, USA) and R; and p-values of ≤ 0.05 were considered significant.

4.3. Results

4.3.1. Samples available for multiplex PCR testing

Of the 71 patients considered for additional PCR testing, CSF samples were available from 64, while throat swab, plasma, urine and rectal swab samples were available from 65, 56, 34 and 44, respectively. Because of the availability of the resources, I prioritised the analysis for CSF and throat swabs, followed by plasma and the other non-sterilised samples as outlined in Figure 4.2.

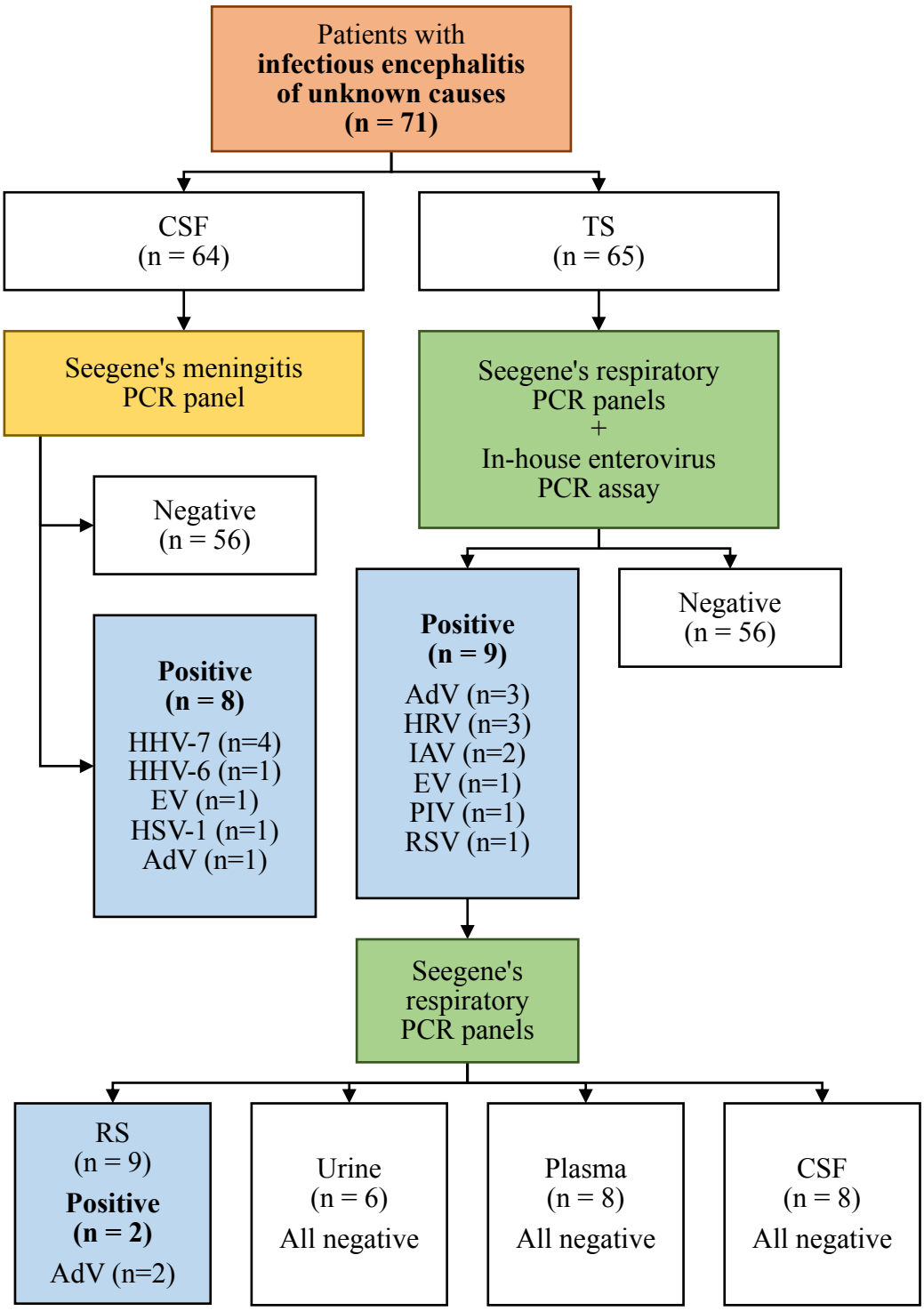


Figure 4.2. Enhanced PCR analysis for further detection of pathogenic using biological samples from paediatric encephalitis of unknown origin

Note: CSF: cerebrospinal fluid, TS: throat swab, RS: rectal swab, AdV: adenovirus, IAV: influenza A virus, EV: enterovirus, HHV: human herpesvirus, HRV: human rhinovirus, HSV: herpes simplex virus, PIV: parainfluenza virus, RSV: respiratory syncytial virus.

4.3.2. PCR analysis of samples from encephalitis patients of unknown origin

Of the 64 CSF samples included for PCR testing, the evidence of a viral pathogen was detected in 8 CSF samples (12.5%), while none of the targeted bacterial pathogen was detected. The detected viruses included four human herpesvirus 7 (HHV-7), one human herpesvirus 6 (HHV-6), one enterovirus (EV), one herpes simplex virus 1 (HSV-1), and one adenovirus (AdV) (Figure 4.2 and Table 4.4). Of note, the HSV-1 positive CSF sample was not requested for HSV diagnosis as part of routine care.

Of the 65 throat swabs, the evidence of viral pathogens was found in 9 throat swabs (13.8%), including three AdV, three human rhinovirus (HRV), two influenza A virus (IAV), one EV, one parainfluenza virus (PIV), and one respiratory syncytial virus (RSV) (Figure 4.2 and Table 4.4).

From the 9 throat swab samples tested positive above, additional PCR testing of the other sample types (8 CSF, 8 plasma, 6 urine, and 9 rectal swabs) available from the corresponding patients could identify only AdV in 2/9 rectal swabs. Of these, one came from a patient who also had a throat swab positive for AdV (Figure 4.2 and Table 4.4).

4.3.3. Confirmed aetiology after routine diagnosis and RT-PCR testing

Of the 89 patients with infectious encephalitis, routine diagnosis and additional RT-PCR testing combined, covering 27 pathogens, could identify a confirmed pathogen in 26/89 (29.2%) patients, (Figure 4.3 and Table 4.4). The detected viruses included JEV (n=14, 53.8%), HSV-1 (n=5, 19.2%), HHV-7 (n=4, 15.4%), HHV-6 (n=1, 3.8%), EV (n=1, 3.8%), and AdV (n=1, 3.8%). The AdV infected patients also had HRV detected in the throat swab. Details of pathogens detected by additional multiplex PCR with Ct value, infected patient count, and co-infection count can be found in Appendix 4.1.

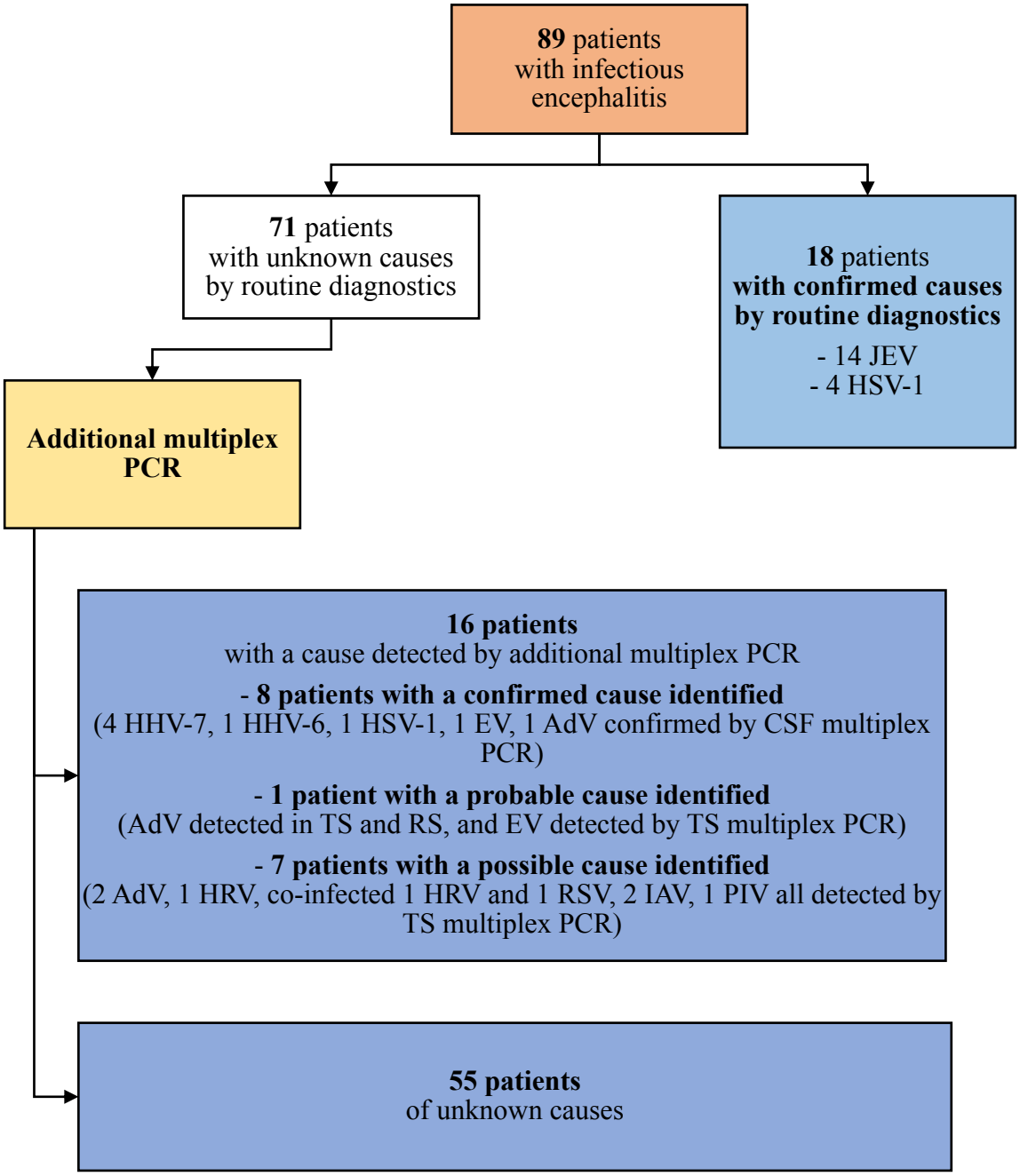


Figure 4.3. The results of additional multiplex PCR: confirmed, probable, and possible causes of infectious encephalitis

Note: CSF: cerebrospinal fluid, TS: throat swab, RS: rectal swab, HSV: herpes simplex virus, JEV: Japanese encephalitis virus, AdV: adenovirus, IAV: influenza A virus, EV: enterovirus, HHV: human herpesvirus, HRV: human rhinovirus, PIV: parainfluenza virus, RSV: respiratory syncytial virus.

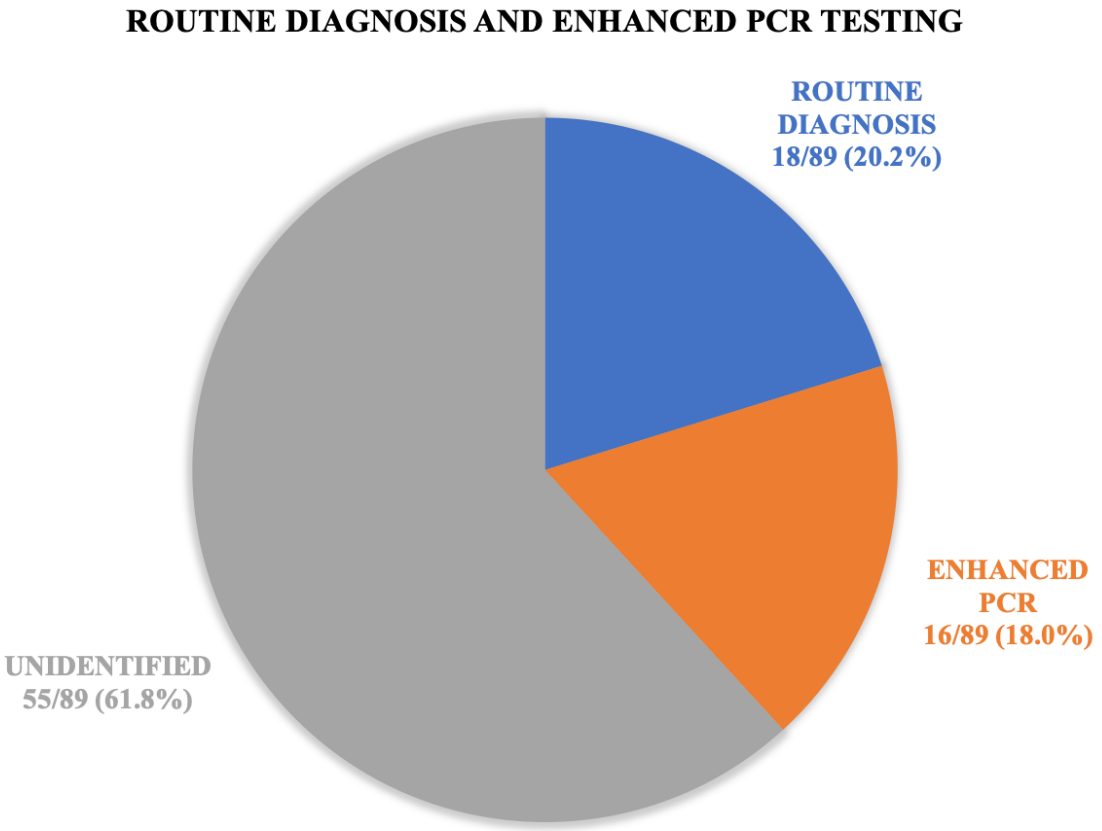


Figure 4.4. Routine diagnosis and enhanced PCR testing for detection of viruses causing encephalitis

Table 4.4. Results of PCR analysis of CSF and non-CSF samples from patients with infectious encephalitis of unknown origin

ID	CSF PCR (Ct values)	Throat swab PCR (Ct values)	Rectal swab PCR (Ct values)	Confirmed cause	Probable cause	Possible cause
P022		AdV (28.77)				
P036		HRV (26.95)				
P044	AdV (41.87)	HRV (17.25)	AdV (30.55)			
P065		IAV (29.42)				
P073		IAV (29.91)				
P076	HHV-7 (39.80)					
P113		AdV (37.38) EV (31.01)	AdV (35.25)			
P119	HSV-1 (28.45)					
P126	HHV-7 (39.32)					
P141	HHV-7 (39.55)					
P194	EV (38.16)					
P197		PIV (24.36)				
P200		RSV (22.38) HRV (39.44)				
P222	HHV-6 (39.59)					
P262		AdV (39.23)				
P272	HHV-7 (40.13)					

Note:
CSF: cerebrospinal fluid, AdV: adenovirus, IAV: influenza A virus, EV: enterovirus, HHV: human herpesvirus, HRV: human rhinovirus, HSV: herpes simplex virus, PIV: parainfluenza virus, RSV: respiratory syncytial virus.

4.3.4. Probable and possible causes

A probable cause of infectious encephalitis after RT-PCR testing was established in 1 patient who had AdV detected in both rectal and throat swabs. This patient also has EV detected in the throat swab (Figure 4.3, Figure 4.4, and Table 4.4). A possible cause after PCR testing was identified in 7 patients (7/89, 7.9%), with all having a virus detected in throat swabs, including 3 AdV, 3 HRV, 2 IAV, and EV, PIV and RSV 1 each (Figure 4.3, Figure 4.4, and Table 4.4). Of these, co-detection in throat swabs was evidenced in 3 patients, including AdV and HRV, AdV and EV, and RSV and HRV in one each (Figure 4.3, Figure 4.4, and Table 4.4).

Collectively, after excluding co-detection, routine diagnosis and extensive multiplex RT-PCR testing combined could identify a confirmed pathogen in 26/89 (29.2%) patients, probable causes in 1/89 (1.1%), and possible causes in 7/89 (7.9%); leaving 55/89 (61.8%) patients having no causes identified (Figure 4.3, Figure 4.4, and Table 4.4). CSF samples from these 55 patients will be further analysed using metagenomics (Chapter 5).

4.3.5. Clinical features and outcomes of patients with and without a confirmed cause

The clinical features, laboratory findings and outcomes of patients with and without a confirmed cause are presented in Table 4.5. In details, patients with confirmed causes established mostly came from outside of HCMC, had higher temperature, longer duration of fever, more severe level of coma, higher proportion of neurological deficits, higher CSF white cell count, higher CSF protein, higher CSF lactate, more affected thalamus and mid-brain on MRI imaging, higher proportion of sequelae, and more severe mRS compared to patients without confirmed causes. Detailed information for patients with a probable or possible cause combined are shown in Table 4.5. However, the number (n = 8) of cases are small, making detailed comparison not informative.

Table 4.5. Comparisons of clinical features and outcomes of patients with confirmed and un-confirmed viral encephalitis

	All infectious encephalitis cases (n = 89)	Cases with a confirmed cause (n = 26)	Cases without a confirmed cause (n = 63)	Cases with a probable or possible cause (n = 8)
Demographic features				
Female	37 (41.6)	8 (30.8)	29 (46.0)	4 (50.0)
Male	52 (58.4)	18 (69.2)	34 (54.0)	4 (50.0)
Age (years)	9.0 (4.5 – 12.0)	8 (7 – 13)	6 (2 – 12)	9 (7 – 11)
Ho Chi Minh city	22 (24.7)	2 (7.7)	20 (31.7)	3 (37.5)
Other provinces	67 (75.3)	24 (92.3)	43 (68.3)	5 (62.5)
Clinical features				
Illness days before admission	4 (3 – 6)	4 (3 – 6)	4 (3 – 6)	3 (3 – 5)
Fever	71 (79.8)	22 (84.6)	49 (77.8)	8 (100.0)
Highest body temperature during hospitalisation (^oC)	39.0 (38.5 – 39.6)	39.0 (39.0 – 40.0)	38.8 (38.5 – 39.5)	39.5 (38.6 – 40.0)
Duration of fever (days)	5.0 (3.0 – 7.0)	6.5 (5.0 – 10.0)	4.0 (3.0 – 6.0)	5.5 (3.5 – 8.0)
Paediatric Glasgow coma scale (GCS)	11 (10 – 13)	9 (7 – 11)	12 (10 – 13)	11 (10 – 12)
Seizure	54 (60.7)	15 (57.7)	39 (61.9)	6 (75.0)
Neurological deficits	18 (20.2)	10 (38.5)	8 (12.7)	0 (0.0)
Abnormal muscular tone	21 (23.6)	8 (30.8)	13 (20.6)	2 (25.0)
Psychiatric symptoms	17 (19.1)	2 (7.7)	15 (23.8)	2 (25.0)
Cognitive dysfunction	14 (15.7)	1 (3.9)	13 (20.6)	2 (25.0)
Language changes	24 (27.0)	4 (15.4)	20 (31.7)	3 (37.5)
Delirium	12 (13.5)	0 (0.0)	12 (19.0)	2 (25.0)
Abnormal movement	22 (24.7)	8 (30.8)	14 (22.2)	0 (0.0)
Dyskinesia	11 (12.4)	4 (15.4)	7 (11.1)	0 (0.0)
CSF				
WBC count (cells/mm^3)	12 (2 – 69)	47 (4 – 126)	4 (2 – 48)	33 (14 – 120)
Neutrophil (%)	70.0 (60.0 – 76.0)	62.0 (60.0 – 68.0)	73.0 (70.0 – 80.0)	80.0 (73.0 – 80.0)
Monocyte (%)	69.0 (58.0 – 73.0)	70.0 (55.0 – 74.0)	65.5 (58.0 – 70.0)	78.0 (65.0 – 90.0)
Protein (g/L)	0.4 (0.3 – 0.6)	0.6 (0.3 – 0.8)	0.4 (0.2 – 0.5)	0.6 (0.2 – 2.2)
Glucose (mmol/L)	3.9 (3.4 – 4.6)	3.8 (3.4 – 4.5)	3.9 (3.5 – 4.6)	3.7 (3.2 – 3.9)
CSF / Plasma glucose	0.7 (0.6 – 0.8)	0.7 (0.6 – 0.8)	0.7 (0.6 – 0.8)	0.8 (0.7 – 0.9)
CSF Lactate (mmol/L)	1.7 (1.5 – 2.1)	1.9 (1.7 – 2.1)	1.6 (1.4 – 2.0)	1.8 (1.2 – 2.5)

MRI findings				
Cerebral cortex	30 (33.7)	8 (30.8)	22 (34.9)	4 (50.0)
Limbic system	13 (14.6)	3 (11.5)	10 (15.9)	2 (25.0)
Thalamus	17 (19.1)	15 (57.7)	2 (3.2)	0 (0.0)
Mid-brain	6 (6.7)	4 (15.4)	2 (3.2)	0 (0.0)
Cerebellum	9 (10.1)	4 (15.4)	5 (7.9)	0 (0.0)
Brain stem	5 (5.6)	3 (11.5)	2 (3.2)	0 (0.0)
EEG abnormality				
Delta brush	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Slow waves	12 (13.5)	3 (11.5)	9 (14.3)	1 (12.5)
Spike waves	3 (3.4)	0 (0.0)	3 (4.8)	1 (12.5)
Beta waves	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Brain dysfunction	10 (11.2)	5 (19.2)	5 (7.9)	1 (12.5)
Seizure	1 (1.1)	0 (0.0)	1 (1.6)	0 (0.0)
Fast activity	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Slow baseline activity	6 (6.7)	1 (3.9)	5 (7.9)	0 (0.0)
Low voltage	2 (2.2)	1 (3.9)	1 (1.6)	0 (0.0)
Treatment				
Methylprednisolone	16 (18.0)	5 (19.2)	11 (17.5)	0 (0.0)
Immunoglobulin	6 (6.7)	2 (7.7)	4 (6.4)	0 (0.0)
Cyclophosphamide	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Rituximab	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Acyclovir	72 (80.9)	20 (76.9)	52 (82.5)	7 (87.5)
Mannitol	55 (61.8)	18 (69.2)	37 (58.7)	7 (87.5)
Sodium chloride 3%	15 (16.9)	6 (23.1)	9 (14.3)	3 (37.5)
Outcomes				
Length of hospital stay (days)	12 (8 – 16)	12 (10 – 22)	12 (8 – 16)	12 (10 – 17)
Mortality	2 (2.2)	0 (0.0)	2 (3.2)	0 (0.0)
Sequelae	26 (29.2)	16 (61.5)	10 (15.9)	0 (0.0)
Modified Rankin scale (mRS)	0 (0 – 1)	1 (0 – 2)	0 (0 – 0)	0 (0 – 0)

Note: Data are presented as n (%) or median (IQR).

4.3.6. Clinical features and outcomes of patients with HHV-6&7

The clinical features, laboratory findings and outcomes of patients with HHV-6&7 infections are presented in Table 4.6. The clinical features of two patients with confirmed AdV and EV infection are shown in Appendix 4.2.

Table 4.6. Clinical features and outcomes of patients with encephalitis caused by HHV-6&7

	HHV-6&7 encephalitis (n = 5)
Demographic features	
Female	1 (20.0)
Male	4 (80.0)
Age (years)	2 (2 – 9)
Ho Chi Minh city	2 (40.0)
Other provinces	3 (60.0)
Clinical features	
Illness days before admission	3 (3 – 4)
Fever	2 (40.0)
Highest temperature during hospitalisation (^oC)	39.5 (39.0 – 40.0)
Duration of fever (days)	7 (1 – 13)
Paediatric Glasgow coma scale (GCS)	11 (10 – 14)
Seizure	2 (40.0)
Neurologic deficits	0 (0.0)
Abnormal muscular tone	2 (40.0)
Psychiatric symptoms	1 (20.0)
Cognitive dysfunction	0 (0.0)
Language changes	2 (40.0)
Delirium	0 (0.0)
Abnormal movement	2 (40.0)
Dyskinesia	1 (20.0)
Cerebral spinal fluid (CSF)	
White cells count	4 (1 – 28)
Neutrophil (%)	55.0 (55.0 – 55.0)
Monocyte (%)	69.0 (69.0 – 69.0)
Protein (g/L)	0.5 (0.2 – 0.6)
Glucose (mmol/L)	3.5 (3.4 – 4.9)
CSF / Plasma glucose	0.7 (0.6 – 0.7)
CSF Lactate (mmol/L)	1.8 (1.3 – 1.8)
MRI findings	
Cerebral cortex	2 (40.0)
Limbic system	0 (0.0)
Thalamus	1 (20.0)
Mid-brain	0 (0.0)
Cerebellum	0 (0.0)
Brain stem	0 (0.0)
EEG abnormality	
Delta brush	0 (0.0)
Slow waves	0 (0.0)
Spike waves	0 (0.0)
Beta waves	0 (0.0)
Brain dysfunction	1 (20.0)
Seizure	0 (0.0)
Fast activity	0 (0.0)
Slow baseline activity	0 (0.0)

Low voltage	0 (0.0)
Treatment	
Methylprednisolone	2 (40.0)
Immunoglobulin	1 (20.0)
Cyclophosphamide	0 (0.0)
Rituximab	0 (0.0)
Acyclovir	4 (80.0)
Mannitol	2 (40.0)
Sodium chloride 3%	0 (0.0)
Outcomes	
Length of hospital stay (days)	11 (7 – 11)
Mortality	0 (0.0)
Sequelae	3 (60.0)
Modified Rankin scale (mRS)	1 (0 – 1)

Note: Data are presented as n (%) or median (IQR).

4.3.7. Seasonal distribution of viruses causing encephalitis

Temporally, the distribution of infectious encephalitis cases peaked in June 2020, December 2020, and July 2022 (Figure 4.5). For specific viruses, JEV cased were mostly detected in the second half of the year in 2020, particularly in December 2020 with 4 cases (Figure 4.5). HSV-1 was detected throughout the study period in July 2020, October 2020, February 2021, September 2021, January 2022 with 1 case for each month (Figure 4.5). Four cases of HHV-7 can be observed in October 2020, February 2021, March 2021, and December 2022 (Figure 4.5).

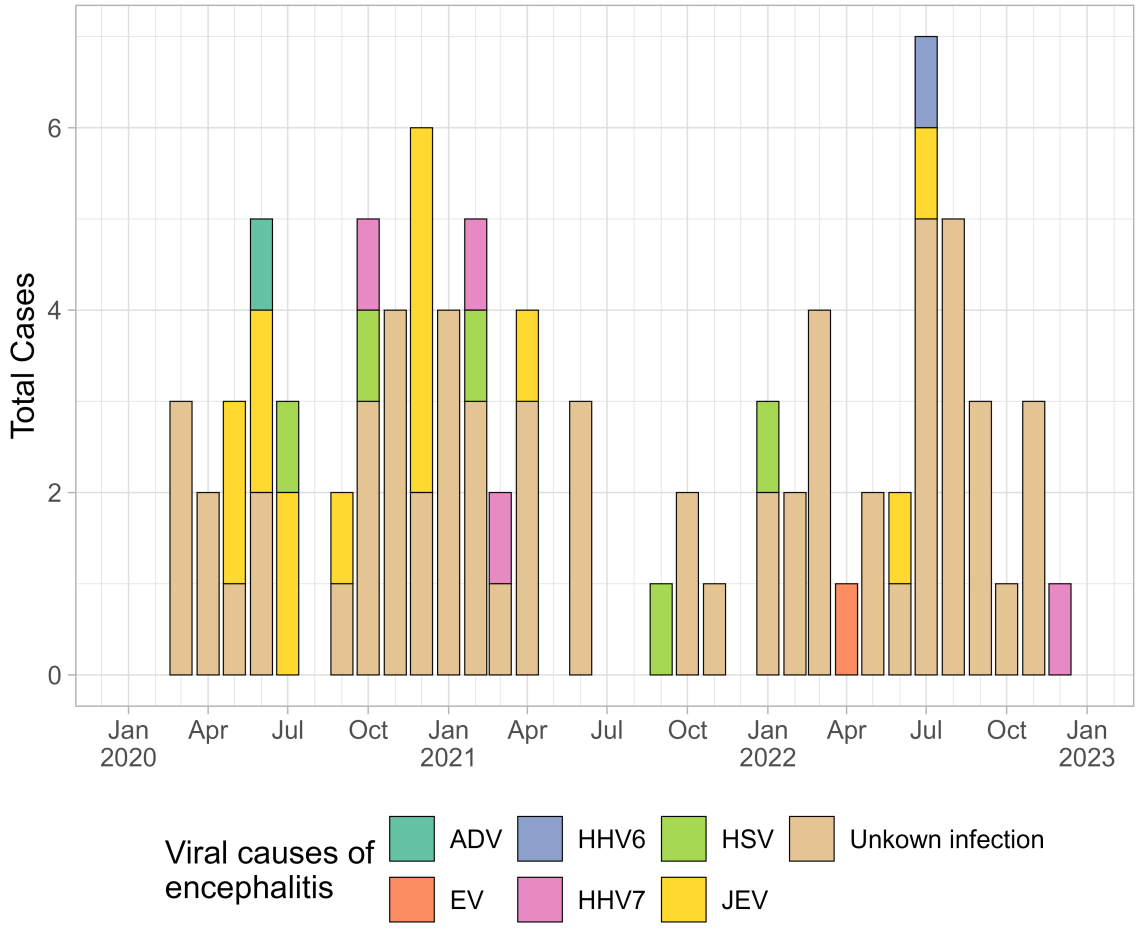


Figure 4.5. The seasonal distribution of all infectious encephalitis cases enrolled per month

Note:
HSV: herpes simplex virus, JEV: Japanese encephalitis virus, AdV: adenovirus, EV: enterovirus, HHV: human herpesvirus

4.3.8. Geographic distribution of patients with a confirmed cause identified

The geographic distribution of patients with a confirmed cause is presented in Figure 4.6. JEV was found in only from patients coming from Mekong Delta, especially in Tra Vinh where 5/14 (36%). None JEV case came from Ho Chi Minh City. The remaining viruses were sporadically distributed across Southern Vietnam.

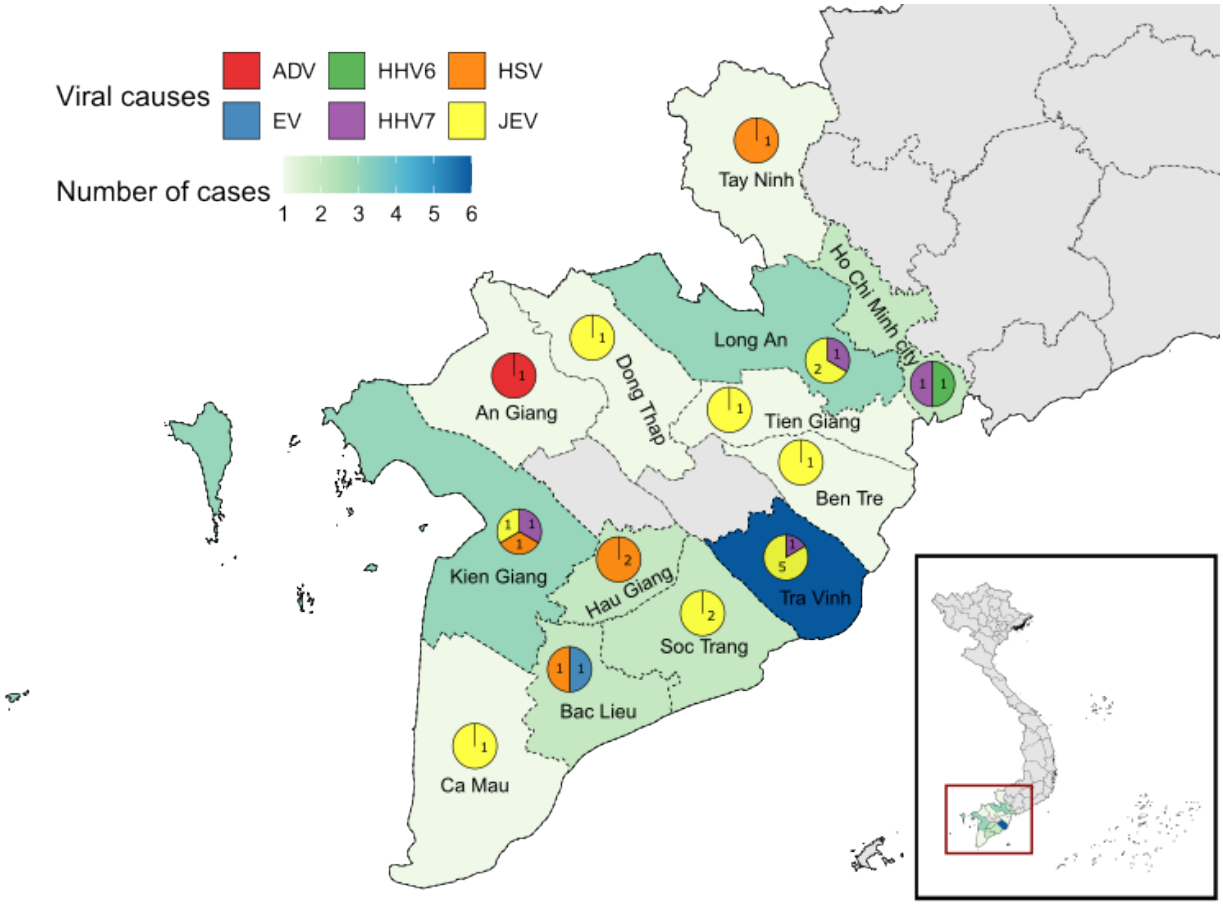


Figure 4.6. The geographic distribution of confirmed viral causes of encephalitis in children

Note:
HSV: herpes simplex virus, JEV: Japanese encephalitis virus, AdV: adenovirus, EV: enterovirus, HHV: human herpesvirus

4.4. Discussion

In this Chapter, I focused my analysis on 89 patients with clinically suspected infectious encephalitis. As outlined in Chapter 3, routine diagnosis targeting HSV and JEV could reveal a pathogen in 18/89 (20.2%). To gain further insights into the causes of infectious encephalitis, I therefore applied multiplex RT-PCR to look for a total of 25 viruses and 6 bacteria that can be associated with central nervous system infections in available CSF and non-CSF samples collected from patients with clinical suspected infectious encephalitis of unknown origin.

Despite extensive analysis, a causative agent (confirmed, probable or possible) could only be detected in 34 patients, including confirmed in 26 (76.5%) patients, probable in 1 (2.9%) patient and possible in 7 (20.6%) patients. This corresponds to an increase in the yield from 18 patients (18/89, 20.2%) after routine diagnosis to 34 patients (34/89, 38.2%). The detected viruses including JEV, HSV, HHV-6&7, AdV, IAV, PIV and RSV belonged to 5 families including *Flaviviridae, Herpesviridae, Adenoviridae, Orthomyxoviridae*, and *Paramyxoviridae*. Collectively, the data emphasizes that diverse pathogens can be responsible for infectious encephalitis, and it remains a challenge to establish a plausible cause in patients presenting with infectious encephalitis, supporting previous reports (104,184,193). Although in encephalitis patients, antivirals are unavailable for most of the viruses, knowledge about the causative agents is clinical and public health importance. Therefore, my findings further emphasize that testing for a wide range of viruses is needed to identify a possible cause in patients with encephalitis, which could help inform clinical management strategies and public health response to local outbreaks.

In terms of the detected viral pathogens, JEV was the leading cause with more cases being detected in the second half of the year, the rainy season in Southern Vietnam, and none

coming from Ho Chi Minh City. JEV transmission cycles involve mosquitoes and vertebrate hosts, especially pigs with humans being the dead-end hosts. These findings are consistent with a recent report conducted in Northern Vietnam and other countries in Southeast Asia (Cambodia, Laos and Myanmar) showing that JEV remained the leading cause of childhood encephalitis (36). JEV is endemic in Asia and is responsible for over 50,000 reported infections annually, despite the availability of an effective vaccine. Likewise, since 2014, JEV vaccine has been included in the national vaccination programme in Vietnam. These findings emphasize that further research is urgently needed to assess the public awareness and vaccine acceptance in JEV endemic countries, critical to inform vaccination campaign.

HSV is an important cause of encephalitis worldwide, with poor outcome if not timely treated. HHV-6 and 7 can occasionally cause encephalitis (39). However, the detection HHV-6&7 in CSF samples may merely reflect an incidental finding (11,31,32). Of note, CSF samples positive for HHV-6 or 7 had a Ct value range of 39-40, the lower limit of detection of a PCR assay (i.e. low viral load) as compared to that of the HSV-1 patient (Ct value: 28.45). Information about Ct values of CSF samples positive for HSV by routine diagnosis was not available. During the latency, HHV-6 genome is integrated into the host genome in ~1% of the immunocompetent patient which is termed chromosomally integrated HHV-6 (ciHHV-6), and individuals with this condition have HHV-6 DNA present in the telomeres of all nucleated cells (195). Therefore, a CSF PCR result positive for HHV-6 might not reflect on ongoing infection. A quantitative PCR to confirm viral load and to demonstrate a reduction in viral copies in combination with clinical improvement with appropriate antiviral medications, measuring intrathecal antibodies against HHV-6, and the presence or absence of chromosomally integrated HHV-6 DNA

can contribute to the strength of diagnosis; however, there is currently no consensus on the diagnostic approach for HHV-6 encephalitis (195).

EV and PEV are the leading cause of meningitis in young children the UK and in developed countries (186), but have been rarely detected in patients with encephalitis in Vietnam, in agreement of my findings (196,197). In contrast, in Southeast Asia, enterovirus A71 has emerged and caused large outbreaks of severe hand foot and mouth disease (198). And outbreaks of enterovirus A71 have rarely been detected outside of Southeast Asia. Collectively, the ecological factors contributing the emergence of picornaviruses across the world should be further studied.

Respiratory pathogens (IAV, AdV, RSV and PIV) can occasionally be associated with central nervous system infections (199–202). However, care should be taken in interpretation of findings of a virus in respiratory samples as it may merely reflect carriage. Nevertheless, in the absence of other pathogens detected in CSF, the results pointed to a possible contribution of those viruses to the pathology of encephalitis. Unlike CSF, which requires an invasive sampling procedure, collection of throat swab is non-invasive, and therefore should be considered for routine care alongside CSF samples where the resources are available. The recognition of clinical and sub-clinical features associated with specific pathogens reflects the tropism of those pathogens (203). Therefore, they can be useful for diagnostic model development. However, this would require follow-up studies with sufficient sample size.

4.4.1. Strengths and limitations

I used multiplex RT-PCR which offers high sensitivity (from 89.4% to 100%) and high specificity (from 92.2% to 100%) for identifying causes of encephalitis in CSF, allowing the simultaneous detection of multiple pathogens of encephalitis in a single sample (204,205). However, there are several factors that might contribute to the low diagnostics

yield in patients with encephalitis. Firstly, the clearance of viruses from CSF at the time of clinical onset as for the case of JEV, which can be one of the contributing factors. Secondly, I was unable to thoroughly search for all the possible infectious causes of encephalitis in children owing to the resource constraint. The untested pathogens such as *Orientia tsutsugamushi*, *Leptospira* spp, Rabies virus, *Rickettsia* spp, Rubella virus, *Listeria monocytogenes*, *Treponema pallidum*, and measles virus, were overlooked (36). Thirdly, previously unidentified causes can be another factor. Last but not least, patients with non-infectious encephalitis might have been missed included in the study. A part from the factors mentioned above, additional limitations include i) patient recruitment was undertaken at only one referral paediatric hospital in Ho Chi Minh, and ii) patient recruitment was conducted during the COVID-19 pandemics. Of note, the impacts of COVID-19 lockdown on the epidemiology of infectious diseases, including meningitis, have recently been reported. (184,202). These factors might have limited the generalizability of my findings.

4.5. Conclusion

In this chapter, I have shown that despite extensive RT-PCR diagnostic work up, a viral pathogen was detected in only 38.2% (34/89) of patients with infectious encephalitis. Of the identified pathogens, JEV remains the leading cause of paediatric encephalitis in Vietnam, although effective JEV vaccines have been introduced into the Vietnamese national vaccination programme since 2014. Study assessing vaccine acceptance and public awareness should be done to inform vaccination campaign. As encephalitis can also be caused by other pathogens, including known viruses not covered in this chapter as well as previously uncharacterised viruses, in the next chapter I will apply metagenomics to further identify the viral causes in the CSF of remaining patients.

Chapter 5. Metagenomics next-generation sequencing analysis of biological samples from children presenting with encephalitis of unknown origin

Chapter 5. Metagenomics next-generation sequencing analysis of biological samples from children presenting with encephalitis of unknown origin

5.1. Introduction

In this Chapter, I applied an in-house mNGS workflow, which has extensively been used for pathogen discovery a whole-genome sequencing of emerging viral pathogens in CSF and non-CSF samples (203–210), to reveal the viral contents in 64 patients with clinically suspected infectious encephalitis. Those were the patients who had no aetiology identified after routine diagnostics (Chapter 3). Their CSF samples were also tested by real time PCRs described in Chapter 4. Therefore, by testing the same set of CSF samples from the same group of patients using both methods, I could assess the potential utility of mNGS in terms of detecting a broad range of encephalitis pathogens.

My contribution: I established the research questions, selected the samples from enrolled patients for analysis, and interpreted the laboratory findings in the clinical context. For technical aspects of the work, I collaborated with the molecular microbiologists from the Emerging Infections Group at OUCRU to perform the laboratory experiments. v

5.2. Materials and Methods

5.2.1. Patient samples

As outlined in Chapter 4, of the 89 patients with clinically suspected infectious encephalitis, after routine diagnosis, 71/89 patients (79.8%) had no causative organisms identified. Of those 71 patients, 64 had CSF samples available for both RT-PCR (Chapter 4), and mNGS analyses (this Chapter). Additionally, where available, plasma (n = 56) and urine samples (n = 34) from those 64 selected patients were also subjected to mNGS analysis (Figure 5.1).

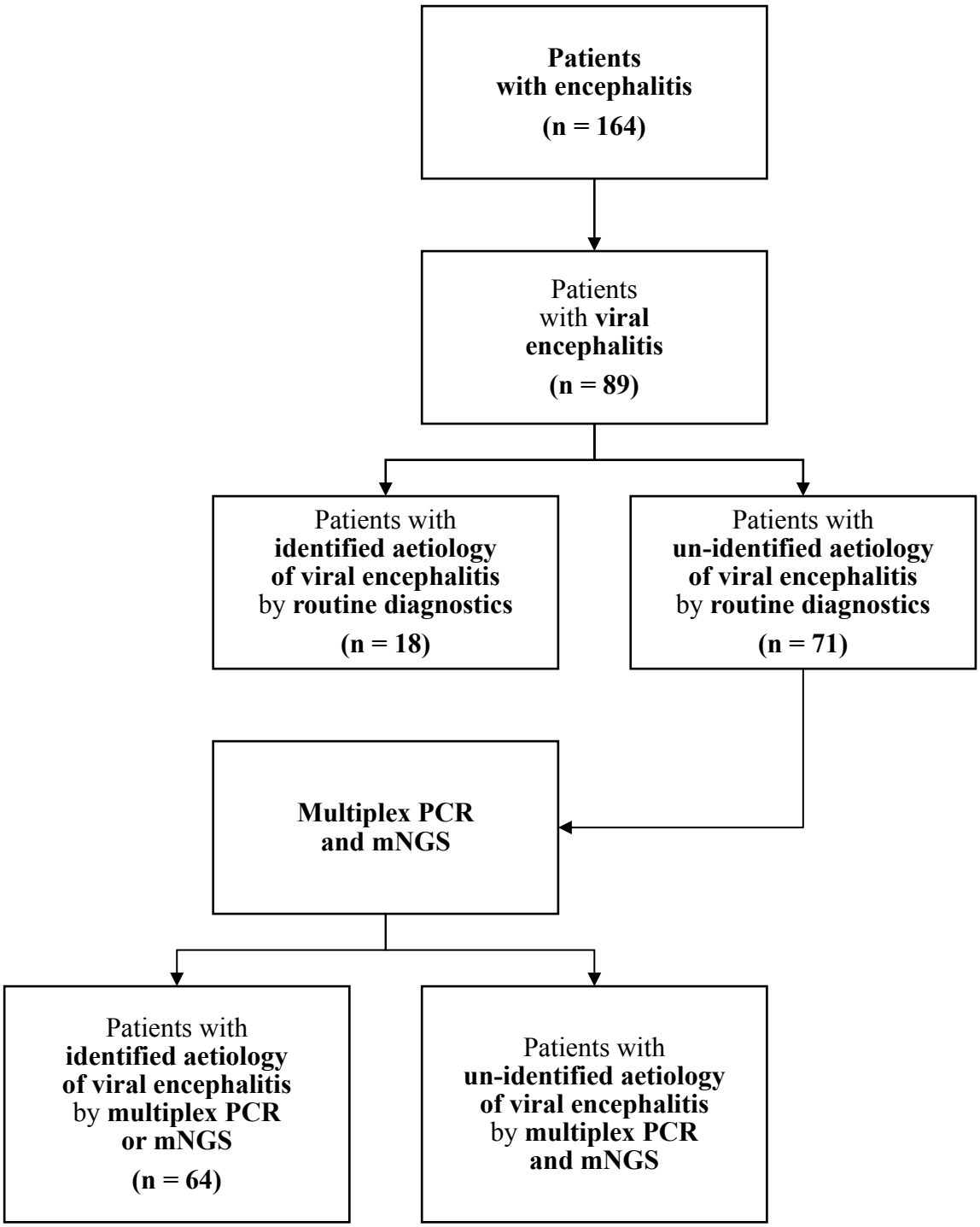


Figure 5.1. Routine diagnostics and additional analyses (multiplex RT-PCR and mNGS) for potential causes of infectious encephalitis

5.2.2. mNGS workflow

An overview of the workflow of the mNGS assay is shown in Figure 5.2. Each tested sample was analysed individually, and for each run, a positive control mixture consisting of 10 µl culture materials derived from non-human viruses (Equine arteritis virus (EAV) and Phocid herpes virus (PhHV), equivalent to Ct=30 and Ct=27, respectively) was used. It was pragmatically defined that a mNGS run was technically successful if evidence of EAV or PhHV was detected.

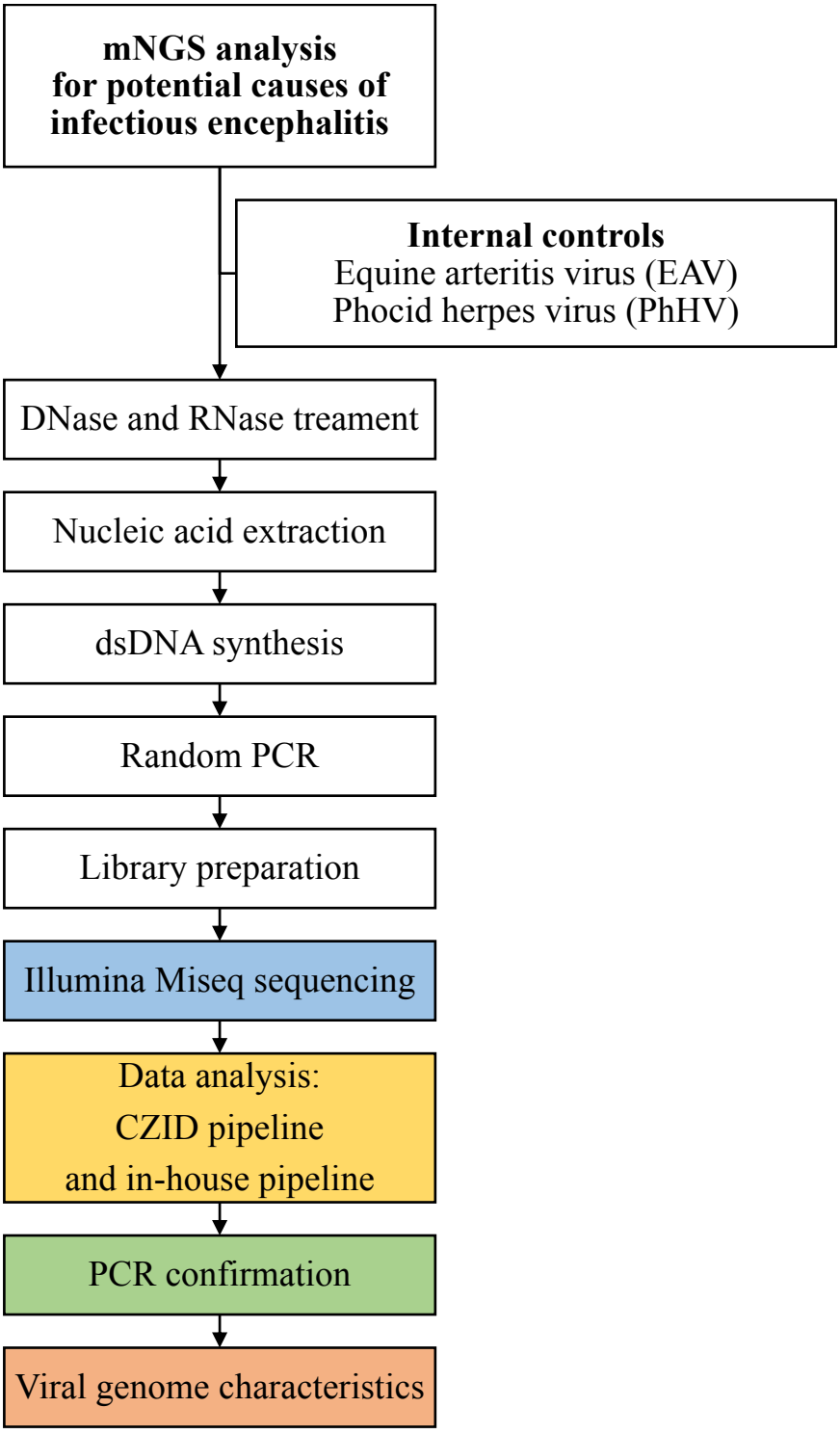


Figure 5.2. The work flow of mNGS analysis

5.2.3. Sample pre-treatments and nucleic acid isolation

Prior to nucleic acid (NA) isolation, 100µl of clinical sample was treated with 2U/µl of turbo DNase and 0.4U/µl RNase I (Ambion, Life Technology, Carlsbad, 130 CA, US) at 37°C for 30 min. This step aimed to eliminate unwanted background of DNA and RNA deriving from the hosts. The treated sample was then proceeded to a viral NA isolation step using QIAamp viral RNA kit (QIAgen GmbH, Hilden, Germany), following manufacture's instruction, and finally recovered in 50µl of the elution buffer provided with the extraction kit.

5.2.4. Double stranded DNA synthesis and sequencing

The isolated NA was subjected to a double stranded DNA synthesis step using a set of 96 non- ribosomal random primer (Appendix 5.1), followed by a random amplification step to enrich for viral NA prior to sequencing. Finally, the amplified products were sequenced on an Illumina MiSeq platform (Illumina, San Diego, CA, US) available at the laboratory of OUCRU. In details, 10µl of extracted NA was firstly mixed with 2µl of non-ribosomal random primer mixture (Appendix 5.1) and 1µl of dNTPs (10mM each) (Roche Diagnostics GmbH, Mannheim, Germany). The mixture was incubated at 85^{o}C for 2 min, and was then immediately chilled on ice for 1 min. Next, 7µl of a reaction mix containing 200U of Super Script III reverse transcriptase (Invitrogen, Carlsbad, CA, US), 40U of RNase OUT (Invitrogen), 0.1M DTT (Invitrogen) and 5X first strand buffer (Invitrogen) was added into the first reaction mixture. The reaction was then continued at 25^{o}C for 10 min, 37^{o}C for 1 min and 94^{o}C for 2 min, and immediately chilled on ice for 2 min. Subsequently, 5U of exo-Klenow fragment (Ambion) and 10U of Ribonuclease H (Ambion) were then added into the reaction mixture. The mixture was subjected to a thermal condition consisting of 25^{o}C for 5 min, 37^{o}C for 1h and 94^{o}C for 2 min, and then

immediately chilled on ice for 2 min. This exo-Klenow fragment associated step was repeated once more time with the second thermal condition consisting of 25^{o}C for 5 min, 37^{o}C for 1h and 75^{o}C for 10 min.

Finally, 5µl of the resulting dsDNA was pre-amplified using FR20RV primer (5'-GCCGGAGCTCTGCAGATATC-3'). Random PCR (rPCR) was carried out in a total reaction volume of 25µl consisting of 2µl of dsDNA, 1µl of primer FR20RV at a final concentration of 40nM and 22µl of Platinum PCR supermix (Invitrogen). The thermal cycling condition consisted of 94^{o}C for 2 min and followed by 40 cycles of 94^{o}C for 30s, 55^{o}C for 30s and 72^{o}C for 3min and 1 cycle of 72^{o}C for 2min. List of reagents and thermal conditions used for these procedures are summarized in Table 5.1.

The obtained random PCR product was subjected to library preparation using Illumina COVIDseq assay (Illumina, San Diego, CA, US), following the manufacturer's instruction. Prior to sequencing, the quantity of the prepared library was measured by using Qubit dsDNA HS kit (Invitrogen) and Tapstation analysis (Agilent Technologies). The prepared library was sequenced by using MiSeq reagent kit v3 (150 cycles) (Illumina, San Diego, CA, US) in a MiSeq platform (Illumina, San Diego, CA, US). For each run, samples were multiplexed and differentiated by double indexes using Nextera XT Index Kit (Illumina, San Diego, CA, US).

Table 5.1. List of reagents and thermal conditions of the pre-treatment, dsDNA synthesis and random amplification procedures

Procedures	Reagents	Concentration	Used volume (per reaction)	Thermal cycling condition
Pre-treatment	Turbo DNase	2 U/μl	10 μl	37°C for 30min
	RNase I	100 U/μl	1 μl	
	DNase buffer	10X	12 μl	
Double strand DNA synthesis	Non-ribosomal 1μM primer mixture	1 μM	2 μl	85°C for 2 min
	dNTPs	10 μM	1 μl	
	Super Script III reverse transcriptase	200 U/μl	1 μl	25°C for 10 min, 37°C for 1 min and 94°C for 2 min
	RNase OUT	40 U/μl	1 μl	
	DTT	0.1 M	1 μl	
	First strand buffer	5X	4 μl	
	exo-Klenow fragment	5 U/μl	0.5 μl	25°C for 5 min, 37°C for 1h and 94°C for 2 min 25°C for 5 min, 37°C for 1h and 75°C for 10 min
	Ribonuclease H	10 U/μl	0.5 μl	
Random amplification	FR20RV primer	10 μM	1 μl	94°C for 2 min, 40 cycles of 94°C for 30s, 55°C for 30s and 72°C for 3min and 1 cycle of 72°C for 2min
	Platinum PCR supermix		22 μl	

5.2.5. Sequence analysis

The obtained sequencing data was analysed using two independent pipelines: an in-house pipeline (211), and a publicly available cloud-based platform called CZ ID (https://czid.org/), enabling the detection of both known and genetically diverse viruses. Findings generated by both platforms were compiled and reported as combined results.

The CZ ID is a free, open-source, user-friendly, no-code analysis platform for analysing NGS data. The in-house pipeline is operated on a 36-node Linux cluster, employing a multiple-step process, which could detect sequences of genetically diverse viruses (212–214). In brief, after duplicate reads and reads belonging to human or bacterial genomes were filtered out, the remaining reads were assembled de novo. The resulting contigs and singlet reads were then aligned against a customized viral proteome database by using an approach based on BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Next, the candidate viral reads were aligned against a nonredundant non-virus protein database to remove any false-positive reads (i.e., reads with expected values higher than those against viral protein databases). Any virus-like sequence with an expected value <0.00001 was considered a significant hit.

A reference-based mapping approach available in Geneious 8.1.5; Biomatters was then used to obtain the consensus of the corresponding detected viruses from the original mNGS datasets. Paired-wise sequence alignment was carried out to assess the levels of sequence identity between the obtained virus genomes and the reference genomes retrieved from the NCBI (https://www.ncbi.nlm.nih.gov/nucleotide/).

5.2.6. PCR confirmatory testing of mNGS results

A viral mNGS result was considered positive only if it was subsequently confirmed by specific PCR analysis of the original samples (Figure 5.2). Accordingly, where appropriate, viral specific PCR was conducted to confirm mNGS hits identified from the viral mNGS pipeline. For this analysis, newly extracted NA from the original samples was used.

5.2.7. Genomic characteristics

Where mNGS generated sufficient sequence data, further genetic characterization using phylogenetics based approached was conducted. This involved the generation of the consensus sequences from mNGS data set, using a reference-based mapping approach available in Geneious 8.1.5. Sequence alignment of the obtained sequences was then conducted using MAFFT v7.520 (215). To assess the evolutionary history of the pathogen, I reconstructed maximum likelihood phylogenetic trees based on the obtained sequences and representatives of global sequences, using IQ tree (216).

5.2.8. GenBank accession number

The metagenomics data obtained in this study have been deposited in GenBank with accession numbers PRJNA1183618.

5.3. Results

5.3.1. Overview of mNGS results

A total of 154 samples (64 CSF samples, 56 plasma samples, 34 urine samples) were sequenced in five Miseq runs, generating a total of 124,453,695 reads (median read per sample, 714,724; range 309 to 2,016,319). Viral reads only constituted a small proportion of the total reads obtained from each run, ranging from 0.07% to 0.68% per run. In terms of viral sequences detected according to sample types, CSF samples had the lowest number of viral reads, accounting for only 0.08% (37,013/48,007,445) of the total reads obtained from all CSF samples, as compared to 0.54% (303,906/56,189,822) and 0.15% (30,929/20,256,428) of the total reads obtained from plasma and urine, respectively (Figure 5.3).

Of the obtained viral reads, evidence of sequences related to 22 viral species belonging to 12 families was detected in 30/64 (46.9%) the included patients. However, not all the detected viruses were those known to cause human infections. Some viruses were those with unknown pathogenicity, or those that have previously been reported to be contaminants found in mNGS data sets or that have not been reported in human samples (data not shown). Here, I focused my analysis on viruses that are known to cause or potentially cause human infection, in particular CNS infections.

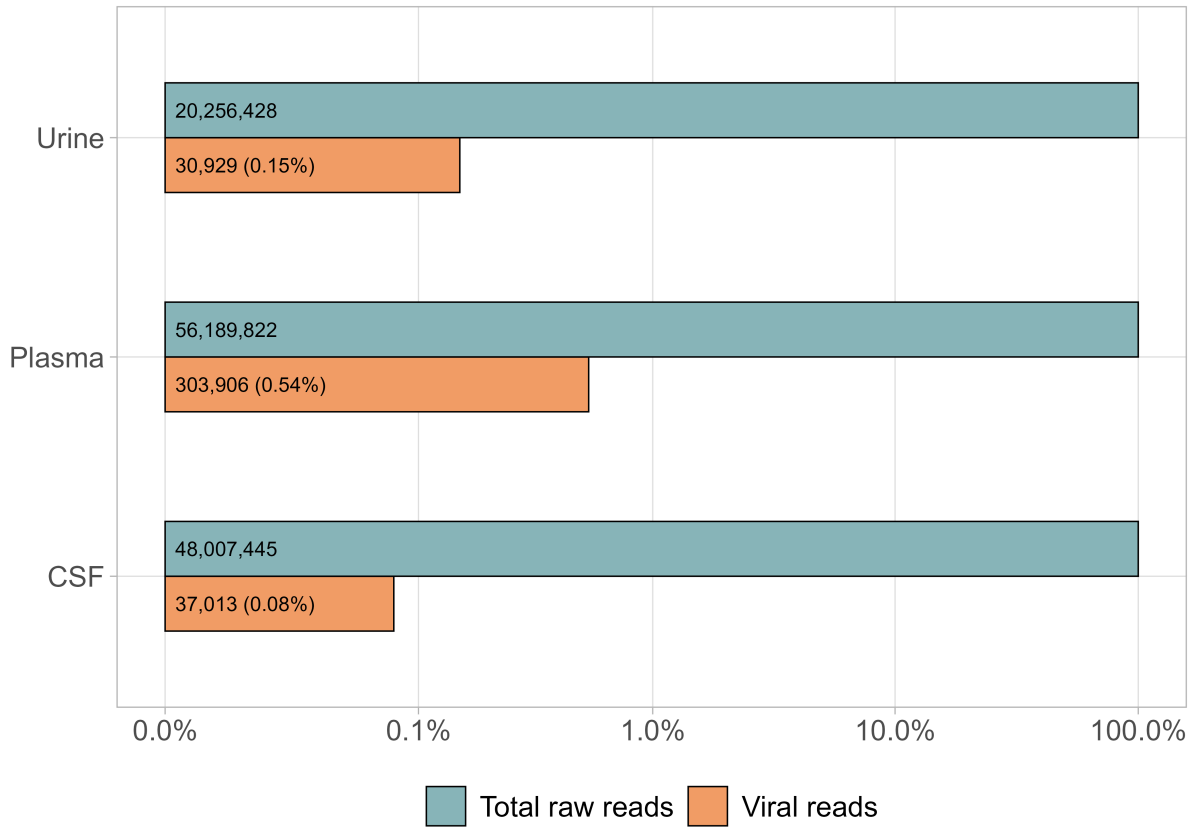


Figure 5.3. The total raw reads and the viral reads obtained from the tested CSF, plasma, and urine samples using mNGS

5.3.2. Detection of known human viral pathogens by mNGS

An overview about the frequency of specific viruses detected by mNGS is shown in Figure 5.4A&B. After confirmatory PCR testing, evidence of HSV-1 and DENV was detected in CSF of two patients (n=1 each, Figure 5.4B). The patient with DENV detected in CSF also had their plasma sample positive for DENV by mNGS. PCR confirmatory testing was also able to confirm the presence of CMV and EBV in urine sample of two patients (one each) (Figure 5.5). The CMV positive patient also had CSF positive for HSV-1. Otherwise, the majority of mNGS findings were not replicated by subsequent confirmatory PCR testing, in particular in case of DENV (Figure 5.4B). PCR testing for human polyomavirus 2 (HPyV-2) and human papillomavirus (HPV) was not done due to the unavailability of the assays. Those viruses however unlikely cause encephalitis, and they were detected by mNGS in urine. Collectively, after confirmatory PCR testing, evidence of a neurotropic virus was detected in 2/64 (3.1%) tested CSF samples, 1/56 (1.8%) plasma samples and 2/34 (5.9%) urine samples (Figure 5.4B). Accordingly, after removing co-detection, a confirmed diagnosis was established in 2/64 (3.1%) CSF samples (HSV1 and DENV, on each), and a probable diagnosis in 1/34 (2.9%) urine samples (EBV), Figure 5.5.

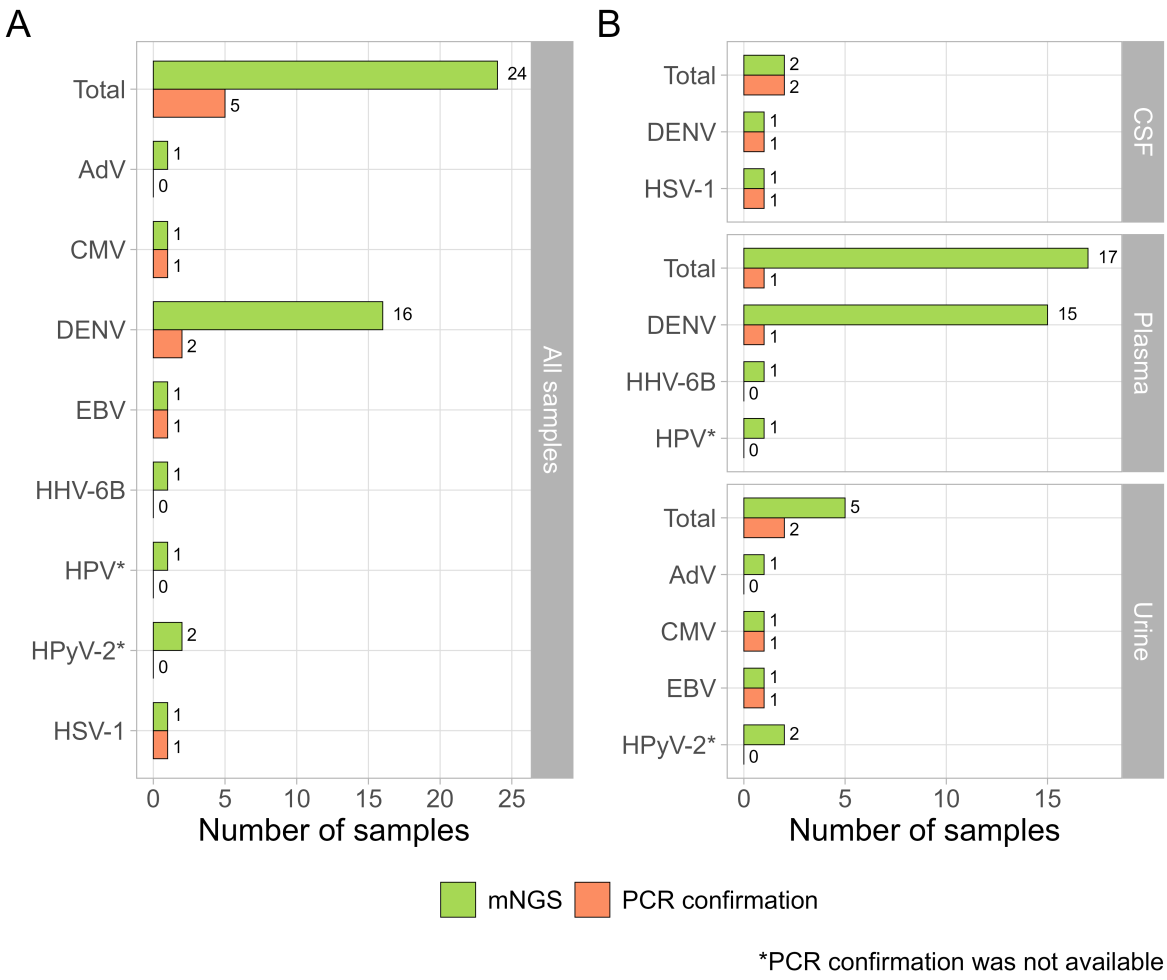


Figure 5.4. Bar chart showing the frequency of known viral pathogens that were detected by mNGS and subsequently confirmed by PCR confirmatory testing

Note:
A. Known viral pathogens detected by mNGS followed by PCR confirmatory analysis.
B. Known viral pathogens detected by mNGS followed by PCR confirmation in specific type of samples including CSF, plasma, and urine.
Green: viral pathogens were detected by mNGS alone.
Orange: viral pathogens were detected by mNGS and subsequently confirmed by PCR confirmatory testing.
*PCR assay was not available for confirmatory testing
HSV-1 (herpes simplex virus-1), EBV (Epstein-Barr virus), CMV (cytomegalovirus), DENV (Dengue virus), AdV (adenovirus), HHV-6B (human herpes virus-6B), HPV (human papillomavirus), and HPyV-2 (human polyomavirus 2)

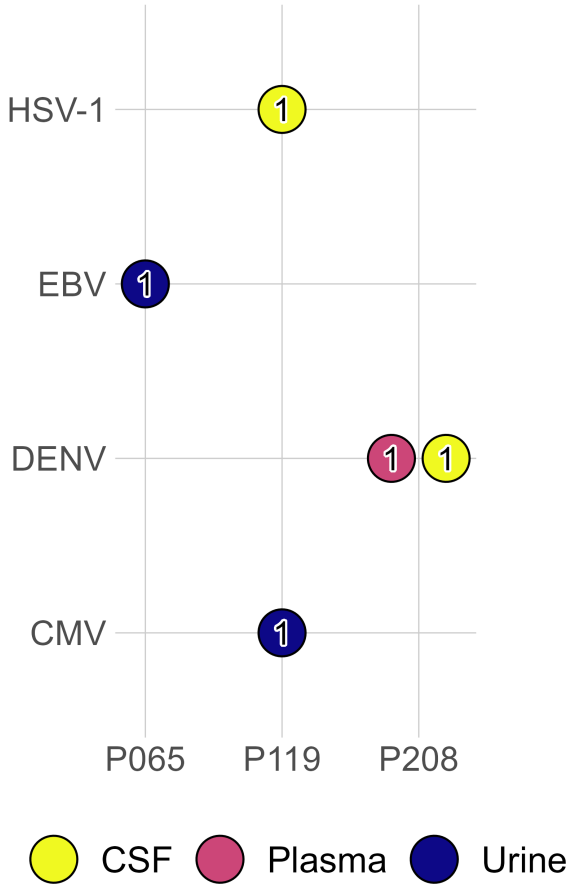


Figure 5.5. Viruses detected by mNGS, which were then confirmed by specific virus PCR testing, in different clinical samples of three patients

5.3.3. Detection of pathogens of unknown pathogenicity or contaminants

mNGS analysis revealed evidence of human associated gemykibivirus 2 (HuGkV-2) in the CSF sample of an encephalitis patient of unknown origin. Sequences related to a recently discovered gemycircularvirus were also identified by mNGS in one urine sample. The pathogenicity and neurotropic property of HuGkV-2 and gemycircularvirus remain unknown, but their sequences have previously been detected by mNGS in human biological samples (217,218).

Sequences related to viruses of unknown pathogenicity or known contaminants of mNGS dataset were also detected by mNGS. Those were sequences related to species of the families *Circoviridae* family (4/16, 25.0%), *Partitiviridae* and *Picobirnaviridae* (both 3/16, 18.8%), followed by *Chrysoviridae* and *Genomoviridae* families (both 2/16, 12.5%), and *Iflaviridae* family, and *Reoviridae* (1/16, 6.3% for each virus). In terms of sample types, CSF was the most frequent clinical sample in which these viruses were detected (10/16, 62.5%), followed by urine samples (4/16, 25.0%), and plasma samples (2/16, 12.5%) (Table 5.2).

Table 5.2. Viruses with unknown pathogenicity or contaminant viruses detected by mNGS

Virus family	Species	Ref.	Number of samples	Type of samples	Number of patients	E-value	Number of hits
Chrysoviridae	Penicillium chrysogenum virus	(219)	1	CSF	1	5,30E-08	273
Chrysoviridae	Aspergillus fumigatus chrysovirus	(220)	1	CSF	1	7,70E-07	335
Circoviridae	Circovirus-like NI/2007-3	(221)	1	CSF	1	2,40E-08	38
Circoviridae	Sichuan tick-associated circovirus 4	(222)	1	Plasma	1	4,50E-07	5
Circoviridae	Torque teno virus 5	(223)	1	CSF	1	5,70E-10	151
Circoviridae	Torque teno virus 11	(223)	1	CSF	1	3,70E-09	360
Genomoviridae	Gemycircular virus	(218)	1	Urine	1	0,00047	2
Genomoviridae	Human associated gemykibivirus 2	(217)	1	CSF	1	2,60E-10	743
Iflaviridae	Iflavirus	(224)	1	Plasma	1	0,00021	80
Partitiviridae	Aspergillus nidulans partitivirus 1	(225)	1	CSF	1	1,00E-06	34
Partitiviridae	Botryosphaeria dothidea partitivirus 1	(226)	1	CSF	1	5,90E-07	234
Partitiviridae	Ustilaginoidea virens partitivirus 2	(227)	1	CSF	1	1,50E-05	110
Picobirnaviridae	Picobirnavirus	(228)	1	Urine	1	2,70E-09	70
Picobirnaviridae	Picobirnavirus HK-2014	(228)	1	Urine	1	2,00E-07	4
Picobirnaviridae	Porcine picobirnavirus	(228)	1	Urine	1	6,30E-09	2
Reoviridae	Kadipiro virus	(229)	1	CSF	1	5,30E-08	8

5.3.4. Genetic characterization of DENV

As outlined above, mNGS successfully detected DENV in CSF and plasma of one patient. Subsequent genome sequence assembly could recover fragments of DENV sequences with 20% and 99.7% of genome coverage from the data sets of the CSF and plasma samples, respectively. Pairwise analysis show that these two obtained sequences were 100% identical, suggesting that the patient was infected with the same virus. Phylogenetic analysis of the complete E-gene coding protein sequence demonstrated that the isolate obtained from the urine samples belonged to DENV-1 serotype, and was closely related to DENV-1 strains, circulating in Vietnam in 2022 (Figure 5.6). Analysis of the CSF sequence was un-informative because of the low genome coverage of the obtained sequence.

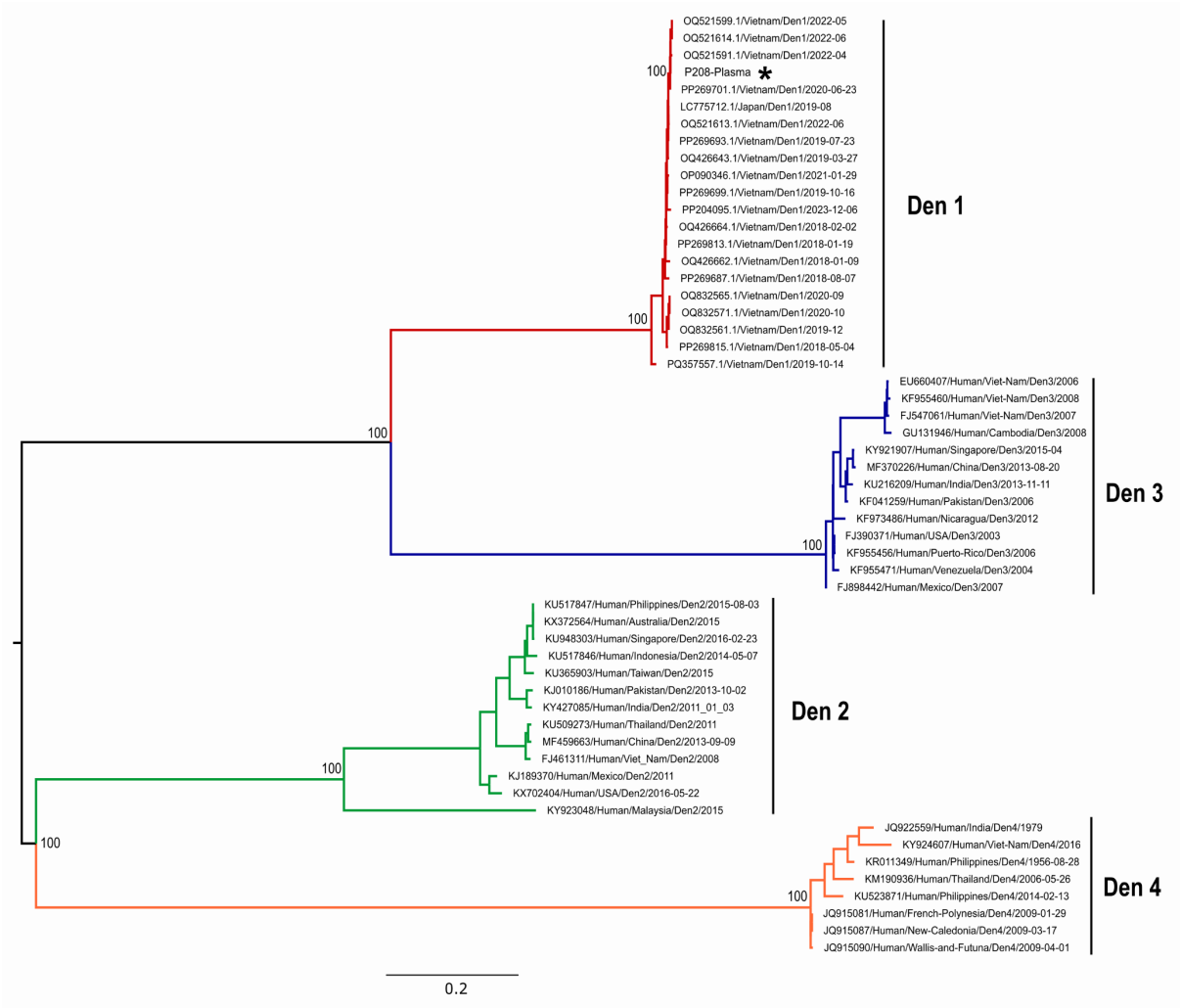


Figure 5.6. Reconstructed maximum likelihood tree illustrating the relatedness between DENV strain recovered in this study by mNGS and representative DENV serotypes

Note: Maximum likelihood tree based on 55 complete E gene sequences of DENV (1,485bp) illustrating the relatedness between DENV strain recovered in the present study by mNGS (black asterisk) and representative DENV serotypes

5.3.5. Comparison between real-time RT-PCR and mNGS

As outlined above, the 64 included CSF samples were analysed by both real time RT-PCR (Chapter 4) and mNGS (this Chapter), allowing back-to-back comparison in terms of diagnostic yields. An overview about the viruses detected by both workflows in the 64 CSF is presented in Table 5.3.

The overlap between Seegene PCR assays and mNGS assay was achieved for HSV-1 in 1 CSF sample (Table 5.3). For the CSF positive for DENV, subsequent PCR testing confirmed the presence of DENV in the mNGS positive CSF, but DENV was not covered by the PCR workflow under Chapter 4. mNGS failed to detected a virus in seven CSF samples that were positive by PCR. In those cases, CSF viral loads were low as reflected by the obtained PCR values (39.32 – 41.87, Table 5.3). Higher Ct values is correlated lower viral loads in the tested sample.

Table 5.3. Summary of viruses detected in CSF by extended RT-PCR and mNGS

Patient ID	Seegene's PCR and in-house PCR assays	mNGS followed by PCR confirmation
	CSF (Ct values)	CSF (Ct values)
P044	AdV (41.87)	
P076	HHV-7 (39.80)	
P119	HSV-1 (28.45)	HSV-1 (34.82)
P126	HHV-7 (39.32)	
P141	HHV-7 (39.55)	
P194	EV (38.16)	
P208	Not requested as part of routine diagnosis nor included in the PCR workflow	DENV (35.00)
P222	HHV-6 (39.59)	
P272	HHV-7 (40.13)	

5.3.6. Viral causes of infectious encephalitis established after routine diagnosis, PCR and mNGS analyses combined

The combined results of routine diagnostics (Chapter 3), PCR testing (Chapter 4), and mNGS (this Chapter) of different clinical sample types from 89 patients with suspected infectious are shown in Figure 5.7. Collectively, I identified a total of 43 viruses of 13 types of viral pathogens in 37 tested samples (Figure 5.7), including 27 in 23 CSF, 1 in 1 plasma, 2 in 2 urines, 11 in 9 throat swabs, and 2 in 2 rectal swabs from 35 patients.

JEV was the most common causes, detected in 14/89 patients (15.7%), followed by HSV (5/89, 5.6%). Other viruses sporadically detected included DENV, HHV6, HHV7, Adv, EV, CMV, EBV, and respiratory viruses (HRV, PIV, RSV, and influenza A). The frequency of those viruses detected in specific sample types are shown in Figure 5.7.

Overall, the diagnostic yield increased from 18/89 (20.2%) after routine diagnosis to 34/89 (38.2%) after extensive PCR testing and to 36/89 (40.4%) after mNGS (Figure 5.8). In my study, five patients (P052, P077, P119, P166, P178) were confirmed to have HSV-1 encephalitis based on HSV-1 detection in cerebrospinal fluid (CSF). Of these, four patients (P052, P077, P166, P178) had HSV-1 identified through routine PCR testing, while one patient (P119) had HSV-1 detected by both extended PCR and mNGS.

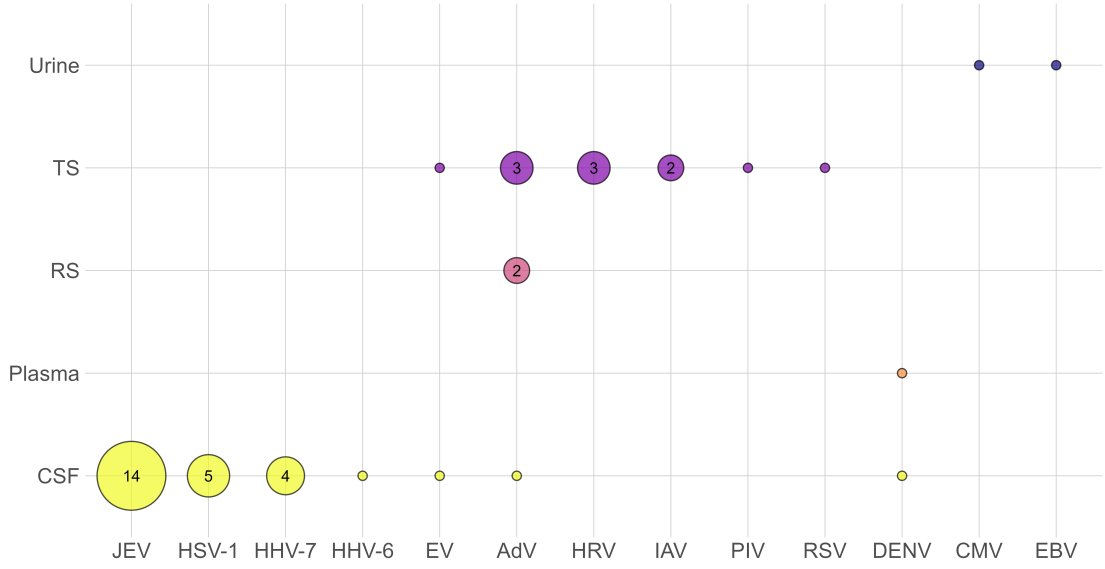


Figure 5.7. Numbers of viral pathogens in encephalitis detected by all means in different samples

Note:
CSF: cerebrospinal fluid, RS: rectal swab, TS: throat swab
AdV: adenovirus, CMV: cytomegalovirus, DENV: Dengue virus, EV: enterovirus, Epstein-Barr virus: EBV, HHV: human herpesvirus, HRV: human rhinovirus, HSV: herpes simplex virus, IAV: influenza A virus, JEV: Japanese encephalitis virus, PIV: parainfluenza virus, RSV: respiratory syncytial virus

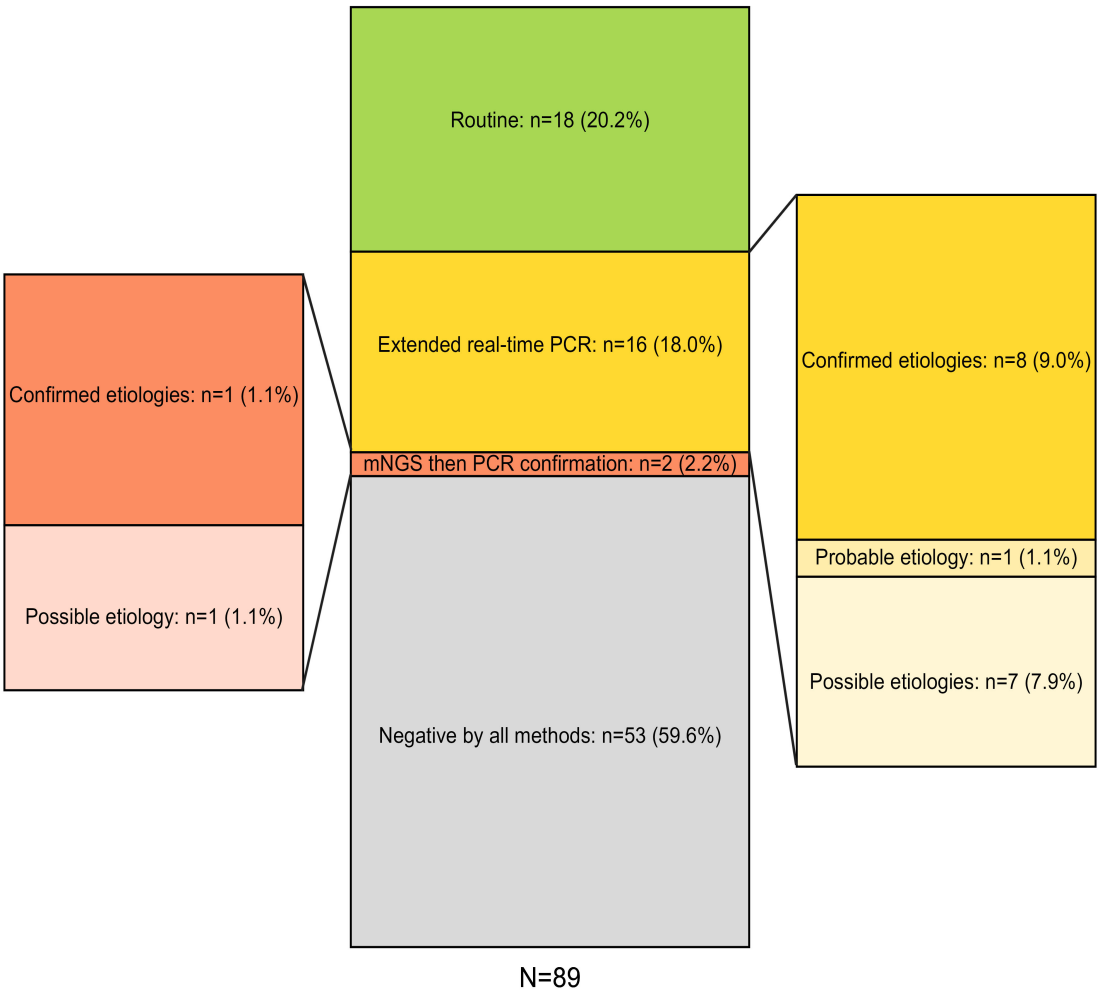


Figure 5.8. The number and proportion of encephalitis patients having viral pathogens detected by routine diagnostics, PCR testing, and mNGS

Note: The clinical features of encephalitis caused by specific pathogens are described in previous Chapters (Chapter 3 and Chapter 4). As mNGS could only identify one additional patient with a confirmed cause and one with a possible cause, additional analysis is deemed uninformative.

5.4. Discussion

In this chapter, I report the results of mNGS analysis of biological samples from 64 Vietnamese children with encephalitis of unknown origin recruited into the clinical study (Chapter 3). mNGS analysis could identify six viruses including HSV-1, DENV, EBV, CMV, HPV, and HPyV-2 in the tested samples. Of these, only HSV and DENV (1 each) are established causes of encephalitis. As outlined in the previous chapter, the detection of EBV and CMV may merely reflect an incidental finding and those viruses can only be associated with encephalitis in specific circumstance, e.g. in immunocompromised individuals (6). Therefore, their detection in urine samples as shown in this chapter can only be considered as possible diagnosis. Additionally, the contribution HPV ad HpyV-2 the pathology of encephalitis remains to be determined, and PCR confirmation was not carried out due to the unavailability of the resources.

Collectively, routine diagnosis, extensive PCR testing targeted at 30 pathogens and mNGS analysis combined could reveal evidence of a confirmed cause in 27/89 (30.3%) and a probable/possible cause in 9/89 (10.1%) patients with clinically suspected encephalitis. While my findings are in line with previous reports (26,46,157,205,206,230,231), the data further emphasize it remains a challenge to establish a causative agent in patients with encephalitis, and that testing for a wide range of pathogens using serological and molecular methods combined is crucial to improve the diagnostic yield.

Compared with PCR, mNGS failed to replicate the detection of a virus in 7 cases. In those samples, viral loads as reflected by the Ct values were low, suggesting that mNGS is less sensitive than PCR (209,236,237). Because viral reads only accounted for a small proportion of total mNGS reads, increasing the sequencing depth per samples may increase the sensitivity of mNGS but this approach would also increase the cost for

mNGS. However, a high PCR Ct value might merely reflect a non-specific signal or contaminations.

5.4.1. Strengths and limitations

The strength of mNGS is that it is a sequence-independent method and therefore in a single assay, it could detect a wide range of viruses in the tested samples without the requirement of pathogen specific PCR primers. In addition, mNGS can generate pathogen sequence data, enabling further characterisation of the detected pathogens, including evolutionary analysis, vaccine development and detections of drug-resistance conferring mutations. Because of those collective factors, mNGS should be used after ruling out common causes using conventional assays (serology and PCR), or in an outbreak situation that might be associated with uncommon or novel causes.

Using mNGS, I also found human associated gemykibivirus 2 (HuGkV-2) in the CSF sample of an encephalitis patient but this virus has not yet been established as a pathogen of encephalitis (238). HuGkV-2 belongs to the *Genomoviridae* family which are viruses having single stranded DNA genomes of ~2.1–2.2 kb that encode a capsid protein and a replication associated protein and have been detected in a variety of plants, insects, animals, and humans (238). In Nepal, a gemykibivirus was identified in the CSF of a male child with encephalitis in 2020 using mNGS, and subsequently gemykibivirus DNA was detected in CSF or serum of 12 more encephalitis patients by real-time PCR, showing a possibility that gemykibivirus might be a candidate pathogenic agent of encephalitis in Nepal (217). While the detection of viruses in sterile CSF supports the hypothesis that they could be causative organism of encephalitis, additional research to fulfil Koch's postulates are needed to definitively establish causality.

5.5. Conclusion

In summary, using mNGS followed by PCR confirmation testing, I identified two patients having HSV-1 and DENV as confirmed causes of encephalitis. As the HSV case was detected by the extensive PCR panel under Chapter 4, mNGS analysis could only establish a confirmed diagnosis in one patient due to DENV infection. The findings further emphasize that diverse viral pathogens can be associated with infectious encephalitis and over half of the patients with infectious encephalitis having no cause identified despite extensive diagnostics.

Chapter 6. A cost of illness analysis of children with encephalitis presenting to a major hospital in Vietnam

Chapter 6. A cost of illness analysis of children with encephalitis presenting to a major hospital in Vietnam

6.1. Introduction

A previous cost of illness analysis of Japanese encephalitis in Vietnam estimated a mean total cost of US$3,371 per acute episode (147). This showed that Japanese encephalitis patients and their families in Vietnam suffer notable medical, economic, and social hardship (147). Whilst this study has provided valuable insight into the costs of one cause of encephalitis in Vietnamese children, knowledge regarding the economic burden of paediatric encephalitis more generally is lacking, but essential to inform local policymakers in planning and prioritizing resources for diagnosis and treatment and public health interventions. For this reason, I carried out this study, conducted analysis to estimate the costs attributed to paediatric encephalitis in Southern Vietnam, using the data captured under the clinical study described in Chapter 2.

My contribution: In this chapter, I gathered comprehensive economic data from hospital records and patient families to assess the cost of encephalitis in children. I partnered with Dr. Hugo C. Turner, a health economist from Imperial College London, to conduct rigorous cost-effectiveness and statistical analyses. His expertise ensured robust and reliable economic findings. And I led the manuscript writing, editing and submission for publication in the American Journal of Tropical Medicine and Hygiene (PMID: 39561390).

6.2. Methods

6.2.1. Cost evaluation

The economic data for the cost-of-illness analysis were collected as part of the same clinical study on paediatric encephalitis described in Chapters 2–5, ensuring consistency in the study population and data collection framework. The questionnaire for the cost of illness analysis, which can be found as Appendix 6.4, was developed in collaboration with a health economist and tailored to the context of southern Vietnam to ensure cultural and economic relevance.

The cost-of-illness data were collected by the same study team responsible for the clinical assessments, comprising general paediatricians and paediatricians specialising in neurology, as outlined in Section 2.7.2 (Economic data collection). To ensure accuracy and standardisation, team members were trained in administering the questionnaire consistently.

The total cost of illness consisted of direct medical costs, direct non-medical costs and productivity costs. The costs were collected from a societal perspective. Data on the cost of illness was collected capturing the period prior to hospital admission, during hospitalization, and seven days after discharge.

The direct medical costs consist of the costs directly associated with the use of medical resources/goods/services (such as the costs associated with diagnostic tests and drugs etc.). These costs were calculated based on the hospital invoices and the information obtained from the face-to-face interviews. The direct medical costs attributed to hospitalization were collected based on the official invoices within the patients' in-hospital medical records. I had official invoices for all prescribed medical items during hospitalization. These costs could be stratified by the cost covered by the government's health insurance programme and the family's out-of-pocket payments. The direct medical

costs incurred before hospital admission and up to seven days after discharge were captured through interviews. However, because these were not based on official invoices it was not possible to stratify these costs depending on whether they were paid by the families themselves or covered by the health insurance programme.

The direct non-medical costs represent the costs related to the use of non-medical resources. These include the patients'/caregivers' travel costs and other expenses related to the care of the patient. Data on direct non-medical costs was collected through face-to-face interviews.

The productivity costs represent the value of monetized productivity losses resulting from lost paid and unpaid work due to an illness or an intervention. Within this study, only the productivity losses of the caregivers were considered and evaluated. The productivity losses of children (such as from missed school days) were not evaluated. Data on the caregivers' productivity losses incurred was collected through face-to-face interviews by asking about the number of days they lost due to caregiving before hospital admission, during hospitalization, and up to seven days after discharge. These productivity losses were evaluated based on the activities the caregivers reported giving up. For those who reported giving up paid employment, their losses were evaluated based on their reported monthly salary. For those who reported losing unpaid work, their losses were evaluated based on the minimum wage. As the minimum wage in Vietnam varies across the different provinces (Appendix 6.2) (239), I used the monthly minimum wage that corresponded to the address (province) of the patient. The monthly salary and minimum wage values were adjusted to a daily value based on the caregivers' reported number of working days per month (Appendix 6.3). The productivity costs were calculated based on this daily value multiplied by the reported number of days lost.

Due to the local COVID-19 regulations during the study, only one registered caregiver (usually the father or mother) was allowed to stay with and take care of the child and the caregiver did not change during the hospitalization period. Therefore, the productivity costs were estimated only for one registered caregiver of a patient. No excess in-hospital death was considered in the calculation of productivity costs.

Under the government health insurance scheme in Vietnam, children under 6 years old are provided with free health care services. For children from 6 to 14 years old, there are two main health insurance programs, which are operated by the Vietnam Health Insurance Organization (VHI) on a non-profit and public basis. However, the families have to pay for medical services that are not listed in the health insurance directory issued by the Ministry of Health (240).

My cost data were highly skewed based on skewness and kurtosis test for normality. Comparisons between groups were made using the statistical non-parametric test which was the Wilcoxon rank-sum test. I summarized all values of illness costs as median and interquartile range (IQR) in US dollars (US$) with a conversion rate of US$1 equivalent to 23,271.2 dong (the exchange rate between Vietnamese Dong and US dollars for the year 2022) based on the database from the World Bank. Costs incurred in 2020 and 2021 were adjusted to 2022 prices using GDP deflators for Vietnam using the approach outlined within Turner et al (241).

6.3. Results

6.3.1. Baseline features of children with encephalitis

As described in previous Chapter 4, 23/164 patients (14.0%) had confirmed NMDAR-antibody encephalitis, while 26/164 cases (15.9%) had a confirmed diagnosis of viral encephalitis established at the time of analysis outlined in this Chapter. The additional

DENV case after analysis conducted under Chapter 5 was not available at the time of analysis conducted under this Chapter. Those two patient groups (23 with NMDAR-antibody encephalitis and 26 with infectious encephalitis) are the focus of this Chapter, while details about the key clinical features of patients with NMDAR-antibody encephalitis and infectious encephalitis are shown in Chapters 3 and 4. However, a short summary is shown in Table 6.1.

Table 6.1. Clinical features of the patients

	NMDAR-antibody encephalitis (n = 23)	**Confirmed viral encephalitis (n = 26)**
General features		
Gender		
Male*	4 (17.4)	18 (69.2)
Female*	19 (82.6)	8 (30.8)
Age (years)†	10 (9–13)	8 (3–12)
Residence		
Ho Chi Minh city*	7 (30.4)	2 (7.7)
Other provinces*	16 (69.6)	24 (92.3)
Clinical features		
Paediatric Glasgow coma scale (GCS)†	11 (9–12)	9 (7–11)
Paediatric GCS < 9* (more severe)	4 (17.4)	13 (50.0)
Paediatric GCS ≥ 9* (less severe)	19 (82.6)	13 (50.0)
Acute extra-neurological symptoms/signs*	18 (78.3)	15 (57.7)
Outcomes		
Respiratory support with ventilation*	6 (26.1)	13 (50.0)
Modified Rankin scale (mRS) score†	2 (0–3)	1 (0–2)
mRS < 3* (less severe)	16 (69.6)	23 (88.5)
mRS ≥ 3* (more severe)	7 (30.4)	3 (11.5)
Sequelae*	13 (56.5)	16 (61.5)
In-hospital death*	1 (4.3)	0 (0.0)
Duration of hospital stay (days)†	38 (15–53)	12 (10–22)

Note: Abbreviation: IQR, interquartile range.
*Two groups of paediatric GCS and mRS score outputs are presented as n (%).
†The paediatric GCS and mRS score outputs are presented as median (IQR).

6.3.2. The informal caregivers' characteristics

Details are shown in Table 6.2. The most common normal activities the caregivers reported giving up to provide care for the child were paid employment (77/164, 46.9%), housework (36/164, 22.0%) and subsistence farming (32/164, 19.5%). The median number of days lost was 28 days (IQR: 20–43 days). The productivity losses were stratified depending on whether they were incurred before hospitalization, during hospitalization, or after hospitalization. The median monthly income of the caregivers was US$201 (IQR: US$156–US$430) (Table 6.2).

Table 6.2. Information regarding the caregivers

Characteristics (N = 164*)	**n (%) or median (IQR)**
Residence (n, (%))	
Ho Chi Minh City	43 (26.2)
Other provinces	121 (73.8)
Relationship (n, (%))	
Mother	118 (72.0)
Father	46 (28.0)
Normal activities (n, (%))	
Paid employment	77 (46.9)
Business ownership	19 (11.6)
Subsistence farming (unpaid work)	32 (19.5)
Housework (unpaid work)	36 (22.0)
Working days per month (median, IQR)	26 (24–26)
Working days per month of paid workers	26 (24–26)
Working days per month of unpaid workers	26 (22–28)
Days of work lost (median, IQR)	28 (20–43)
Days lost before hospitalization	4 (3–7)
Days lost during hospitalization	14 (9–23)
Days lost after hospitalization	7 (7–7)
Individual income per month – US$ (median, IQR)	201 (156–430)

Note: Abbreviation: IQR, interquartile range.
*Only one registered caregiver could stay with the child during the hospitalization period.

6.3.3. An overview of the costs of illness attributed to paediatric encephalitis

A summary of the estimated costs associated with encephalitis in children is presented in Table 6.3. The total median cost of illness was US$1,859 (IQR: US$1,273–US$3,128). The direct costs constituted the majority (83.9%) of this total cost of illness with a median of US$1,560 (IQR: US$975–US$2,460) of which the direct medical cost incurred during hospitalization was found to be the main component/driver, with a median of US$1,044 (IQR: US$615–US$1,906). Importantly, in Vietnam, the government's health insurance programme does not cover all the medical costs and in this study, the patients had to pay 30.2% (US$316) of the direct medical costs incurred during hospitalization. The median direct non-medical cost was US$335 (IQR: US$226–US$505) – 37.0% of which were related to transportation. The remaining accounted for other types of direct non-medical costs – such as costs related to the care of the patient. The productivity costs during hospitalization were the dominant contributor to the total productivity costs (US$138 of US$275).

Table 6.3. Summary of the estimated costs of illness of paediatric encephalitis cases

Costs type	Median US$ (IQR)
Direct medical cost	1,202 (698–2,087)
Direct medical costs before hospital admission	34 (0–134)
Direct medical costs during hospitalization	1,044 (615–1,906)
Direct medical costs up to seven days after discharge	43 (21–46)
Direct medical cost paid by the patients	
Direct medical costs before hospitalization paid by the patients	NA
Direct medical costs during hospitalization paid by the patients	316 (154–623)
Direct medical costs during hospitalization paid by the patients < 6 years of age (n = 41)	223 (86–645)
Direct medical costs during hospitalization paid by the patients ≥ 6 years of age (n = 123)	326 (183–602)
Direct medical costs up to seven days after discharge paid by the patients	NA
Direct medical cost paid by the health insurance	
Direct medical costs before hospitalization paid by the health insurance	NA
Direct medical costs during hospitalization paid by the health insurance	631 (330–1,117)
Direct medical costs during hospitalization paid by the health insurance for patients < 6 years of age (n = 41)	873 (488–1,364)
Direct medical costs during hospitalization paid by the health insurance for patients ≥ 6 years of age (n = 123)	570 (261–1,034)
Direct medical costs up to seven days after discharge paid by the health insurance	NA
Direct non-medical cost	335 (226–505)
Transportation costs before hospital admission	9 (0–43)
Transportation costs during hospitalization	92 (45–138)
Transportation costs up to seven days after discharge	23 (9–46)
Other expenses*	172 (92–275)
Total productivity cost	275 (154–474)
Productivity costs before hospital admission	42 (23–89)
Productivity costs during hospitalization	138 (77–280)
Productivity costs up to seven days after discharge	68 (42–116)
Total direct cost	1,560 (975–2,460)
Total costs of illness (societal perspective)	1,859 (1,273–3,128)

Note: Abbreviations: IQR, interquartile range; NA, Not available.
*Such as other costs related to the care of the patient.

6.3.4. The valuation of productivity costs

Paid work constituted 58.5% (96/164) of the normal activities of the caregivers. The median productivity cost for caregivers who lost paid work was estimated to be US$368 based on their reported wages. For those who lost unpaid work, the median productivity costs were calculated as US$188 based on the daily minimum wage. Consequently, the productivity costs of paid workers are estimated to be twice as much as caregivers performing unpaid work (Table 6.4). The productivity loss during hospitalization contributed to approximately half of the total productivity loss of caregivers (Table 6.4).

Table 6.4. The valuation of the caregivers' productivity costs

	Caregivers losing paid work*	Caregivers losing unpaid work†
	Median (IQR)	Median (IQR)
Monthly income - US$	344 (236–430)	156 (156–201)
Working days per month	26 (24–26)	26 (22–28)
Daily value of a lost day‡ - US$	15 (10–17)	7 (6–8)
Days lost before hospitalization	5 (3–7)	4 (3–7)
Days lost during hospitalization	15 (9–23)	12 (8–24)
Days lost after hospitalization	7 (7–7)	7 (7–7)
Total days lost	29 (20–43)	26 (19–43)
Productivity cost before hospitalization - US$	53 (33–105)	28 (19–51)
Productivity cost during hospitalization - US$	185 (114–329)	90 (53–157)
Productivity cost after hospitalization - US$	103 (58–125)	49 (39–68)
Total productivity cost - US$	368 (229–642)	188 (122–305)

Note: Abbreviations: IQR, interquartile range.

*For those that reported losing paid work, their productivity losses were evaluated based on the monthly salary (n = 96).

†For those that reported losing unpaid work, their productivity losses were evaluated based on the monthly minimum wage (n = 68).

‡Calculated by dividing the monthly income (salary for lost paid work and minimum wage for lost unpaid work) by the reported number of working days per month (Appendix 6.2). The productivity cost was calculated by multiplying this daily value by the number of days lost.

6.3.5. Costs of illness of encephalitis in children by geographic location, diagnosis, severity, and outcomes

Ventilated patients had higher total costs of illness than that of non-ventilated patients (median, US$3,162 vs median, US$1,754) ($p < 0.0001$). Assessing the costs by the degree of disability or dependence in their daily activities, the total costs of illness at discharge of more severely affected patients (mRS ≥ 3) were much higher than that of less severe patients (mRS < 3) (median, US$6,193 compared to US$1,763) ($p < 0.0001$). Similarly, patients with sequelae had higher total costs compared to those of patients without sequelae (median, US$2,359 vs US$1,721) ($p = 0.0007$) (Table 6.5). I identified a number of factors that influenced the projected costs of illness (Table 6.5). Patients with confirmed NMDAR-antibody encephalitis were associated with higher costs of illness (median, US$2,823) compared to other categories of encephalitis in children ($p = 0.0003$). As expected, in terms of level of consciousness, the total costs of illness of more severe patients were higher than that of less severe patients (median, US$2,685 vs median, US$1,775) ($p = 0.0006$). Patients with acute extra-neurological symptoms/signs had higher costs of illness compared to those who did not have acute extra-neurological symptoms/signs (median, US$2,497 vs median, US$1,344) ($p < 0.0001$).

Table 6.5. Estimated costs of illness stratified by different patient groupings

Patient groups*	Direct medical costs (median US$ (IQR))	Direct non-medical costs (median US$ (IQR))	Total direct costs (median US$ (IQR))	Total productivity costs (median US$ (IQR))	Total costs of illness (median US$ (IQR))	P-values†, #
All patients (n = 164)	1,211 (698–2,087)	335 (226–505)	1,560 (975–2,460)	275 (154–474)	1,859 (1,273–3,128)	ND
Age of patients						
< 6 years of age (n = 41)	1,364 (780–2,217)	460 (290–602)	1,885 (1,172–2,558)	259 (151–532)	2,018 (1,466–3,144)	0.2081
≥ 6 years of age (n = 123)	1,160 (692–1,964)	312 (206–438)	1,514 (959–2,425)	284 (161–434)	1,837 (1,169–3,041)	
Residence of patients						
Ho Chi Minh City (n = 43)	1,205 (652–2,551)	258 (215–390)	1,460 (882–2,941)	298 (116–469)	1,754 (1,141–3336)	0.6309
Other provinces (n = 121)	1,199 (718–1,964)	374 (250–536)	1,643 (1,034–2,425)	275 (163–481)	1,935 (1,309–3,041)	
Diagnosis of paediatric encephalitis						
NMDAR-antibody encephalitis (n = 23)	1,760 (1,332–5,746)	580 (298–735)	2,133 (1,830–6,365)	550 (299–925)	2,823 (2,225–7,573)	**0.0003**‡ 0.9175§ **0.0073**¶
Confirmed viral encephalitis (n = 26)	1,363 (780–2,116)	294 (223–394)	1,693 (1,055–2,479)	196 (130–360)	1,998 (1,181–2,759)	
Unknown aetiology encephalitis (n = 115)**	1,106 (661–1,705)	326 (220–455)	1,460 (954–2,029)	266 (157–434)	1,763 (1,201–2,563)	
Acute extra-neurological symptoms/signs***						
With acute extra-neurological symptoms/signs (n = 96)	1,569 (1,085–3,947)	392 (296–597)	1,960 (1,370–4,562)	336 (190–673)	2,497 (1,708–5,288)	**< 0.0001**
Without acute extra-neurological symptoms/signs (n = 68)	771 (572–1,204)	248 (182–363)	1,051 (779–1,547)	242 (134–369)	1,344 (987–1,889)	
Paediatric Glasgow coma scale (GCS)						
Paediatric GCS < 9	2,036 (1,338–6,009)	414 (289–589)	2,409 (1,676–6,634)	321 (161–481)	2,685 (1,839–7,052)	**0.0006**

(more severe) (n = 26)						
Paediatric GCS ≥ 9 (less severe) (n = 138)	1,115 (660–1,705)	323 (220–481)	1,475 (954–2,133)	275 (151–469)	1,775 (1,181–2,700)	
Modified Rankin scale (mRS)						
mRS < 3 (less severe) (n = 141)	1,128 (660–1.684)	309 (223–430)	1,496 (954–2,029)	247 (144–395)	1,763 (1,181–2,437)	**< 0.0001**
mRS ≥ 3 (more severe) (n = 23)	4,227 (1,332–7,267)	619 (509–784)	4,846 (1,983–8,135)	734 (367–1,284)	6,193 (3,144–8,255)	
Respiratory support with ventilators						
Ventilated (n = 31)	2,408 (1,338–6,009)	430 (289–625)	2,752 (1,676–6,634)	323 (161–627)	3,162 (1,839–7,052)	**< 0.0001**
Non-ventilated (n = 133)	1,084 (659–1,621)	312 (220–460)	1,460 (948–2,029)	275 (151–465)	1,754 (1,169–2,563)	
In-hospital death						
Died (n = 3)	1,205 (721–6,009)	193 (142–688)	1,398 (863–6,697)	297 (81–319)	1,696 (944–7,016)	0.8492
Survived (n = 161)	1,199 (692–2,057)	344 (236–501)	1,564 (976–2,440)	275 (157–479)	1,861 (1,284–3,111)	
Sequelae						
With sequelae (n = 64)	1,393 (1,036–3,846)	379 (264–595)	1,883 (1,350–4,479)	377 (219–719)	2,359 (1,664–5,288)	**0.0007**
Without sequelae (n = 100)	1,072 (652–1,784)	317 (211–455)	1,348 (904–2,165)	236 (139–380)	1,721 (1,143–2,397)	

Note: Abbreviation: IQR, interquartile range; ND, not done

*Data are presented as median (IQR).

†Comparisons of total costs of illness between groups are made using Wilcoxon rank-sum test.

‡Comparisons of total costs of illness between NMDAR-antibody encephalitis and other categories of encephalitis.

§Comparisons of total costs of illness between confirmed viral encephalitis and other categories of encephalitis.

¶Comparisons of total costs of illness between unknown aetiology encephalitis and other categories of encephalitis.

#P-values < 0.05 are considered to be statistically significant and are highlighted in bold.

** including those with probable/possible causes, grouping into this category because of the uncertainty in diagnostic outcome and a small sample size.

***Acute extra-neurological symptoms/signs refer to acute clinical manifestations occurring concurrently with encephalitis that are not directly related to central nervous system dysfunction

6.4. Discussion

Despite the clear presence and clinical threat of paediatric encephalitis in Vietnam, limited information regarding its economic burden is available to support policymakers and physicians in prioritizing the resources for the improvement and execution of intervention strategies. Here, I describe the results of a prospective hospital-based study during 2020–2022, estimating the cost of illness of encephalitis in children. Among 458 patients with clinical/suspected cases of paediatric encephalitis, only 164 cases (35.8%) fulfilled the diagnostic criteria of encephalitis were all enrolled into the study. This may reflect the significant contribution of encephalitis (35.8%) to the diagnosis profile of patients with initial signs and symptoms of central nervous system infections at my centre during the study period, or the stricter definition used for my study compared to hospital diagnostic coding.

The results show that paediatric encephalitis cases are associated with a substantial economic burden in Vietnam. Direct costs were the main cost driver, accounting for 84.6% of the total cost, particularly the direct medical cost during hospitalization. In terms of the direct non-medical costs the transportation cost during hospitalization and expenses related to childcare arrangements were the main drivers. Patients with NMDAR-antibody encephalitis, more severely ill patients, and patients with sequelae had higher total costs. This may be due to the longer length of stay seen in these patients (Table 6.1). My analysis has revealed that cases with acute extra-neurological symptoms/signs had higher costs than those without acute extra-neurological symptoms/signs. However, I believe that this reflects multisystemic disease (i.e. more severe disease) and would be expected to be associated with higher costs.

Importantly, the total median direct medical costs associated with non-ventilated and ventilated children suffering from encephalitis in my study were approximately 7.0 times

and 15.6 times higher respectively than Vietnam's annual average per capita health care spending in 2020 (US$154.2, 2020 prices) (242). The direct medical costs during hospitalization paid by the patients themselves (not covered by government health insurance) was approximately 1.6 times higher than the income per month. I also found that families incur notable direct non-medical costs (with a median of US$335). These direct non-medical costs are not covered by insurance, and therefore, the families have to pay for these expenses themselves. These numbers are concerning and highlight the risk of families incurring catastrophic health expenditures. In addition, the productivity cost during the hospitalization period was also much higher than the minimum earnings of the parent, posing an important loss to the family and society.

6.4.1. Strengths and limitations

This is the first study to estimate the costs attributed to paediatric encephalitis in Southern Vietnam. However, my study only looked at the acute phase of encephalitis. Persistent neurologic effects are common following encephalitis, for example personality change, behavioural disorders, movement disorders, intellectual disability, learning disorders, blindness, paresis, and sleeping problems (22,23). Such disorders are likely to have long-lasting economic impacts to individuals, families and society. In addition, my study has other limitations. Firstly, it only investigated patients admitted to one hospital in Ho Chi Minh City, Vietnam. These cost estimates can not necessarily be generalized to every encephalitis case in Vietnam. For example, as a specialist centre, it is possible that the severity of cases and association with long-term sequelae may be higher in my sample. It is important to note that the current/standard approach to monetize productivity losses remains an area of debate, particularly regarding the valuation of unpaid work and whether or not to include the valuation of the productivity losses of children (243–245). In addition, it is possible that some cost items could have been missed (such as any direct

medical costs that were not on the hospital invoices). The local COVID-19 regulations during the study period likely reduced the number of caregivers per patient and the associated costs associated with the caregivers. During the COVID-19 pandemic, the number of patients with encephalitis admitted to my hospital was reduced. Therefore, COVID-19 pandemic may have affected the representativeness of the patients enrolling into my study (e.g. less referrals from rural areas because of the lock down, which may have had an impact on the epidemiological findings and the generalizability of the results of this study). Finally, the cost data estimates calculated within this thesis were based directly on the charges from the patients' hospital bills and the costs related to the staff time were assumed to be captured by the charges for the different services. However, these charges do not necessarily reflect the economic value of the resources utilized for their care (246–248). In order to try and capture economic costs within this context, a cost-to-charge ratio is commonly applied to the charges (which is based on the ratio of the hospital's (or department's) expenses and what they charge) (254,255) but the data were not available to do this adjustment within this study.

6.5. Conclusion

My results show that the cost of illness of encephalitis in children is considerable and higher in more severe patients, patients with sequelae, and ventilated patients. Notably, I found that despite high health insurance coverage, patients and families still incur significant costs. Of note, many of the children in my study suffered from JEV (14/164, 8.5%), a vaccine-preventable disease, indicating the potential of preventative public health measures to impact and reduce these cost outcomes.

Chapter 7. General discussion

Chapter 7. General discussion

Encephalitis is defined as an inflammation of cerebral parenchyma associated with neurological dysfunction, and is associated with high morbidity and mortality worldwide, but especially in LMICs (2). There are however more than 100 pathogens, including emerging viruses, that can cause encephalitis. Additionally, over the last decades, antibody-mediated encephalitis has emerged as an important differential diagnosis in patients with encephalitis. Because of those collective factors, routine diagnosis fails to establish the causative agents in around 70% of patients (251). Yet, clinical outcomes of encephalitis are highly dependent on the identification of the causative agents. As a consequence, the disease causes a significant burden in both children and adults worldwide, especially in LMICs.

The emergence of new pathogens causing encephalitis, the recognition of antibody-mediate encephalitis, the wide spectrum of known viruses responsible for this devastating condition emphasise that addressing the diagnostic challenge in patients with encephalitis requires a holistic approach, applying a combination method, in particular molecular- and antibody-based assays. However, few such studies, especially in children, have been systematically conducted in LIMCs, while such knowledge is crucial to update national guidelines. It is also of equal importance to assess the illness costs associated with encephalitis to inform the policymakers in planning and prioritizing resources for development of strategic programmes to reduce the burden of encephalitis.

Therefore, my PhD aims to:

1. To unravel the epidemiology, clinical profiles, and in-hospital outcomes of NMDAR-antibody encephalitis in children in Southern Vietnam;

2. To comprehensively identify the infectious causes of paediatric encephalitis in Southern Vietnam;
3. To estimate the illness costs attributed to NMDAR-antibody encephalitis and infectious encephalitis in children in Southern Vietnam.

My PhD findings have therefore revealed novel insights into the epidemiology of encephalitis in children in Southern Vietnam, providing first real-world evidence about the prevalence of NMDAR-antibody encephalitis, utility of molecular methods, in particular mNGS in the diagnosis of paediatric encephalitis, and illness costs. Herein I summarise my research findings and offer some associated future research directions.

7.1. Summary of main results

To lay the foundation for my PhD research, I first set up a clinical study, recruiting children presenting with encephalitis admitted to my hospital, the CH1 in HCMC, Vietnam between March 2020 and December 2022. During this study period, a total of 164 children with a clinical diagnosis of encephalitis including infectious and autoimmune encephalitis were enrolled. Meta-clinical data, biological samples and illness costs were collected alongside the hospital outcome. I then applied a combination of serology and molecular assays (including PCR and mNGS) to establish the infectious causes, and antibody-mediated encephalitis causes in the enrolled patients. In parallel, I applied descriptive statistics to assess the associated illness costs. These collective analyses, to the best of my knowledge, represent the first comprehensive body of work on the epidemiology of encephalitis in children ever conducted in Vietnam, and as a result have led to four result Chapters 3 – 6. Herein, I provide a summary of my main findings. Details are presented in corresponding thesis chapters.

7.1.1. NMDAR antibodies: an important cause of encephalitis in Vietnamese children

Of the 164 children with a clinical diagnosis of encephalitis enrolled in my study, 75 (45.7%) had signs and symptoms meeting the predefined criteria of probable autoimmune encephalitis, while 89 fulfilled a clinical diagnosis of infectious encephalitis. CSF examination for antibodies against NMDAR (in all) and AMPA-R1/R2, $GABA_{A}/_{B1}/_{B2}R$, LGI1, CASPR2 or DPPX (in 22) revealed that NMDAR antibodies were detected in 23/75 (30.7%), but antibodies against the remaining antigens were not detected.

Of the remaining 89 patients with a clinical diagnosis of infectious encephalitis, routine diagnosis established JEV and HSV in 14 (15.7%) and 4 (4.5%), respectively. Collectively, of the 164 enrolled patients, NMDAR-antibody encephalitis was detected in 23 (14.0%), which was higher that the detection rate of JEV or HSV combined, 11.0% (18/164). These important observations may be attributable to several factors. Firstly, widespread JEV vaccination campaigns in southern Vietnam have significantly reduced JEV incidence, particularly in children, by providing robust herd immunity (252). Secondly, societal changes, such as urbanization and improved sanitation, have likely decreased exposure to JEV, which is transmitted via mosquitoes in rural settings with pig farming (44). Thirdly, referral patterns to my central specialist hospital may preferentially select complex cases, such as NMDAR-antibody encephalitis, which often present with distinctive neuropsychiatric symptoms requiring advanced diagnostics and management, unlike JEV cases that may be managed locally (105). This referral bias could inflate the proportion of NMDAR cases in my cohort. Fourthly, increased awareness and diagnostic capacity for autoimmune encephalitis, including NMDAR-antibody testing, may enhance detection rates, making it appear more common (79). And finally, the testing strategies I

applied as described in Chapter 3, for which only admission samples were collected for testing.

7.1.2. Differences between patients with NMDAR-antibody encephalitis and those with JEV encephalitis

As JEV encephalitis is the most common cause of paediatric encephalitis in Vietnam and Southeast Asia, I conducted back-to-back comparison between JEV encephalitis and NDMAR-antibody encephalitis groups. In patients with NMDAR-antibody encephalitis, females were predominant (82.6%; 19/23) as compared on 2/14 (14.3%) in those with JEV encephalitis. Patients with NMDAR-antibody encephalitis were older than those with JEV encephalitis and they were more likely came from outside of Ho Chi Minh City than JEV encephalitis patients.

In terms of clinical presentation, all JEV encephalitis patients had fever recorded at enrolment as compared to just over half of NMDAR-Ab encephalitis patients, and they had a longer fever duration during hospitalisation (median days (IQR): 7 (5 – 10) vs. 3 (3 – 7), p=0.014). Additionally, JEV encephalitis patients were associated a lower level of consciousness than those with NMDAR-antibody encephalitis; median paediatric GCS (IQR): 8 (8-11) vs. 11 (9-12). In contrast, patients with NMDAR-antibody encephalitis patients commonly presented with signs and symptoms such as psychiatric disorder, cognitive dysfunction, language changes, abnormal movement, and dyskinesias, which were almost absent in those with JEV encephalitis.

The prevalence of movement disorders in JEV encephalitis varies across studies but is significant, particularly in severe cases (38,43,252,253). Based on available data, movement disorders are common neurological sequelae, especially in children and survivors of acute encephalitis (38,43,252,253). A study analysing 17 JE patients found

movement disorders in 14 (82%), with parkinsonism being the most frequent, alongside dystonia, chorea, and tremors (253). In my study, psychiatric disorder, delirium, and cognitive dysfunction were exclusively found in NMDAR-antibody encephalitis patients, whereas language changes, abnormal movement, and dyskinesias were less frequently recorded in JE patients. This can be explained by several factors. Firstly, the early timing of CSF sampling within 24–48 hours of admission likely captured JEV cases during the acute phase, where systemic symptoms (e.g., fever) and neurological deficits (e.g., seizures, altered consciousness) predominate, potentially before neuropsychiatric symptoms fully manifest (38,42,43). Secondly, subtler neuropsychiatric symptoms, which are less commonly reported in children with JEV compared to adults, may have been underrecognized due to challenges in assessing these features in paediatric populations, particularly in young children with limited verbal communication (38,42,43).

Remarkable differences between JEV encephalitis and NMDAR-antibody encephalitis patients were also observed for CSF laboratory findings, with the latter having lower white cell counts, protein and lactate levels.

MRI findings were also different between the two groups. In NMDAR-antibody encephalitis patient, limbic system and cerebral cortex were commonly affected, documented in over 50%, whereas in JEV encephalitis patients, abnormal findings of the thalami were documented in all.

In terms of outcome, NMDAR-antibody encephalitis patients had a longer duration of hospital stay than JEV encephalitis patients did. Neurological sequelae assessed at discharge was comparable between the two group, but decease was documented in one with NMDAR-antibody encephalitis, but in none of the JEV encephalitis patients.

Collectively, these data have provided the first real-world evidence that in Vietnamese children the prevalence of NMDAR-antibody encephalitis surpassed that of JE, the most common cause of infectious encephalitis in Vietnamese children.

7.1.3. Multiplex real-time PCR analysis of possible viral and bacterial causes of encephalitis and CNS infections

In my hospital and more broadly in Vietnam, routine diagnosis of viral cause of encephalitis only covers JEV, and HSV. Because there are over 100 pathogens can cause encephalitis, I therefore apply multiplex real-time PCRs to test for 13 viruses and 6 bacteria that can potentially cause encephalitis and CNS infections in children. In total 64 CSF samples from 89 patients with clinically suspected infectious encephalitis were available for testing. Subsequently, evidence of a viral pathogen was detected in 8 (12.5%), but none had evidence of bacterial infection. The detected viruses included HHV7 in four; and HHV6, EV, HSV1, and AdV in one for each virus.

To further explore the possible viral culprits in the 65 undiagnosed patients, whose CSF were available for testing, I also analysed their throat swabs for respiratory pathogens. Subsequently, evidence of a viral pathogen was found in 9 (13.8%) patients. The detected viruses included AdV in three, HRV in three, IAV in two, and EV, PIV, and respiratory RSV in one for each virus. In these 9 patients with a throat swab positive for a respiratory virus, subsequent PCR testing of their available CSF (n=8), plasma (n=8), urine (n=6), and rectal swabs (n=9) could only detect AdV in 2/9 rectal swabs. Of these, one came from a patient who also had a throat swab positive for AdV. Collectively, extensive RT-PCR diagnostic work up could established a confirmed diagnosis in 8/89 (9.0%) patients, a probable in 1/89 (1.1%), and a possible cause in 7/89 (7.9%), respectively. This corresponded to an increase of the confirmed diagnostic yield from 18 patients (18/89,

20.2%) after routine diagnosis to 34 patients (34/89, 38.2%) after extensive PCR testing and routine diagnosis combined.

7.1.4. Identification of viruses causing encephalitis in clinical samples using mNGS analysis

Previous studies, including those conducted by my research group, showed the utility potential of mNGS in establishing the causative agents, especially in adults with severe diseases such as sepsis and CNS infections. I therefore applied mNGS to study the viral causes in CSF, plasma and urine of 64 patients that were negative by routine diagnosis and were also tested by multiplex real-time PCR detailed above. In total, combination method of routine test, Seegene's PCR and mNGS revealed 43 viruses in the tested samples, including 27 in 23 CSF, 1 in 1 plasma, 2 in 2 urine, 11 in 9 throat swabs, and 2 in 2 rectal swabs. Subsequent PCR confirmatory testing replicated mNGS findings in 5, including 2 CSF samples positive for HSV1 and DENV (on each), plasma sample positive for DENV, and 1 urine samples positive for CMV. The case of DENV encephalitis was subjected to phylogenetics analysis and it was indicated that the strain belonged DENV1 serotype and was closely related with a Vietnamese DENV-1 cluster detected in 2022.

Since CSF of those 64 patients with unknown causes after routine diagnosis were tested by both multiplex PCR and mNGS, this enabled me to assess the utility potential of both methods. Accordingly, multiplex real-time PCR could detect a virus in 16 CSF, of these two were also concomitantly detected by mNGS (including virus HSV-1 and EBV). In one instance, mNGS revealed evidence of DENV in one CSF. However, multiple real-time PCR panel did not cover DENV therefore it initially failed to detect DENV in this CSF samples. Overall, the diagnostic yield increased from 18/89 (20.2%) after routine diagnosis to 34/89 (38.2%) after extensive PCR testing and to 36/89 (40.4%) after mNGS.

7.1.5. Costs of illness attributed to paediatric encephalitis and the evaluation of productivity costs.

My analysis showed that (in-)direct and productivity costs of illness attributable to encephalitis in children are considerable, significant differences between patient groups, and vary between geographic locations of the patients. In particular, while the total median cost of illness was US$1,859 (IQR: US$1,273–US$3,128), the direct costs constituted the majority (83.9%) of this total cost of illness, with the direct medical cost incurred during hospitalization being the main component. Of the direct costs, 30.2% equivalent to US$316 was paid by parents as national insurance does not cover all the direct medical costs incurred during hospitalization. The median direct non-medical cost was US$335 (IQR: US$226–US$505) – 37.0% of which were related to transportation. The productivity costs during hospitalization were the dominant contributor to the total productivity costs (US$138 of US$275).

As expected, ventilated patients had higher total costs of illness than that of non-ventilated patients (median, US$3,162 vs median, US$1,754) ($p < 0.0001$). Likewise, the total costs of illness at discharge of more severely affected patients (mRS ≥ 3) were much higher than that of less severe patients (mRS < 3) (median, US$6,193 compared to US$1,763) ($p < 0.0001$). And, patients with sequelae had higher total costs compared to those of patients without sequelae (median, US$2,359 vs US$1,721, $p = 0.0007$). I also identified a number of factors that influenced the projected costs of illness. Patients with confirmed NMDAR-antibody encephalitis were associated with higher costs of illness (median, US$2,823) compared to other categories of encephalitis in children ($p = 0.0003$).

7.2. Implications of my PhD findings

7.2.1. Clinical implications

My PhD findings have shown for the first time that NMDAR-antibody encephalitis has become a major clinical entity in Vietnamese children presenting with encephalitis. Together with previous findings in Vietnamese adults with encephalitis (163), NMDAR-antibody encephalitis therefore should now be considered as an important differential diagnosis in patients presenting with encephalitis in Vietnam and perhaps beyond. Because of those findings, including my PhD's, NMDAR-antibody encephalitis has now been included in diagnostic and management guidelines of hospitals in Vietnam, including my hospital.

7.2.2. Diagnosis and public health implications

The data emphasise that testing for a wide range of viral pathogens is needed in patients with encephalitis, especially in Southeast Asia a hotspot of emerging infectious diseases, and highlight the utility potential of PCR and mNGS. However, the results also demonstrate that it remains a challenge to establish a possible viral culprit in this group of patients with severe disease.

Yet, despite the availability of effective vaccines, it is unacceptable to witness that JEV remains the most common cause of encephalitis in Vietnam and many countries in Asia. These findings together with the considerable illness costs associated with encephalitis present compelling evidence emphasizing that much effort is needed to assess the JEV vaccination coverage and the affected factors in the endemic countries. Notably, in 2024 Vietnam experienced an explosive outbreak of measles, causing over 20,000 suspected measles cases, including nearly 5,000 confirmed cases and 7 deaths across the countries. This was associated with a low vaccination coverage.

7.3. Limitations of my PhD study

Despite novel findings with potential implications outlined above, my research has some limitations. Owing to the nature of a single hospital-based study, the obtained results may not accurately capture overall epidemiological picture of encephalitis in Vietnamese children. Additionally, the COVID-19 lockdown during the study period, in particular between 2020 and 2021, could be a potential bias toward the admission of more severe patients from outside of HCMC, resulting an overestimation of disease burden. My study was also limited by the intrinsic difficulties in defining undiagnosed encephalitis because of the absence of microbiological and immunological confirmations. Encephalitis cases in my study have been defined based on compatible manifestations of autoimmune encephalitis or viral encephalitis suggested by guidelines. Therefore, it cannot be ruled out that patients with other conditions, e.g. toxicity, were missed included. Finally, the cost data estimates calculated were based directly on the charges from the patients' hospital bills and the costs related to the staff time were assumed to be captured by the charges for the different services. In addition, it is possible that some cost items could have been missed such as any direct medical costs that were not on the hospital invoices. And long-term follow up was not conducted.

I acknowledge the potential for interobserver variability when multiple assessors conduct the paediatric GCS and mRS, as these scales involve some degree of subjective interpretation, particularly in young children with varying developmental baselines. To mitigate this, assessments were cross-verified by at least two team members, including a neurologist, whenever possible, and discrepancies were resolved through discussion in multidisciplinary team meetings. However, I recognize that interobserver reliability remains a potential limitation, as formal inter-rater reliability testing (e.g., using kappa statistics) was not performed due to resource constraints. Future studies should

incorporate formal inter-rater reliability assessments to further validate these scales' application in paediatric populations.

Developmental delay, while an important outcome in paediatric encephalitis, was not assessed in my study due to the lack of personnel trained in administering standardised developmental assessment tools and the absence of age-appropriate testing protocols for my cohort. This is a significant limitation, as developmental outcomes are critical for understanding the long-term impact of conditions like JEV and NMDAR-antibody encephalitis. Standardized tools such as the Bayley Scales of Infant and Toddler Development (Bayley-III/4, ages 1–42 months), Wechsler Preschool and Primary Scale of Intelligence (WPPSI-IV, ages 2.5–7 years), Wechsler Intelligence Scale for Children (WISC-V, ages 6–16 years), Ages and Stages Questionnaires (ASQ-3, ages 1 month–5.5 years), and Vineland Adaptive Behaviour Scales (Vineland-3, birth to adulthood) are commonly used to evaluate cognitive, motor, language, and adaptive skills in children (254–256). However, due to the absence of trained examiners and standardised protocols, I did not implement these assessments, and no uniform battery of tests was applied across age groups. To address this gap, I recommend that future studies incorporate standardized, age-appropriate developmental assessments to ensure consistent evaluation across participants within specific age brackets.

7.4. Future directions

While my findings further emphasise that NMDAR-antibody is the most common cause of autoimmune encephalitis in children, future research should comprehensively look at all the 23 possible autoantibodies recently recognized as potential causes of autoimmune encephalitis. The recognition of clinical and sub-clinical features associated with specific pathogens reflects the tropism of those pathogens. Therefore, they can be useful for

diagnostic model development. However, this would require follow-up studies with sufficient sample size. Although in encephalitis patients, antivirals are unavailable for most of the viruses, knowledge about the causative agents is of clinical and public health importance. Therefore, a critical question to address is the extent to which molecular methods, especially sequence-independent assays such as mNGS could benefit routine care and public health in LMICs. An important aspect of having mNGS implemented in a clinical laboratory in LMICs is its ability to detect emerging viruses as they emerge. This would in turn offer an early warning signal for outbreak response, mitigating the public health impact caused by disease outbreaks. In parallel, study addressing the regulatory framework required for mNGS to be implemented as a routine diagnostic assay should be performed. Yet, given the current high costs to conduct mNGS in LIMCs and its current performance, mNGS should be considered as a complementary diagnostic approach when all routine investigations could not identify a possible cause in encephalitis patients. In term of treatment, future studies should also explore how to best manage the encephalitis patients, especially those with autoimmune. Trials on diverse therapeutics in different clinical settings may be essential to answer the questions. In Vietnam, JEV vaccine was introduced into the national expanded vaccination program in 2015, and could have minimised the incidence of JEV encephalitis. Therefore, future studies should also look into JEV vaccination coverage and the affected factors such as public awareness and acceptance. These analyses are critical to inform vaccination campaign in the future. It is important for future studies to bring more insightful approach to the monetisation of productivity losses, particularly regarding the valuation of unpaid work and whether or not to include the valuation of the productivity losses of children.

References

1. Venkatesan A, Tunkel AR, Bloch KC, Lauring AS, Sejvar J, Bitnun A, et al. **Case Definitions, Diagnostic Algorithms, and Priorities in Encephalitis: Consensus Statement of the International Encephalitis Consortium**. *Clinical Infectious Diseases*. 2013 Oct 15;**57**(8):1114–28.
2. Bloch KC, Glaser C, Gaston D, Venkatesan A. **State of the Art: Acute Encephalitis**. *Clinical Infectious Diseases*. 2023 Sep 11;**77**(5):e14–33.
3. Cellucci T, Van Mater H, Graus F, Muscal E, Gallentine W, Klein-Gitelman MS, et al. **Clinical approach to the diagnosis of autoimmune encephalitis in the pediatric patient**. *Neurol Neuroimmunol Neuroinflamm*. 2020 Apr;**7**(7):e730.
4. Graus F, Titulaer MJ, Balu R, Benseler S, Bien CG, Cellucci T, et al. **A clinical approach to diagnosis of autoimmune encephalitis**. *Lancet Neurol*. 2016 Apr;**15**(4):391–404.
5. Venkatesan A, Geocadin RG. **Diagnosis and management of acute encephalitis: A practical approach**. *Neurol Clin Pract*. 2014 Jun 1;4(3):206–15.
6. Olie SE, Staal SL, van de Beek D, Brouwer MC. **Diagnosing infectious encephalitis: a narrative review**. *Clinical Microbiology and Infection*. 2025 Apr;**31**(4):522–8.
7. Boucher A, Herrmann JL, Morand P, Buzelé R, Crabol Y, Stahl JP, et al. **Epidemiology of infectious encephalitis causes in 2016**. *Med Mal Infect*. 2017 May;**47**(3):221–35.
8. Iro MA, Sadarangani M, Nickless A, Kelly DF, Pollard AJ. **A Population-based Observational Study of Childhood Encephalitis in Children Admitted to Pediatric Intensive Care Units in England and Wales**. *Pediatric Infectious Disease Journal*. 2019 Jul;**38**(7):673–7.

9. Bagdure D, Custer JW, Rao S, Messacar K, Dominguez S, et al. **Hospitalized Children With Encephalitis in the United States: A Pediatric Health Information System Database Study**. *Pediatr Neurol*. 2016 Aug;**61**:58–62.

10. Kakoti G, Dutta P, Ram Das B, Borah J, Mahanta J. **Clinical Profile and Outcome of Japanese Encephalitis in Children Admitted with Acute Encephalitis Syndrome**. *Biomed Res Int*. 2013;**2013**:1–5.

11. De Tiège X, Rozenberg F, Héron B. **The spectrum of herpes simplex encephalitis in children**. *European Journal of Paediatric Neurology*. 2008 Mar;**12**(2):72–81.

12. Messacar K, Fischer M, Dominguez SR, Tyler KL, Abzug MJ. **Encephalitis in US Children**. *Infect Dis Clin North Am*. 2018 Mar;**32**(1):145–62.

13. Thompson C, Kneen R, Riordan A, Kelly D, Pollard AJ. **Encephalitis in children**. *Arch Dis Child*. 2012 Feb;**97**(2):150–61.

14. Koskiniemi M, Rautonen J, Lehtokoski-Lehtiniemi E, Vaheri A. **Epidemiology of encephalitis in children: A 20-Year survey**. *Ann Neurol*. 1991;**29**(5):492–7.

15. Britton PN, Khoury L, Booy R, Wood N, Jones CA. **Encephalitis in Australian children: contemporary trends in hospitalisation**. *Arch Dis Child*. 2016 Jan;**101**(1):51–6.

16. Koskiniemi M, Korppi M, Mustonen K, Rantala H, Muttilainen M, Herrgård E, et al. **Epidemiology of encephalitis in children. A prospective multicentre study**. *Eur J Pediatr*. 1997 Jun 26;**156**(7):541–5.

17. Guerrero MP, Romero AF, Luengas M, Dávalos DM, Mesa-Monsalve JG, Vivas-Trochez R, et al. **Etiology and Risk Factors for Admission to the Pediatric Intensive Care Unit in Children with Encephalitis in a Developing Country**. *Pediatric Infectious Disease Journal*. 2022 Oct 14;**41**(10):806–12.

18. Palmas G, Duke T. **Severe encephalitis: aetiology, management and outcomes over 10 years in a paediatric intensive care unit**. *Arch Dis Child*. 2023 Nov;**108**(11):922–8.

19. DuBray K, Anglemyer A, LaBeaud AD, Flori H, Bloch K, Joaquin KS, et al. Epidemiology, **Outcomes and Predictors of Recovery in Childhood Encephalitis**. *Pediatric Infectious Disease Journal*. 2013 Aug;**32**(8):839–44.

20. Rautonen J, Koskiniemi M, Vaheri A. **Prognostic factors in childhood acute encephalitis**. *Pediatr Infect Dis J*. 1991 Jun;**10**(6):441–5.

21. Elenga N, Roux A, Cuadro-Alvarez E, Martin E, Kallel H, Defo A. **Etiology and prognosis of encephalitis in French Guianese children: a retrospective record-based study**. *J Infect Public Health*. 2020 Jul;**13**(7):1051–3.

22. Fowler Å, Stödberg T, Eriksson M, Wickström R. **Long-term Outcomes of Acute Encephalitis in Childhood**. *Pediatrics*. 2010 Oct 1;**126**(4):e828–35.

23. Michaeli O, Kassis I, Shachor-Meyouhas Y, Shahar E, Ravid S. **Long-term Motor and Cognitive Outcome of Acute Encephalitis**. *Pediatrics*. 2014 Mar 1;**133**(3):e546–52.

24. Zhou Y, Zhu Y, Cao L, Shi Y, Shen J. **High mortality rates and long-term complications in children with infectious brainstem encephalitis: A study of sixteen cases**. *PLoS One*. 2025 Feb 4;**20**(2):e0318818.

25. Glaser CA, Honarmand S, Anderson LJ, Schnurr DP, Forghani B, et al. **Beyond Viruses: Clinical Profiles and Etiologies Associated with Encephalitis**. *Clinical Infectious Diseases*. 2006 Dec 15;**43**(12):1565–77.

26. Fowler Å, Stödberg T, Eriksson M, Wickström R. **Childhood encephalitis in Sweden: Etiology, clinical presentation and outcome**. *European Journal of Paediatric Neurology*. 2008 Nov;**12**(6):484–90.

27. Galanakis E, Tzoufi M, Katragkou A, Nakou I, Roilides E. **A Prospective Multicenter Study Of Childhood Encephalitis In Greece**. *Pediatr Infect Dis J*. 2009 Aug;**28**(8):740–2.

28. Rice P. **Viral meningitis and encephalitis**. *Medicine*. 2013 Dec;**41**(12):678–82.

29. Ho Dang Trung N, Le Thi Phuong T, Wolbers M, Nguyen Van Minh H, Nguyen Thanh V, Van MP, et al. **Aetiologies of Central Nervous System Infection in Viet Nam: A Prospective Provincial Hospital-Based Descriptive Surveillance Study**. *PLoS One*. 2012 May 25;**7**(5):e37825.

30. Davis LE, Beckham JD, Tyler KL. **North American Encephalitic Arboviruses**. *Neurol Clin*. 2008 Aug;**26**(3):727–57.

31. Petersen LR, Brault AC, Nasci RS. **West Nile Virus: Review of the Literature**. *JAMA*. 2013 Jul 17;**310**(3):308.

32. Piantadosi A, Solomon IH. **Powassan Virus Encephalitis**. *Infect Dis Clin North Am*. 2022 Sep;**36**(3):671–88.

33. Normandin E, Solomon IH, Zamirpour S, Lemieux J, Freije CA, Mukerji SS, et al. **Powassan Virus Neuropathology and Genomic Diversity in Patients With Fatal Encephalitis**. *Open Forum Infect Dis*. 2020 Oct 1;**7**(10):ofaa392.

34. Srivastava KS, Jeswani V, Pal N, Bohra B, Vishwakarma V, Bapat AA, et al. **Japanese Encephalitis Virus: An Update on the Potential Antivirals and Vaccines**. *Vaccines (Basel)*. 2023 Mar 27;**11**(4):742.

35. Quan TM, Thao TTN, Duy NM, Nhat TM, Clapham H. **Estimates of the global burden of Japanese encephalitis and the impact of vaccination from 2000-2015**. *Elife*. 2020 May 26;**9**:e51027.

36. Pommier JD, Gorman C, Crabol Y, Bleakley K, Sothy H, Santy K, et al. **Childhood encephalitis in the Greater Mekong region (the SouthEast Asia Encephalitis**

Project): a multicentre prospective study. *Lancet Glob Health*. 2022 Jul;**10**(7):e989–1002.

37. Furuya-Kanamori L, Gyawali N, Mills DJ, Hugo LE, Devine GJ, Lau CL. **The Emergence of Japanese Encephalitis in Australia and the Implications for a Vaccination Strategy**. *Trop Med Infect Dis*. 2022 May 29;**7**(6):85.

38. Misra UK, Kalita J. **Overview: Japanese encephalitis**. *Prog Neurobiol*. 2010 Jun;**91**(2):108–20.

39. Liang GD, Huanyu W. **Epidemiology of Japanese encephalitis: past, present, and future prospects**. *Ther Clin Risk Manag*. 2015 Mar;**11**:435–48.

40. Pendrey CGA, Martin GE. **Japanese encephalitis clinical update: Changing diseases under a changing climate**. *Aust J Gen Pract*. 2023 May 1;**52**(5):275–80.

41. Pichl T, Wedderburn CJ, Hoskote C, Turtle L, Bharucha T. **A systematic review of brain imaging findings in neurological infection with Japanese encephalitis virus compared with Dengue virus**. *International Journal of Infectious Diseases*. 2022 Jun;**119**:102–10.

42. Turtle L, Solomon T. **Japanese encephalitis—the prospects for new treatments**. *Nat Rev Neurol*. 2018 May 26;**14**(5):298–313.

43. Ooi MH, Lewthwaite P, Lai BF, Mohan A, Clear D, Lim L, et al. **The Epidemiology, Clinical Features, and Long-Term Prognosis of Japanese Encephalitis in Central Sarawak, Malaysia, 1997–2005**. *Clinical Infectious Diseases*. 2008 Aug 15;**47**(4):458–68.

44. Solomon T. **Neurological aspects of tropical disease: Japanese encephalitis**. *J Neurol Neurosurg Psychiatry*. 2000 Apr 1;**68**(4):405–15.

45. Mai NTH, Phu NH, Nhu LNT, Hong NTT, Hanh NHH, Nguyet LA, et al. **Central Nervous System Infection Diagnosis by Next-Generation Sequencing: A Glimpse Into the Future?** *Open Forum Infect Dis*. 2017 Apr 1;**4**(2):ofx046.

46. Jain S, Patel B, Bhatt GC. **Enteroviral encephalitis in children: clinical features, pathophysiology, and treatment advances**. *Pathog Glob Health*. 2014 Jul 31;**108**(5):216–22.

47. Chau NVV, Thuong TC, Hung NT, Hong NTT, Quy DT, Thien TB, et al. **Emerging Enterovirus A71 Subgenogroup B5 Causing Severe Hand, Foot, and Mouth Disease, Vietnam, 2023**. *Emerg Infect Dis*. 2024 Feb;**30**(2):363–7.

48. Elbers JM, Bitnun A, Richardson SE, Ford-Jones EL, Tellier R, Wald RM, et al. **A 12-Year Prospective Study of Childhood Herpes Simplex Encephalitis: Is There a Broader Spectrum of Disease?** *Pediatrics*. 2007 Feb 1;**119**(2):e399–407.

49. Whitley RJ, Kimberlin DW. **Herpes simplex: Encephalitis children and adolescents**. *Semin Pediatr Infect Dis*. 2005 Jan;**16**(1):17–23.

50. Kimberlin DW, Lin CY, Jacobs RF, Powell DA, Corey L, Gruber WC, et al. **Safety and Efficacy of High-Dose Intravenous Acyclovir in the Management of Neonatal Herpes Simplex Virus Infections**. *Pediatrics*. 2001 Aug 1;**108**(2):230–8.

51. Bibert S, Quinodoz M, Perriot S, Krebs FS, Jan M, Malta RC, et al. **Herpes simplex encephalitis due to a mutation in an E3 ubiquitin ligase**. *Nat Commun*. 2024 May 10;**15**(1):3969.

52. Anzivino E, Fioriti D, Mischitelli M, Bellizzi A, Barucca V, Chiarini F, et al. **Herpes simplex virus infection in pregnancy and in neonate: status of art of epidemiology, diagnosis, therapy and prevention**. **Virol J**. 2009 Dec 6;**6**(1):40.

53. Doja A, Bitnun A, Ford Jones EL, Richardson S, Tellier R, Petric M, et al. **Pediatric Epstein-Barr Virus—Associated Encephalitis: 10-Year Review**. J *Child Neurol*. 2006 May 2;21(5):384–91.

54. Chouliaras G, Spoulou V, Quinlivan M, Breuer J, Theodoridou M. **Vaccine-Associated Herpes Zoster Opthalmicus and Encephalitis in an Immunocompetent Child**. *Pediatrics*. 2010 Apr 1;**125**(4):e969–72.

55. Amin R, Ford-Jones E, Richardson SE, MacGregor D, Tellier R, Heurter H, et al. **Acute Childhood Encephalitis and Encephalopathy Associated With Influenza**. *Pediatric Infectious Disease Journal*. 2008 May;**27**(5):390–5.

56. Arnold JC, Singh KK, Milder E, Spector SA, Sawyer MH, Gavali S, et al. **Human Metapneumovirus Associated With Central Nervous System Infection in Children**. *Pediatric Infectious Disease Journal*. 2009 Dec;**28**(12):1057–60.

57. Park A, Suh S il, Son GR, Lee YH, Seo HS, Eun BL, et al. **Respiratory syncytial virus-related encephalitis: magnetic resonance imaging findings with diffusion-weighted study**. *Neuroradiology*. 2014 Feb 13;**56**(2):163–8.

58. Tian J, Wang X, Zhang L, Li Q, Feng G, Zeng Y, et al. **Clinical epidemiology and disease burden of adenoviral encephalitis in hospitalized children in China: A nationwide cross-sectional study**. *Pediatr Investig*. 2023 Dec 25;**7**(4):247–53.

59. Ang BSP, Lim TCC, Wang L. **Nipah Virus Infection**. *J Clin Microbiol*. 2018 Jun;**56**(6):e01875–17.

60. Islam MS, Sazzad HMS, Satter SM, Sultana S, Hossain MJ, Hasan M, et al. **Nipah Virus Transmission from Bats to Humans Associated with Drinking Traditional Liquor Made from Date Palm Sap, Bangladesh, 2011–2014**. *Emerg Infect Dis*. 2016 Apr;**22**(4):664–70.

61. Ching PKG, de los Reyes VC, Sucaldito MN, Tayag E, Columna-Vingno AB, Malbas FF, et al. **Outbreak of Henipavirus Infection, Philippines, 2014**. *Emerg Infect Dis*. 2015 Feb;**21**(2):328–31.

62. Middleton D. **Hendra Virus**. *Veterinary Clinics of North America: Equine Practice*. 2014 Dec;**30**(3):579–89.

63. Hoffmann B, Tappe D, Höper D, Herden C, Boldt A, Mawrin C, et al. **A Variegated Squirrel Bornavirus Associated with Fatal Human Encephalitis**. *New England Journal of Medicine*. 2015 Jul 9;**373**(2):154–62.

64. Cadar D, Allendorf V, Schulze V, Ulrich RG, Schlottau K, Ebinger A, et al. **Introduction and spread of variegated squirrel bornavirus 1 (VSBV-1) between exotic squirrels and spill-over infections to humans in Germany**. *Emerg Microbes Infect*. 2021 Jan 1;**10**(1):602–11.

65. Andrews N, Pebody RG, Berbers G, et al. **The European Sero-Epidemiology Network: standardizing the enzyme immunoassay results for measles, mumps and rubella**. *Epidemiol Infect*. 2000 Aug;**125**(1):S0950268899004173.

66. Lam T, Ranjan R, Newark K, Surana S, Bhangu N, Lazenbury A, et al. **A recent surge of fulminant and early onset subacute sclerosing panencephalitis (SSPE) in the United Kingdom: An emergence in a time of measles**. *European Journal of Paediatric Neurology*. 2021 Sep;**34**:43–9.

67. Patel M, Lee AD, Redd SB, Clemmons NS, McNall RJ, Cohn AC, et al. **Increase in Measles Cases — United States, January 1–April 26, 2019**. *MMWR Morb Mortal Wkly Rep*. 2019 May 3;**68**(17):402–4.

68. Shukla A, Gangwar M, Rastogi S, Nath G. **Viral Encephalitis: A Hard Nut to Crack**. *Ann Natl Acad Med Sci*. 2019 Apr 2;**55**(02):098–109.

69. Gharpure R, Gleason M, Salah Z, Blackstock AJ, Hess-Homeier D, Yoder JS, et al. **Geographic Range of Recreational Water-Associated Primary Amebic Meningoencephalitis, United States, 1978–2018**. *Emerg Infect Dis*. 2021 Jan;**27**(1):271–4.

70. Maciver SK, Piñero JE, Lorenzo-Morales J. **Is Naegleria fowleri an Emerging Parasite?** *Trends Parasitol*. 2020 Jan;**36**(1):19–28.

71. Langelier C, Reid MJ, Halabi C, Witek N, LaRiviere A, Shah M, et al. **Baylisascaris procyonis–Associated Meningoencephalitis in a Previously Healthy Adult, California, USA**. *Emerg Infect Dis*. 2016 Aug;**22**(8):1480–4.

72. Dalmau J, Graus F. **Antibody-Mediated Encephalitis**. *New England Journal of Medicine*. 2018 Mar;**378**(9):840–51.

73. Dalmau J. **NMDA receptor encephalitis and other antibody-mediated disorders of the synapse**. *Neurology*. 2016 Dec 6;**87**(23):2471–82.

74. Scheer S, John RM. **Anti-N-Methyl-D-Aspartate Receptor Encephalitis in Children and Adolescents**. *J Pediatr Health Care*. 2016;**30**(4):347–58.

75. Goldberg EM, Titulaer M, de Blank PM, Sievert A, Ryan N. **Anti–N-methyl-D-aspartate Receptor-Mediated Encephalitis in Infants and Toddlers: Case Report and Review of the Literature**. *Pediatr Neurol*. 2014 Feb;**50**(2):181–4.

76. Ryan N. **Anti-N-Methyl-d-Aspartate Receptor-Mediated Encephalitis: Recent Advances in Diagnosis and Treatment in Children**. *Curr Probl Pediatr Adolesc Health Care*. 2016 Feb;**46**(2):58–61.

77. Hacohen Y, Wright S, Waters P, Agrawal S, Carr L, Cross H, et al. **Paediatric autoimmune encephalopathies: Clinical features, laboratory investigations and outcomes in patients with or without antibodies to known central nervous system autoantigens**. *J Neurol Neurosurg Psychiatry*. 2013;**84**(7):748–55.

78. Varley JA, Strippel C, Handel A, Irani SR. **Autoimmune encephalitis: recent clinical and biological advances**. *J Neurol*. 2023 Aug 28;**270**(8):4118–31.

79. Dalmau J, Lancaster E, Martinez-Hernandez E, Rosenfeld MR, Balice-Gordon R. **Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis**. *Lancet Neurol*. 2011 Jan;**10**(1):63–74.

80. Dale RC, Irani SR, Brilot F, Pillai S, Webster R, Gill D, et al. **N-methyl-D-aspartate receptor antibodies in pediatric dyskinetic encephalitis lethargica**. *Ann Neurol*. 2009;**66**(5):704–9.

81. Davies G, Irani SR, Coltart C, Ingle G, Amin Y, Taylor C, et al. **Anti-N-methyl-D-aspartate receptor antibodies: A potentially treatable cause of encephalitis in the intensive care unit**. *Crit Care Med*. 2010 Feb;**38**(2):679–82.

82. Al-Diwani A, Handel A, Townsend L, Pollak T, Leite MI, Harrison PJ, et al. **The psychopathology of NMDAR-antibody encephalitis in adults: a systematic review and phenotypic analysis of individual patient data**. *Lancet Psychiatry*. 2019 Mar;**6**(3):235–46.

83. Varley JA, Webb AJS, Balint B, Fung VSC, Sethi KD, Tijssen MAJ, et al. **The Movement disorder associated with NMDAR antibody-encephalitis is complex and characteristic: an expert video-rating study**. *J Neurol Neurosurg Psychiatry*. 2019 Jun;**90**(6):724–6.

84. Nosadini M, Thomas T, Eyre M, Anlar B, Armangue T, Benseler SM, et al. **International Consensus Recommendations for the Treatment of Pediatric NMDAR Antibody Encephalitis**. *Neurol Neuroimmunol Neuroinflamm*. 2021 Sep;**8**(5):e1052.

85. Xu CL, Liu L, Zhao WQ, Li JM, Wang RJ, Wang SH, et al. **Anti-N-methyl-D-aspartate receptor encephalitis with serum anti-thyroid antibodies and IgM**

antibodies against Epstein-Barr virus viral capsid antigen: a case report and one- year follow-up. *BMC Neurol*. 2011 Dec 29;**11**(1):149.

86. Armangue T, Petit-Pedrol M, Dalmau J. **Autoimmune Encephalitis in Children**. *J Child Neurol*. 2012;**27**(11):1460–9.

87. Gable MS, Sheriff H, Dalmau J, Tilley DH, Glaser CA. **The Frequency of Autoimmune N-Methyl-D-Aspartate Receptor Encephalitis Surpasses That of Individual Viral Etiologies in Young Individuals Enrolled in the California Encephalitis Project**. *Clinical Infectious Diseases*. 2012 Apr 1;**54**(7):899–904.

88. Titulaer M, McCracken L, Gabilondo I, Armangue T, Martinez-Hernandez E, Glaser C, et al. **Clinical features, treatment, and outcome of 568 patients with anti-NMDA receptor encephalitis**. *J Neuroimmunol*. 2012;**253**(1–2):34–5.

89. Florance NR, Davis RL, Lam C, Szperka C, Zhou L, Ahmad S, et al. **Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis in children and adolescents**. *Ann Neurol*. 2009 Jul;**66**(1):11–8.

90. Varley J, Taylor J, Irani SR. **Autoantibody-mediated diseases of the CNS: Structure, dysfunction and therapy**. *Neuropharmacology*. 2018 Apr;**132**:71–82.

91. Varley JA, Strippel C, Handel A, Irani SR. **Autoimmune encephalitis: recent clinical and biological advances**. *J Neurol*. 2023 Aug 28;**270**(8):4118–31.

92. Iizuka T, Sakai F, Ide T, Monzen T, Yoshii S, Iigaya M, et al. **Anti-NMDA receptor encephalitis in Japan: Long-term outcome without tumor removal**. *Neurology*. 2008 Feb 12;**70**(7):504–11.

93. Flanagan EP, Geschwind MD, Lopez-Chiriboga AS, Blackburn KM, Turaga S, Binks S, et al. **Autoimmune Encephalitis Misdiagnosis in Adults**. *JAMA Neurol*. 2023 Jan 1;**80**(1):30.

94. Dalmau J, Gleichman AJ, Hughes EG, Rossi JE, al et. **Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies**. *Lancet Neurol*. 2008 Dec;**7**(12):1091–8.

95. Tunkel AR, Glaser CA, Bloch KC, Sejvar JJ, Marra CM, Roos KL, et al. **The Management of Encephalitis: Clinical Practice Guidelines by the Infectious Diseases Society of America**. *Clinical Infectious Diseases*. 2008 Aug 1;**47**(3):303–27.

96. Huttunen P, Lappalainen M, Salo E, Lönnqvist T, Jokela P, et al. **Differential diagnosis of acute central nervous system infections in children using modern microbiological methods**. *Acta Paediatr*. 2009 Aug;**98**(8):1300–6.

97. DeBiasi RL, Tyler KL. **Molecular Methods for Diagnosis of Viral Encephalitis**. *Clin Microbiol Rev*. 2004 Oct;**17**(4):903–25.

98. Leber AL, Everhart K, Balada-Llasat JM, Cullison J, Daly J, Holt S, et al. **Multicenter Evaluation of BioFire FilmArray Meningitis/Encephalitis Panel for Detection of Bacteria, Viruses, and Yeast in Cerebrospinal Fluid Specimens**. *J Clin Microbiol*. 2016 Sep;**54**(9):2251–61.

99. Vandendriessche S, Padalko E, Wollants E, Verfaillie C, Verhasselt B, Coorevits L. **Evaluation of the Seegene Allplex™ Respiratory Panel for diagnosis of acute respiratory tract infections**. *Acta Clin Belg*. 2019 Nov 2;**74**(6):379–85.

100. Atchison S, Shilling H, Balgovind P, Machalek DA, Hawkes D, Garland SM, et al. **Evaluation of the Roche MagNA Pure 96 nucleic acid extraction platform for the Seegene Anyplex II HPV28 detection assay**. *J Appl Microbiol*. 2021 Nov 16;**131**(5):2592–9.

101. Wang S, Lei H, Huang K, Wu J, Wang J, Yu C, et al. **Pathogenesis of Enterovirus 71 Brainstem Encephalitis in Pediatric Patients: Roles of Cytokines and**

Cellular Immune Activation in Patients with Pulmonary Edema. *J Infect Dis*. 2003 Aug 15;**188**(4):564–70.

102. Le Doare K, Menson E, Patel D, Lim M, Lyall H, Herberg J. **Managing neonatal and childhood herpes encephalitis**. *Arch Dis Child Educ Pract Ed*. 2015 Apr;**100**(2):58–63.

103. McJunkin JE, de los Reyes EC, Irazuzta JE, Caceres MJ, Khan RR, Minnich LL, et al. **La Crosse Encephalitis in Children**. *New England Journal of Medicine*. 2001 Mar 15;**344**(11):801–7.

104. Granerod J, Tam CC, Crowcroft NS, Davies NWS, Borchert M, Thomas SL. **Challenge of the unknown: A systematic review of acute encephalitis in non-outbreak situations**. *Neurology*. 2010 Sep 7;**75**(10):924–32.

105. Granerod J, Ambrose HE, Davies NW, Clewley JP, Walsh AL, Morgan D, et al. **Causes of encephalitis and differences in their clinical presentations in England: a multicentre, population-based prospective study**. *Lancet Infect Dis*. 2010 Dec;**10**(12):835–44.

106. Qu C, Chen Y, Ouyang Y, Huang W, Liu F, Yan L, et al. **Metagenomics next-generation sequencing for the diagnosis of central nervous system infection: A systematic review and meta-analysis**. *Front Neurol*. 2022 Sep 20;**13**:989280.

107. Wilson MR, Sample HA, Zorn KC, Arevalo S, Yu G, Neuhaus J, et al. **Clinical Metagenomic Sequencing for Diagnosis of Meningitis and Encephalitis**. *New England Journal of Medicine*. 2019 Jun 13;**380**(24):2327–40.

108. Miller S, Naccache SN, Samayoa E, Messacar K, Arevalo S, et al. **Laboratory validation of a clinical metagenomic sequencing assay for pathogen detection in cerebrospinal fluid**. *Genome Res*. 2019 May;**29**(5):831–42.

109. Graff K, Dominguez SR, Messacar K. **Metagenomic Next-Generation Sequencing for Diagnosis of Pediatric Meningitis and Encephalitis: A Review**. *J Pediatric Infect Dis Soc*. 2021 Dec 24;**10**(Supplement 4):S78–87.

110. Sansing LH, Tüzün E, Ko MW, Baccon J, Lynch DR, Dalmau J. **A patient with encephalitis associated with NMDA receptor antibodies**. *Nat Clin Pract Neurol*. 2007 May;**3**(5):291–6.

111. Leonard JR, Moran CJ, Cross DT, Wippold FJ, Schlesinger Y, Storch GA. **MR Imaging of Herpes Simplex Type I Encephalitis in Infants and Young Children**. *American Journal of Roentgenology*. 2000 Jun;**174**(6):1651–5.

112. Koelfen W, Freund M, G□ckel F, Rohr H, Schultze C. **MRI of encephalitis in children: comparison of CT and MRI in the acute stage with long-term follow-up**. *Neuroradiology*. 1995 Dec 1;**38**(1):73–9.

113. Bykowski J, Kruk P, Gold JJ, Glaser CA, Sheriff H, Crawford JR. **Acute Pediatric Encephalitis Neuroimaging: Single-Institution Series as Part of the California Encephalitis Project**. *Pediatr Neurol*. 2015 Jun;**52**(6):606–14.

114. Ilias A, Galanakis E, Raissaki M, Kalmanti M. **Childhood Encephalitis in Crete, Greece**. *J Child Neurol*. 2006 Oct 1;**21**(10):910–2.

115. Rao S, Elkon B, Flett KB, Moss AFD, Bernard TJ, Stroud B, et al. **Long-Term Outcomes and Risk Factors Associated With Acute Encephalitis in Children**. *J Pediatric Infect Dis Soc*. 2017 Mar 1;**6**(1):20–7.

116. Beattie GC, Glaser CA, Sheriff H, Messenger S, Preas CP, Shahkarami M, et al. **Encephalitis With Thalamic and Basal Ganglia Abnormalities: Etiologies, Neuroimaging, and Potential Role of Respiratory Viruses**. *Clinical Infectious Diseases*. 2013 Mar 15;**56**(6):825–32.

117. Baskin HJ, Hedlund G. **Neuroimaging of herpesvirus infections in children**. *Pediatr Radiol*. 2007 Oct 22;**37**(10):949.

118. Sansing LH, Tuzun E, Ko MW, Baccon J, Lynch DR, Dalmau J. **A patient with encephalitis associated with NMDA receptor antibodies**. *Nat Clin Pract Neurol*. 2007;**3**:291–6.

119. Hartung TJ, Bartels F, Kuchling J, Krohn S, Leidel J, et al. **MRI findings in autoimmune encephalitis**. *Rev Neurol (Paris)*. 2024 Nov;**180**(9):895–907.

120. Maschke M, Kastrup O, Forsting M, Diener HC. **Update on neuroimaging in infectious central nervous system disease**. *Curr Opin Neurol*. 2004 Aug;**17**(4):475–80.

121. Titulaer MJ, McCracken L, Gabilondo I, Armangué T, Glaser C, Iizuka T, et al. **Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study**. *Lancet Neurol*. 2013 Feb;**12**(2):157–65.

122. Gold JJ, Crawford JR, Glaser C, Sheriff H, Wang S, Nespeca M. **The Role of Continuous Electroencephalography in Childhood Encephalitis**. *Pediatr Neurol*. 2014 Apr;**50**(4):318–23.

123. Castellano J, Glover R, Robinson J. **Extreme Delta Brush in NMDA Receptor Encephalitis**. *Neurohospitalist*. 2017 Jul 14;**7**(3):NP3–4.

124. Steiner I, Budka H, Chaudhuri A, Koskiniemi M, Sainio K, Salonen O, et al. **Viral encephalitis: a review of diagnostic methods and guidelines for management**. *Eur J Neurol*. 2005 May 24;**12**(5):331–43.

125. Nosadini M, Thomas T, Eyre M, Anlar B, Armangue T, Benseler SM, et al. **International Consensus Recommendations for the Treatment of Pediatric**

NMDAR Antibody Encephalitis. *Neurol Neuroimmunol Neuroinflamm*. 2021 Sep;**8**(5):e1052.

126. Abboud H, Probasco JC, Irani S, Ances B, Benavides DR, Bradshaw M, et al. **Autoimmune encephalitis: proposed best practice recommendations for diagnosis and acute management**. *J Neurol Neurosurg Psychiatry*. 2021 Jul;**92**(7):757–68.

127. Nosadini M, Eyre M, Molteni E, Thomas T, Irani SR, Dalmau J, et al. **Use and Safety of Immunotherapeutic Management of N-Methyl-d-Aspartate Receptor Antibody Encephalitis**. *JAMA Neurol*. 2021 Nov 1;**78**(11):1333–44.

128. Irani SR, Vincent A. **The expanding spectrum of clinically-distinctive, immunotherapy-responsive autoimmune encephalopathies**. *Arq Neuropsiquiatr*. 2012 Apr;**70**(4):300–4.

129. Miya K, Takahashi Y, Mori H. **Anti-NMDAR autoimmune encephalitis**. *Brain Dev*. 2014 Sep;**36**(8):645–52.

130. Zhang M, Li W, Zhou S, Zhou Y, Yang H, Yu L, et al. **Clinical Features, Treatment, and Outcomes Among Chinese Children With Anti-methyl-D-aspartate Receptor (Anti-NMDAR) Encephalitis**. *Front Neurol*. 2019 Jun 6;**10**(Jun):596.

131. Bradley L. **Rehabilitation following anti-NMDA encephalitis**. *Brain Inj*. 2015 May 12;**29**(6):785–8.

132. Hoke CH, Vaughn DW, Nisalak A, Intralawan P, Poolsuppasit S, Jongsawas V, et al. **Effect of High-Dose Dexamethasone on the Outcome of Acute Encephalitis Due to Japanese Encephalitis Virus**. *Journal of Infectious Diseases*. 1992 Apr 1;**165**(4):631–7.

133. Makhoul B, Braun E, Herskovitz M, Ramadan R, Hadad S, Krivoy N. **Hyperimmune gammaglobulin for the treatment of West Nile virus encephalitis**. *Israel Medical Association Journal*. 2009;**11**(3):151.

134. Solomon T, Dung NM, Wills B, Kneen R, Gainsborough M, Diet TV, et al. **Interferon alfa-2a in Japanese encephalitis: a randomised double-blind placebo-controlled trial**. *The Lancet*. 2003 Mar;**361**(9360):821–6.

135. Kawano G, Iwata O, Iwata S, Kawano K, Obu K, Kuki I, et al. **Determinants of outcomes following acute child encephalopathy and encephalitis: pivotal effect of early and delayed cooling**. *Arch Dis Child*. 2011 Oct 1;**96**(10):936–41.

136. Shin YW, Lee ST, Park KI, Jung KH, Jung KY, Lee SK, et al. **Treatment strategies for autoimmune encephalitis**. *Ther Adv Neurol Disord*. 2018 Jan 1;**11**:1756285617722347.

137. Martinez-Torres F, Menon S, Pritsch M, Victor N, Jenetzky E, Jensen K, et al. **Protocol for German trial of Acyclovir and corticosteroids in Herpes-simplex-virus-encephalitis (GACHE): a multicenter, multinational, randomized, double-blind, placebo-controlled German, Austrian and Dutch trial**. *BMC Neurol*. 2008 Dec 29;**8**(1):40.

138. Rismanchi N, Gold JJ, Sattar S, Glaser C, Sheriff H, Proudfoot J, et al. **Neurological Outcomes After Presumed Childhood Encephalitis**. *Pediatr Neurol*. 2015 Sep;**53**(3):200–6.

139. Kumar R. **Prevention, diagnosis, and management of Japanese encephalitis in children**. *Pediatric Health Med Ther*. 2014 Sep;**2014**(5):99–110.

140. Sahu RC, Suthar T, Pathak A, Jain K. **Interventions for the Prevention and Treatment of Japanese Encephalitis**. *Curr Infect Dis Rep*. 2022 Nov 23;**24**(11):189–204.

141. Yoshida LM, Toizumi M, Nguyen HAT, Quilty BJ, Lien LT, Hoang LH, et al. **Effect of a Reduced PCV10 Dose Schedule on Pneumococcal Carriage in Vietnam**. *New England Journal of Medicine*. 2024 Nov 28;**391**(21):1992–2002.

142. Piamonte BLC, Easton A, Wood GK, Davies NWS, Granerod J, Michael BD, et al. **Addressing vaccine-preventable encephalitis in vulnerable populations**. *Curr Opin Neurol*. 2023 Jun;**36**(3):185–97.

143. Vora NM, Holman RC, Mehal JM, Steiner CA, Blanton J, Sejvar J. **Burden of encephalitis-associated hospitalizations in the United States, 1998-2010**. *Neurology*. 2014 Feb 4;**82**(5):443–51.

144. Griffiths MJ, Lemon J V., Rayamajhi A, Poudel P, Shrestha P, Srivastav V, et al. **The Functional, Social and Economic Impact of Acute Encephalitis Syndrome in Nepal – a Longitudinal Follow-Up Study**. *PLoS Negl Trop Dis*. 2013 Sep 12;**7**(9):e2383.

145. Touch S, Suraratdecha C, Samnang C, Heng S, Gazley L, Huch C, et al. **A cost–effectiveness analysis of Japanese encephalitis vaccine in Cambodia**. *Vaccine*. 2010 Jun;**28**(29):4593–9.

146. Liu W, Clemens JD, Kari K, Xu ZY. **Cost-effectiveness of Japanese encephalitis (JE) immunization in Bali, Indonesia**. *Vaccine*. 2008 Aug;**26**(35):4456–60.

147. Nguyen ALT, Slavkovsky R, Phan HT, Nguyen HTT, Vannachone S, Le DH, et al. **Estimating the cost of illness of acute Japanese encephalitis and sequelae care in Vietnam and Laos: A cross-sectional study**. *PLOS Global Public Health*. 2023 Jun 13;**3**(6):e0001873.

148. Huong NHT, Toan ND, Quy DT, Khanh TH, Thinh LQ, Nhan LNT, et al. **Study protocol: The clinical features, epidemiology, and causes of paediatric encephalitis in southern Vietnam**. *Wellcome Open Res*. 2022 Sep 13;**6**:133.

149. Le VT, Phan TQ, Do QH, Nguyen BH, Lam QB, Bach VC, et al. **Viral etiology of encephalitis in children in southern Vietnam: results of a one-year prospective descriptive study**. *PLoS Negl Trop Dis*. 2010 Oct 26;**4**(10):e854.

150. Wright S, Hacohen Y, Jacobson L, Agrawal S, Gupta R, Philip S, et al. **N-methyl-D-aspartate receptor antibody-mediated neurological disease: Results of a UK-based surveillance study in children**. *Arch Dis Child*. 2015;**100**(6):521–6.

151. Moore CE, Blacksell SD, Taojaikong T, Jarman RG, Gibbons R V., Lee SJ, et al. **A Prospective Assessment of the Accuracy of Commercial IgM ELISAs in Diagnosis of Japanese Encephalitis Virus Infections in Patients with Suspected Central Nervous System Infections in Laos**. *The American Society of Tropical Medicine and Hygiene*. 2012 Jul 2;**87**(1):171–8.

152. Ruiz-García R, Muñoz-Sánchez G, Naranjo L, Guasp M, Sabater L, Saiz A, et al. **Limitations of a Commercial Assay as Diagnostic Test of Autoimmune Encephalitis**. *Front Immunol*. 2021 Jun 29;**12**:691536.

153. Suh-Lailam BB, Haven TR, Copple SS, Knapp D, Jaskowski TD, Tebo AE. **Anti-NMDA-receptor antibody encephalitis: Performance evaluation and laboratory experience with the anti-NMDA-receptor IgG assay**. *Clinica Chimica Acta*. 2013 Jun;**421**:1–6.

154. O'Connor K, Waters P, Komorowski L, Zekeridou A, Guo CY, Mgbachi VC, et al. **GABA A receptor autoimmunity**. *Neurol Neuroimmunol Neuroinflamm*. 2019 May;**6**(3):e552.

155. Anh NT, Hong NTT, Nhu LNT, Thanh TT, Lau CY, Limmathurotsakul D, et al. **Viruses in Vietnamese Patients Presenting with Community-Acquired Sepsis of Unknown Cause**. *J Clin Microbiol*. 2019 Sep;**57**(9):e00386–19.

156. Anh NT, Nhu LNT, Hong NTT, Phuc TM, Tam PTT, Huong DT, et al. **Viral Metagenomic Analysis of Cerebrospinal Fluid from Patients with Acute Central Nervous System Infections of Unknown Origin, Vietnam**. *Emerg Infect Dis*. 2021 Jan;**27**(1):205–13.

157. Hong NTT, Anh NT, Mai NTH, Nghia HDT, Nhu LNT, Thanh TT, et al. **Performance of Metagenomic Next-Generation Sequencing for the Diagnosis of Viral Meningoencephalitis in a Resource-Limited Setting**. *Open Forum Infect Dis*. 2020 Mar 1;**7**(3):ofaa046.

158. Dubey D, Pittock SJ, Kelly CR, McKeon A, Lopez-Chiriboga AS, Lennon VA, et al. **Autoimmune encephalitis epidemiology and a comparison to infectious encephalitis**. *Ann Neurol*. 2018 Jan;**83**(1):166–77.

159. Kunchok A, McKeon A, Zekeridou A, Flanagan EP, Dubey D, Lennon VA, et al. **Autoimmune/Paraneoplastic Encephalitis Antibody Biomarkers: Frequency, Age, and Sex Associations**. *Mayo Clin Proc*. 2022 Mar;**97**(3):547–59.

160. Nissen MS, Ørvik MS, Nilsson AC, Ryding M, Lydolph M, Blaabjerg M. **NMDA-receptor encephalitis in Denmark from 2009 to 2019: a national cohort study**. *J Neurol*. 2022 Mar 5;**269**(3):1618–30.

161. Pruss H, Dalmau J, Harms L, Holtje M, Ahnert-Hilger G, Borowski K, et al. **Retrospective analysis of NMDA receptor antibodies in encephalitis of unknown origin**. *Neurology*. 2010 Nov 9;**75**(19):1735–9.

162. Li X, Hou C, Wu WL, Liang H, Zheng K, Zhang Y, et al. **Pediatric anti-N-methyl-d-aspartate receptor encephalitis in southern China: Analysis of 111 cases**. *J Neuroimmunol*. 2021 Mar;**352**:577479.

163. Nguyen Thi Hoang M, Nguyen Hoan P, Le Van T, McBride A, Ho Dang Trung N, Tran Tan T, et al. **First reported cases of anti-NMDA receptor encephalitis in Vietnamese adolescents and adults**. *J Neurol Sci*. 2017 Feb;**373**:250–3.

164. Uy CE, Mayxay M, Harrison R, Al-Diwani A, Jacobson L, Rattanavong S, et al. **Detection and significance of neuronal autoantibodies in patients with meningoencephalitis in Vientiane, Lao PDR**. *Trans R Soc Trop Med Hyg*. 2022 Oct 2;**116**(10):959–65.

165. Ouyang S, Tang Z, Duan W, Tang S, Zeng Q, Gu W, et al. **Mapping the global research landscape and trends of autoimmune encephalitis: A bibliometric analysis**. *Heliyon*. 2024 Feb;**10**(4):e26653.

166. Holmes JF. **Performance of the Pediatric Glasgow Coma Scale in Children with Blunt Head Trauma**. *Academic Emergency Medicine*. 2005 Sep 1;**12**(9):814–9.

167. Bigi S, Fischer U, Wehrli E, Mattle H, Boltshauser E, Bürki S, et al. **Differences in risk-factors, aetiology and outcome between children and young adults with acute ischaemic stroke**. *Neuropediatrics*. 2010 Aug 26;**41**(02):V1255.

168. Irani SR, Bera K, Waters P, Zuliani L, Maxwell S, Zandi MS, et al. **N-methyl-d-aspartate antibody encephalitis: temporal progression of clinical and paraclinical observations in a predominantly non-paraneoplastic disorder of both sexes**. *Brain*. 2010 Jun;**133**(6):1655–67.

169. Armangue T, Moris G, Cantarín-Extremera V, Conde CE, Rostasy K, Erro ME, et al. **Autoimmune post–herpes simplex encephalitis of adults and teenagers**. *Neurology*. 2015 Nov 17;**85**(20):1736–43.

170. Kaur S, Juneja M, Mishra D, Jain S. **Anti-N-methyl-D-aspartate receptor encephalitis: A case report and review of the literature**. *J Pediatr Neurosci*. 2014;**9**(2):145.

171. Dalmau J, Armangué T, Planagumà J, Radosevic M, Mannara F, et al. **An update on anti-NMDA receptor encephalitis for neurologists and psychiatrists: mechanisms and models**. *Lancet Neurol*. 2019 Nov;**18**(11):1045–57.

172. Pan J, Ovlyakulov B, Zhou L. **Overlapping anti-N-methyl-d-aspartate receptor (NMDAR) encephalitis with neuromyelitis optica spectrum disorders: a case report**. *Eur J Med Res*. 2022 Dec 9;**27**(1):66.

173. Huang CN, Tian XB, Jiang SM, Chang SH, Wang N, Liu MQ, et al. **Comparisons Between Infectious and Autoimmune Encephalitis: Clinical Signs, Biochemistry, Blood Counts, and Imaging Findings**. *Neuropsychiatr Dis Treat*. 2020 Nov; **16**:2649–60.

174. Zhang F, Xu G, Zhang X, Li Y, Li D, Wang C, et al. **Clinical characteristics and short-term outcomes of Japanese encephalitis in pediatric and adult patients: a retrospective study in Northern China**. *Front Neurol*. 2023 Apr 20;**14**:1135001.

175. Mohammad SS, Fung VSC, Grattan-Smith P, Gill D, Pillai S, Ramanathan S, et al. **Movement disorders in children with anti-NMDAR encephalitis and other autoimmune encephalopathies**. *Movement Disorders*. 2014 Oct;**29**(12):1539–42.

176. Garg D, Mohammad SS, Sharma S. **Autoimmune Encephalitis in Children: An Update**. *Indian Pediatr*. 2020 Jul 29;**57**(7):662–70.

177. de Bruijn MAAM, Bruijstens AL, Bastiaansen AEM, van Sonderen A, Schreurs MWJ, Sillevis Smitt PAE, et al. **Pediatric autoimmune encephalitis**. *Neurol Neuroimmunol Neuroinflamm*. 2020 May;**7**(3):e682.

178. Yakir MJ, Yang JH. **Treatment Approaches in Pediatric Relapsing Autoimmune Encephalitis**. *Curr Treat Options Neurol*. 2024 May 21;**26**(5):139–49.

179. Erickson TA, Muscal E, Munoz FM, Lotze T, Hasbun R, Brown E, et al. **Infectious and Autoimmune Causes of Encephalitis in Children**. *Pediatrics*. 2020 Jun 1;**145**(6):e20192543.

180. Sandweiss AJ, Erickson TA, Jiang Y, Kannan V, Yarimi JM, Levine JM, et al. **Infectious profiles in pediatric anti-N-methyl-d-aspartate receptor encephalitis**. *J Neuroimmunol*. 2023 Aug;**381**:578139.

181. Hu S, Lan T, Bai R, Jiang S, Cai J, Ren L. **HSV encephalitis triggered anti-NMDAR encephalitis: a case report**. *Neurological Sciences*. 2021 Mar 8;**42**(3):857–61.

182. Leypoldt F, Titulaer MJ, Aguilar E, Walther J, Bönstrup M, Havemeister S, et al. **Herpes simplex virus–1 encephalitis can trigger anti-NMDA receptor encephalitis: Case report**. *Neurology*. 2013 Oct 29;**81**(18):1637–9.

183. Nibber A, Wills B, Pettingill P, Fox H, Thuy TTN, Chau NVV, et al. **Evolution and significance of neuronal surface autoantibodies after Japanese encephalitis**. *J Neuroimmunol*. 2025 Sep;**406**:578671.

184. Alam AM, Easton A, Nicholson TR, Irani SR, Davies NWS, Solomon T, et al. **Encephalitis: diagnosis, management and recent advances in the field of encephalitides**. *Postgrad Med J*. 2023 Jul 21;**99**(1174):815–25.

185. Tyler KL. **Acute Viral Encephalitis**. *New England Journal of Medicine*. 2018 Aug 9;**379**(6):557–66.

186. Kadambari S, Abdullahi F, Celma C, Ladhani S. **Epidemiological trends in viral meningitis in England: Prospective national surveillance, 2013–2023**. *Journal of Infection*. 2024 Sep 1;**89**(3):106223.

187. Khanh TH, Sabanathan S, Thanh TT, Thoa LPK, Thuong TC, Hang V thi T, et al. **Enterovirus 71–associated Hand, Foot, and Mouth Disease, Southern Vietnam, 2011**. *Emerg Infect Dis*. 2012 Dec;**18**(12):2002–5.

188. Beld M, Minnaar R, Weel J, Sol C, Damen M, van der Avoort H, et al. **Highly Sensitive Assay for Detection of Enterovirus in Clinical Specimens by Reverse Transcription-PCR with an Armored RNA Internal Control**. *J Clin Microbiol*. 2004 Jul;**42**(7):3059–64.

189. Hue KDT, Tuan TV, Thi HTN, Bich CTN, Anh HH Le, Wills BA, et al. **Validation of an internally controlled one-step real-time multiplex RT-PCR assay for the detection and quantitation of dengue virus RNA in plasma**. *J Virol Methods*. 2011 Nov;**177**(2):168–73.

190. Scheltinga SA, Templeton KE, Beersma MFC, Claas ECJ. **Diagnosis of human metapneumovirus and rhinovirus in patients with respiratory tract infections by an internally controlled multiplex real-time RNA PCR**. *Journal of Clinical Virology*. 2005 Aug;**33**(4):306–11.

191. Corless CE, Guiver M, Borrow R, Edwards-Jones V, Fox AJ, Kaczmarski EB. **Simultaneous Detection of Neisseria meningitidis, Haemophilus influenzae, and Streptococcus pneumoniae in Suspected Cases of Meningitis and Septicemia Using Real-Time PCR**. *J Clin Microbiol*. 2001 Apr;**39**(4):1553–8.

192. Dung TTN, Phat VV, Nga TVT, My PVT, Duy PT, et al. **The validation and utility of a quantitative one-step multiplex RT real-time PCR targeting Rotavirus A and Norovirus**. *J Virol Methods*. 2013 Jan;**187**(1):138–43.

193. Vergadi E, Zacharioudaki M, Raissaki M, Galanakis E. **Challenges in the Diagnosis of Viral Encephalitis in Children: The Case of Two Siblings**. *Infect Dis Rep*. 2022 Feb 11;**14**(1):106–11.

194. Ansari A, Li S, Abzug MJ, Weinberg A. **Human Herpesviruses 6 and 7 and Central Nervous System Infection in Children**. *Emerg Infect Dis*. 2004 Aug;**10**(8):1450–4.

195. Webb G, Leong MYM, Bishop E, Sehu M. **Diagnostic Dilemmas: A Review of Reported Cases of Human Herpesvirus 6 Encephalitis in Immunocompetent Adults**. *Open Forum Infect Dis*. 2024 Aug 30;**11**(9):ofae501.

196. Ai J, Xie Z, Liu G, Chen Z, Yang Y, Li Y, et al. **Etiology and prognosis of acute viral encephalitis and meningitis in Chinese children: a multicentre prospective study**. *BMC Infect Dis*. 2017 Dec 14;**17**(1):494.

197. Chansaenroj J, Vichaiwattana P, Puenpa J, Thatsanathorn T, Sudhinaraset N, Wanlapakorn N, et al. **Human Enterovirus B Is a Significant Cause of Aseptic Meningitis and Sepsis-Like Illness in Young Infants in Thailand**. *Cureus*. 2024 Feb 26;**16**(2):e54997.

198. Chau NVV, Thuong TC, Hung NT, Hong NTT, Quy DT, Thien TB, et al. **Emerging Enterovirus A71 Subgenogroup B5 Causing Severe Hand, Foot, and Mouth Disease, Vietnam, 2023**. *Emerg Infect Dis*. 2024 Feb;**30**(2):363-367.

199. Britton PN, Dale RC, Blyth CC, Macartney K, Crawford NW, Marshall H, et al. **Influenza-associated Encephalitis/Encephalopathy Identified by the Australian Childhood Encephalitis Study 2013–2015**. *Pediatric Infectious Disease Journal*. 2017 Nov;**36**(11):1021–6.

200. Bohmwald K, Gálvez NMS, Ríos M, Kalergis AM. **Neurologic Alterations Due to Respiratory Virus Infections**. *Front Cell Neurosci*. 2018 Oct 26;**12**:386.

201. Morfopoulou S, Brown JR, Davies EG, Anderson G, Virasami A, Qasim W, et al. **Human Coronavirus OC43 Associated with Fatal Encephalitis**. *New England Journal of Medicine*. 2016 Aug 4;**375**(5):497–8.

202. Tian J, Wang X, Zhang L, Li Q, Feng G, Zeng Y, et al. **Clinical epidemiology and disease burden of adenoviral encephalitis in hospitalized children in China: A nationwide cross-sectional study**. *Pediatr Investig*. 2023 Dec 25;**7**(4):247–53.

203. McCall LI. **Quo vadis? Central Rules of Pathogen and Disease Tropism**. *Front Cell Infect Microbiol*. 2021 Feb 25;**11**:640987.

204. Si Y, He W, Guo S, Wang X, Tang M, Ying B, et al. **Multiplex detection of meningitis and encephalitis pathogens: A study from laboratory to clinic**. *Front Neurol*. 2022 Dec 16;**13**:1054071.

205. Syed Hussin SAD, Chen XW, Al-Talib H, Chua AL, Azimi Z, Wang SM. **Diagnostic accuracy of multiplex real-time PCR for detecting viruses associated with encephalitis: A systematic review and meta-analysis protocol**. *PLoS One*. 2025 Mar 13;**20**(3):e0318805.

206. Yeoh DK, Foley DA, Minney-Smith CA, Martin AC, Mace AO, et al. **Impact of Coronavirus Disease 2019 Public Health Measures on Detections of Influenza and Respiratory Syncytial Virus in Children During the 2020 Australian Winter**. *Clinical Infectious Diseases*. 2021 Jun 15;**72**(12):2199–202.

207. Anh NT, Nhu LNT, Van HMT, Hong NTT, Thanh TT, Hang VTT, et al. **Emerging Coxsackievirus A6 Causing Hand, Foot and Mouth Disease, Vietnam**. *Emerg Infect Dis*. 2018 Apr;**24**(4):654–62.

208. Anh NT, Hong NTT, Nhu LNT, Thanh TT, Lau CY, Limmathurotsakul D, et al. **Viruses in Vietnamese Patients Presenting with Community-Acquired Sepsis of Unknown Cause**. *J Clin Microbiol*. 2019 Sep;**57**(9):e00386–19.

209. Hong NTT, Anh NT, Mai NTH, Nghia HDT, Nhu LNT, Thanh TT, et al. **Performance of Metagenomic Next-Generation Sequencing for the Diagnosis of Viral Meningoencephalitis in a Resource-Limited Setting**. *Open Forum Infect Dis*. 2020 Mar;**7**(3):ofaa046.

210. Anh NT, Nhu LNT, Hong NTT, Phuc TM, Tam PTT, Huong DT, et al. **Viral Metagenomic Analysis of Cerebrospinal Fluid from Patients with Acute Central Nervous System Infections of Unknown Origin, Vietnam**. *Emerg Infect Dis*. 2021 Jan;**27**(1):205–13.

211. Nhu LNT, Nhan LNT, Anh NT, Hong NTT, Van HMT, Thanh TT, et al. **Coxsackievirus A16 in Southern Vietnam**. *Front Microbiol*. 2021;**12**:689658.

212. Dung NT, Hung LM, Hoa HTT, Nga LH, Hong NTT, Thuong TC, et al. **Monkeypox Virus Infection in 2 Female Travelers Returning to Vietnam from Dubai, United Arab Emirates, 2022**. **Emerg Infect Dis**. 2023 Apr;**29**(4):778–81.

213. Hoa HTT, Dung NT, Hung LM, Hong NTT, Quy VT, Thao NT, et al. **Emerging Monkeypox Virus Sublineage C.1 Causing Community Transmission, Vietnam, 2023**. *Emerg Infect Dis*. 2024 Nov;**30**(11):2385–90.

214. Li L, Deng X, Mee ET, Collot-Teixeira S, Anderson R, Schepelmann S, et al. **Comparing viral metagenomics methods using a highly multiplexed human viral pathogens reagent**. *J Virol Methods*. 2015 Mar;**213**:139–46.

215. Tu NTK, Deng X, Hong NTT, Ny NTH, Phuc TM, Tam PTT, et al. **Redondoviridae: High Prevalence and Possibly Chronic Shedding in Human Respiratory Tract, But No Zoonotic Transmission**. *Viruses*. 2021 Mar 24;**13**(4):533.

216. Anh NT, Hong NTT, Nhu LNT, Thanh TT, Anscombe C, Chau LN, et al. **Detection and Characterization of Human Pegivirus 2, Vietnam**. *Emerg Infect Dis*. 2018 Nov;**24**(11):2063–7.

217. Thi Kha Tu N, Thi Thu Hong N, Thi Han Ny N, My Phuc T, Thi Thanh Tam P, Doorn HR van, et al. **The Virome of Acute Respiratory Diseases in Individuals at Risk of Zoonotic Infections**. *Viruses*. 2020 Aug 29;**12**(9):960.

218. Katoh K, Standley DM. **MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability**. *Mol Biol Evol*. 2013 Apr 1;**30**(4):772–80.

219. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. **IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies**. *Mol Biol Evol*. 2015 Jan;**32**(1):268–74.

220. Tuladhar ET, Shrestha S, Vernon S, Droit L, Mihindukulasuriya KA, Tamang M, et al. **Gemykibivirus detection in acute encephalitis patients from Nepal**. *mSphere*. 2024 Jul 30;**9**(7):e0021924.

221. da Silva Assis MR, Vieira CB, Fioretti JM, Rocha MS, de Almeida PIN, Miagostovich MP, et al. **Detection and Molecular Characterization of Gemycircularvirus from Environmental Samples in Brazil**. *Food Environ Virol*. 2016 Dec 8;**8**(4):305–9.

222. Jiang D, Ghabrial SA. **Molecular characterization of Penicillium chrysogenum virus: reconsideration of the taxonomy of the genus Chrysovirus**. *Journal of General Virology*. 2004 Jul 1;**85**(7):2111–21.

223. Jamal A, Bignell EM, Coutts RHA. **Complete nucleotide sequences of four dsRNAs associated with a new chrysovirus infecting Aspergillus fumigatus**. *Virus Res*. 2010 Oct;**153**(1):64–70.

224. Wen L, Yin L, Zhu J, Li H, Zhang F, Hu Q, et al. **Nearly 20 Years of Genetic Diversity and Evolution of Porcine Circovirus-like Virus P1 from China**. *Viruses*. 2022 Mar 28;**14**(4):696.

225. Ma R, Zhao M, Wang H, Hou R, Qin K, Qian Y, et al. **Virome of Giant Panda-Infesting Ticks Reveals Novel Bunyaviruses and Other Viruses That Are Genetically Close to Those from Giant Pandas**. *Microbiol Spectr*. 2022 Aug 31;**10**(4):e02034–22.

226. Ssemadaali MA, Effertz K, Singh P, Kolyvushko O, Ramamoorthy S. **Identification of heterologous Torque Teno Viruses in humans and swine**. *Sci Rep*. 2016 May 25;**6**(1):26655.

227. Silva LA, Ardisson-Araujo DMP, Tinoco RS, Fernandes OA, Melo FL, Ribeiro BM. **Complete genome sequence and structural characterization of a novel iflavirus isolated from Opsiphanes invirae (Lepidoptera: Nymphalidae)**. *J Invertebr Pathol*. 2015 Sep;**130**:136–40.

228. Jiang Y, Yang B, Liu J, Wang Q, Zhang Q, Yu W, et al. **A novel mycovirus infecting Aspergillus nidulans that is closely related to viruses in a new genus of the family Partitiviridae**. *Arch Virol*. 2021 Feb 6;**166**(2):659–64.

229. Wang Y, Zhao H, Cao J, Yin X, Guo Y, Guo L, et al. **Characterization of a Novel Mycovirus from the Phytopathogenic Fungus Botryosphaeria dothidea**. *Viruses*. 2022 Feb 6;**14**(2):331.

230. Zhang T, Jiang Y, Huang J, Dong W. **Genomic organization of a novel partitivirus from the phytopathogenic fungus Ustilaginoidea virens**. *Arch Virol*. 2013 Nov 4;**158**(11):2415–9.

231. Ganesh B, Masachessi G, Mladenova Z. **Animal Picobirnavirus**. *Virus disease*. 2014 Jun 2;**25**(2):223–38.

232. Zhang W, Li F, Liu A, Lin X, Fu S, Song J, et al. **Identification and genetic analysis of Kadipiro virus isolated in Shandong province, China**. *Virol J*. 2018 Dec 6;**15**(1):64.

233. Turner P, Suy K, Tan L Van, Sar P, Miliya T, Hong NTT, et al. **The aetiologies of central nervous system infections in hospitalised Cambodian children**. *BMC Infect Dis*. 2017 Dec 29;**17**(1):806.

234. Tan L Van, Qui PT, Ha DQ, Hue NB, Bao LQ, Cam B Van, et al. **Viral etiology of encephalitis in children in southern Vietnam: results of a one-year prospective descriptive study**. *PLoS Negl Trop Dis*. 2010 Oct 26;**4**(10):e854.

235. Tan L Van, Van Doorn HR, Van der Hoek L, Minh Hien V, F. Jebbink M, Quang Ha D, et al. **Random PCR and ultracentrifugation increases sensitivity and throughput of VIDISCA for screening of pathogens in clinical specimens**. *The Journal of Infection in Developing Countries*. 2010 Jul 19;**5**(02):142–8.

236. Chen W, Liu G, Cui L, Tian F, Zhang J, Zhao J, et al. **Evaluation of metagenomic and pathogen-targeted next-generation sequencing for diagnosis of meningitis and encephalitis in adults: A multicenter prospective observational cohort study in China**. *Journal of Infection*. 2024 May;**88**(5):106143.

237. Wilson MR, Sample HA, Zorn KC, Arevalo S, Yu G, Neuhaus J, et al. **Clinical Metagenomic Sequencing for Diagnosis of Meningitis and Encephalitis**. *New England Journal of Medicine*. 2019 Jun 13;**380**(24):2327–40.

238. Varsani A, Krupovic M. **Family Genomoviridae: 2021 taxonomy update**. *Arch Virol*. 2021 Oct 31;**166**(10):2911–26.

239. Doan T, Ha V, Tran T, Yang J. **Dynamics of wage inequality over the prolonged economic transformation: The case of Vietnam**. *Econ Anal Policy*. 2023 Jun;**78**:816–34.

240. Nguyen C. **The impact of health insurance programs for children: evidence from Vietnam**. *Health Econ Rev*. 2016 Dec 5;**6**(1):34.

241. Turner HC, Lauer JA, Tran BX, Teerawattananon Y, Jit M. **Adjusting for Inflation and Currency Changes Within Health Economic Studies**. *Value in Health*. 2019 Sep;**22**(9):1026–32.

242. **Vietnam: Health spending per capita in 2020**. *TheGlobalEconomy.com*. URL: https://www.theglobaleconomy.com. Accessed Date: July 15, 2025.

243. Krol M, Brouwer W, Rutten F. **Productivity Costs in Economic Evaluations: Past, Present, Future**. *Pharmacoeconomics*. 2013 Jul 26;**31**(7):537–49.

244. Jiang S, Wang Y, Si L, Zang X, Gu YY, Jiang Y, et al. **Incorporating productivity loss in health economic evaluations: a review of guidelines and practices worldwide for research agenda in China**. *BMJ Glob Health*. 2022 Aug 17;**7**(8):e009777.

245. Turner HC, Archer RA, Downey LE, Isaranuwatchai W, Chalkidou K, Jit M, et al. **An Introduction to the Main Types of Economic Evaluations Used for Informing Priority Setting and Resource Allocation in Healthcare: Key Features, Uses, and Limitations**. *Front Public Health*. 2021 Aug 25;**9**:722927.

246. Shwartz M, Young DW, Siegrist R. **The ratio of costs to chargees: how good a basis for estimating costs?** *Inquiry*. 1995;**32**(4) :476–81.

247. Finkler SA. **The Distinction Between Cost and Charges**. *Ann Intern Med*. 1982 Jan 1;**96**(1):102–9.

248. Sandmann FG, Robotham J V., Deeny SR, Edmunds WJ, Jit M. **Estimating the opportunity costs of bed-days**. *Health Econ*. 2018 Mar 6;**27**(3):592–605.

249. Anh DD, Riewpaiboon A, Tho LH, Nyambat B. **Treatment Costs of Pneumonia, Meningitis, Sepsis, and Other Diseases among Hospitalized Children in Viet Nam**. *J Health Popul Nutr*. 2010 Oct;**28**(5):436–442.

250. Nguyen TPL, Nguyen TBY, Nguyen TT, Vinh Hac V, Le HH, Schuiling-Veninga C, et al. **Direct costs of hypertensive patients admitted to hospital in Vietnam' a bottom-up micro-costing analysis**. *BMC Health Serv Res*. 2014 Dec 28;**14**(1):514.

251. Granerod J, Tam CC, Crowcroft NS, Davies NWS, Borchert M, Thomas SL. **Challenge of the unknown: A systematic review of acute encephalitis in non-outbreak situations**. *Neurology*. 2010 Sep 7;**75**(10):924–32.

252. Yen NT, Duffy MR, Hong NM, Hien NT, Fischer M, Hills SL. **Surveillance for Japanese encephalitis in Vietnam, 1998-2007**. *Am J Trop Med Hyg*. 2010 Oct;**83**(4):816–9.

253. Misra UK, Kalita J. **Movement disorders in Japanese encephalitis**. *J Neurol*. 1997 Apr 24;**244**(5):299–303.

254. Anderson PJ, Burnett A. **Assessing developmental delay in early childhood — concerns with the Bayley-III scales**. *Clin Neuropsychol*. 2017 Feb 17;**31**(2):371–81.

255. Pandolfi V, Magyar CI. **Vineland-3 Structural Validity and Interpretability of Domain Scores: Implications for Practitioners Assessing Adolescents With Developmental Conditions**. *Am J Intellect Dev Disabil*. 2021 May 1;**126**(3):216–29.

256. Na SD, Burns TG. **Wechsler Intelligence Scale for Children-V: Test Review**. *Appl Neuropsychol Child*. 2016 Apr 2;**5**(2):156–60.

Appendices

Appendix 3.1. The paediatric Glasgow coma scale and the modified Rankin scale for children

	Background	Components	Definitions of more severe outcomes in my study
The paediatric Glasgow coma scale (GCS) (166)	The paediatric Glasgow coma scale (GCS) was used in this study to assess the impaired consciousness and coma in paediatric patients with encephalitis. The paediatric GCS comprises three examinations including eye, verbal and motor responses. The three values separately as well as their sum are considered. The lowest possible sum of the paediatric GCS is 3 (deep coma or death) whilst the highest is 15 (fully awake and aware person).	**Eye Opening (Score: 1–4)** 4: Spontaneous – Eyes open spontaneously without stimulation. 3: To speech – Eyes open in response to verbal stimuli (e.g., calling the child's name). 2: To pain – Eyes open only in response to painful stimuli (e.g., pressure on the nail bed). 1: None – No eye opening, even with painful stimuli. **Verbal Response (Score: 1–5)** ***For pre-verbal children (e.g., infants and toddlers):*** 5: Coos, babbles, or age-appropriate vocalization – Normal, spontaneous vocal sounds or social smiling. 4: Irritable, cries but consolable – Crying or irritable but can be soothed. 3: Cries to pain – Persistent crying, especially in response to painful stimuli. 2: Moans to pain – Only moaning or grunting in response to pain. 1: None – No vocal response, even to pain. ***For verbal children (able to speak):*** 5: Orientated – Age-appropriate conversation, knows name, place, or simple facts. 4: Confused – Words or phrases are used but not fully coherent or appropriate. 3: Inappropriate words – Random or inappropriate words, no conversational exchange. 2: Incomprehensible sounds – Moaning or unintelligible sounds. 1: None – No verbal response. **Motor Response (Score: 1–6)** 6: Obeys commands (or normal spontaneous movements in pre-verbal children) – Follows simple commands (e.g., "raise your arm") or displays purposeful spontaneous movements (e.g., reaching for objects in infants). 5: Localizes pain – Purposeful movement toward a painful stimulus (e.g., attempts to push away the stimulus).	I used the paediatric Glasgow coma scale (GCS) to evaluate the consciousness, and the patients were defined to have more severe condition when the paediatric GCS < 9.

		4: Withdraws from pain – General withdrawal of a limb from painful stimulus. 3: Flexion to pain (decorticate posturing) – Abnormal flexion of limbs (e.g., bending of arms at the elbow) in response to pain. 2: Extension to pain (decerebrate posturing) – Abnormal extension of limbs (e.g., straightening of arms and legs) in response to pain. 1: None – No motor response to pain.	
The modified Rankin scale (mRS) for children (167)	The modified Rankin scale (mRS) for children was used in this study to measure the degree of disability or dependence in the daily activities of children who have suffered encephalitis. The scale comprises seven levels, from 0 to 6, with higher scores indicating greater disability and where 0–2 is generally considered a good outcome with individuals assuming complete functional independence. A modified Rankin score of 6 is often used to denote an individual who is deceased.	**Score 0: No symptoms** The child has no symptoms or functional limitations. They can perform all age-appropriate activities (e.g., play, school tasks, social interactions) without difficulty. **Score 1: No significant disability despite symptoms** The child has minor symptoms (e.g., mild weakness or behavioural changes) but no significant functional impairment. They can carry out all usual activities, including school and play, with minimal or no assistance. **Score 2: Slight disability** The child is unable to perform some age-appropriate activities but can manage most daily activities independently. They may require minor assistance or modifications (e.g., extra time for tasks, mild cognitive or motor limitations) but are largely independent. **Score 3: Moderate disability** The child requires some help with daily activities but can walk without assistance and participate in modified school or social activities. They may have noticeable motor, cognitive, or behavioural impairments that affect performance but do not preclude independent mobility or basic self-care. **Score 4: Moderately severe disability** The child requires significant assistance with daily activities and cannot walk without support. They may be able to perform some self-care tasks (e.g., eating, dressing) with help but have substantial limitations in mobility, communication, or cognition, impacting school and social participation. **Score 5: Severe disability** The child is bedridden or requires constant supervision and assistance for all daily activities. They have severe motor, cognitive, or communication impairments, with minimal or no independent function. **Score 6: Death** The child has passed away.	I used modified Rankin scale (mRS) for children to assess the degree of disability or dependence in the daily activities, and the patients were defined to have more severe condition when the mRS ≥ 3.

Appendix 3.2. Case report form (CRF) for the clinical study of encephalitis in children

33EI - Children encephalitis

DEMOGRAPHICS			**DM (1 / 1)**
Study Identifier	Study Site Identifier	Subject Identifier	Subject Initials
[33EI]	**[001]**	[__|__|__]	[__|__|__|__|__|__]

ADMINISTRATION

1. Sex/Gender: ○ Male ○ Female

2. Date of Birth [__|__]/[__|__]/[__|__] (dd/mm/yy)

Age: months (<5 years) [__|__]

Years: [__|__]

3. Place of residence: ○ HCMC ○ PROVINCES

If Provinces, specify: [_____]

4. Admitted: ○ from home ○ Transferred from other hospital

If Transfer, Specify: [_____]

Stayed from: [__|__]/[__|__]/[__|__] (dd/mm/yy)

to: [__|__]/[__|__]/[__|__] (dd/mm/yy)

5. Date of CH1 admission: [__|__]/[__|__]/[__|__] (dd/mm/yy)

6. Date of CH1 discharge: [__|__]/[__|__]/[__|__] (dd/mm/yy)

Completed by: _____ Date: _____/____/_____

MEDICAL HISTORY **HIST (1 / 1)**

Study Identifier	Study Site Identifier	Subject Identifier	Subject Initials
[33EI]	**[001]**	[__|__|__|__|__|__]	[__|__|__|__|__|__]

1. Hospital of birth: [_____]
2. Gestational age: [__|__] weeks
3. Birth weight: [__|__|__|__] grams
4. Type of delivery: ○ Vaginal ○ C-section ○ Forceps ○ Vacuum
5. Vaccination history: ○ Yes ○ No
   if yes, specify: [_____]

Initial symptoms:

6. Abnormal/Psychiatric behaviour or cognitive dysfunction ○ Yes ○ No
   if yes, specify: [_____]
7. Seizure: ○ Yes ○ No
8. Movement disorder: ○ Yes ○ No
9. Decrease level of consciousness: ○ Yes ○ No

Completed by: _____ Date: _____/____/_____

CLINICAL FEATURES			EXAM (1 / 2)
Study Identifier **[33EI]**	Study Site Identifier **[001]**	Subject Identifier [__|__|__|__|__|__]	Subject Initials [__|__|__|__|__|__]

1. Fever: ○ Yes ○ No
2. Temperature: [__|__] . [__] oC
3. Duration of fever: [__|__|__]
4. Headache: ○ Yes ○ No
5. Weight loss: ○ Yes ○ No
6. Night sweats ○ Yes ○ No

Gastrointestinal features:

7. Nausea: ○ Yes ○ No
8. Vomiting: ○ Yes ○ No
9. Diarrhoea: ○ Yes ○ No

Respiratory features:

10. Coughing: ○ Yes ○ No
11. Sneezing: ○ Yes ○ No
12. Respiratory failure ○ Yes ○ No
13. Abnormal breath: ○ Yes ○ No
14. Hypoventilation: ○ Yes ○ No
15. Hyperventilation: ○ Yes ○ No

16. Skin lesions: ○ Yes ○ No
    - Rash ○ Yes ○ No
    - Petechiae ○ Yes ○ No
    - Purpura ○ Yes ○ No

17. Hand food and mouth syndrome: ○ Yes ○ No
    - Mouth/tongue ulcer: ○ Yes ○ No
    - Maculopapular rashes ○ Yes ○ No
    - Vesicles on palms and soles ○ Yes ○ No

18. Glasgow Coma Scale: [__|__] / 15
19. Level of consciousness:
    - ○ Alert (GCS=14–15)
    - ○ Drowsy (GCS=9–13)
    - ○ Stupor (GCS=6–8)
    - ○ Coma (GCS=3–5)

CLINICAL FEATURES **EXAM (2 / 2)**

20. Seizure: ○ Yes ○ No
    Refractory seizure: ○ Yes ○ No
    Single seizure ○ Yes ○ No
    Status epileptics ○ Yes ○ No

21. Type of seizure:

Focal (partial) seizure: ○ Yes ○ No
    Simple focal seizure ○ Yes ○ No
    Complex focal seizure ○ Yes ○ No

Generalized seizure ○ Yes ○ No
    Absence seizure ○ Yes ○ No
    Atonic seizure ○ Yes ○ No
    Generalized tonic-clonic seizure ○ Yes ○ No
    Myoclonic seizure ○ Yes ○ No

22. Meningitis syndrome: ○ Yes ○ No
    Bulging fontanelle ○ Yes ○ No
    Nuchal rigidity ○ Yes ○ No
    Kernig's sign ○ Yes ○ No
    Brudzinski's sign ○ Yes ○ No

23. Paralysis/Focal neurologic deficits: ○ Yes ○ No
    Paraplegia (lower body) ○ Yes ○ No
    Hemiplegia (one side of body) ○ Yes ○ No
    Quadriplegia/Tetraplegia (whole body) ○ Yes ○ No

24. Abnormal muscular tone: ○ Yes ○ No
    Hypertonia ○ Yes ○ No
    Hypotonia ○ Yes ○ No

25. Psychosis: ○ Yes ○ No if yes, specify: [_____]

26. Language change: ○ Yes ○ No if yes, specify: [_____]

27. Abnormal movement ○ Yes ○ No if yes, specify: [_____]

28. Respiratory distress/failure: ○ Yes ○ No

29. Shock/hypotension: ○ Yes ○ No

30. Other clinical features: ○ Yes ○ No if yes, specify: [_____]

Completed by: _____ Date: _____/____/_____

LABORATORY FINDINGS **LAB (1 / 2)**

Study Identifier	Study Site Identifier	Subject Identifier	Subject Initials
[33EI]	**[001]**	[__|__|__|__|__|__]	[__|__|__|__|__|__]

Haematology:

1. WBC: [__|__|__] . [__] x 10^3/mm^3
2. Neutrophil: [__] . [__] x 10^3/mm^3
3. Lymphocyte: [__] . [__] x 10^3/mm^3
4. Eosinophil: [__] . [__] x 10^3/mm^3
5. Hemoglobin: [__|__] . [__] g/dL
6. Haematocrit: [__|__] %
7. Platelet: [__|__|__] x 10^3/mm^3

Biochemistry:

8. CRP: [__|__|__] . [__|__] mg/L
9. Procalcitonin: [__|__|__] . [__|__] ng/mL
10. Blood glucose: [__|__|__] mg/dL
11. Creatinine: [__|__|__] µmol/L
12. Sodium: [__|__|__] mmol/L
13. Potassium: [__|__|__] mmol/L
14. Calcium: [__|__|__] mmol/L
15. Chloride: [__|__|__] mmol/L
16. AST: [__|__|__|__] IU/L
17. ALT: [__|__|__|__] IU/L
18. Total Bilirubin: [__|__] . [__] µmol/L
19. Direct Bilirubin: [__|__] . [__] µmol/L
20. Indirect bilirubin: [__|__] . [__] µmol/L
21. Lactate: [__|__] . [__] mmol/L

Cerebral spinal fluid (CSF):

22. WBC: [__|__|__|__] cells
23. Neutrophil: [__|__] . [__] %
24. Monocyte: [__|__] . [__] %
25. Protein: [__|__] . [__|__] g/L
26. Glucose: [__|__] . [__|__] mmol/L
27. CSF Glucose / Plasma Glucose: [__] . [__]
28. Lactate: [__|__] . [__] mmol/L
29. CSF Culture: ○ Positive ○ Negative ○ Not Done if positive, specify: [_____]
29. CSF Latex: ○ Positive ○ Negative ○ Not Done if positive, specify: [_____]

LABORATORY FINDINGS **LAB (2 / 2)**

30. PCR HSV: ○ Positive ○ Negative ○ Not Done
31. ELISA IgM JVE - CSF ○ Positive ○ Negative ○ Not Done
32. ELISA IgM JVE - Plasma ○ Positive ○ Negative ○ Not Done
33. PCR EV71 ○ Positive ○ Negative ○ Not Done
34. Anti NMDAR ○ Positive ○ Negative ○ Not Done

35. Blood culture: ○ Positive ○ Negative ○ Not Done if positive, specify: [_____]
36. Coagulation ○ Positive ○ Negative ○ Not Done if positive, specify: [_____]
37. Urinary analysis ○ Normal ○ Abnormal if abnormal, specify: [_____]

Blood gases:

38. pH: [__] . [__]
39. pO2: [__|__|__] mmHg
40. pCO2: [__|__|__] mmHg
41. HCO3: [__|__] mEq/L
42. Base excess = – [__|__] mEq/L
43. Base excess = + [__|__] mEq/L
44. PaO2/FiO2: [__|__|__] mmHg
45. Abnormal blood gases: ○ Yes ○ No
46. Metabolic acidosis: ○ Yes ○ No
47. Respiratory acidosis ○ Yes ○ No
48. Metabolic alkalosis: ○ Yes ○ No
49. Respiratory alkalosis ○ Yes ○ No

Completed by: _____ Date: _____/____/_____

IMAGING STUDIES **IMAGE (1 / 1)**

Study Identifier	Study Site Identifier	Subject Identifier	Subject Initials
[33EI]	**[001]**	[__|__|__|__|__|__]	[__|__|__|__|__|__]

Imaging	Cranial Ultrasound	CT Scan 1	CT Scan 2	MRI 1	MRI 2	EEG
Performing	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
Date of performing (dd/mm/yy)	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__
Result	○ Normal ○ Abn	○ Normal ○ Abn	○ Normal ○ Abn	○ Normal ○ Abn	○ Normal ○ Abn	○ Normal ○ Abn
Subarachnoid effusion	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
Subarachnoid empyema	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
Subdural effusion	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
Subdural empyema	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
Ventricular dilation	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
Description	[_____]	[_____]	[_____]	[_____]	[_____]	[_____]

DIAGNOSIS **DIAG (1 / 2)**

Study Identifier	Study Site Identifier	Subject Identifier	Subject Initials
[33EI]	**[001]**	[__|__|__|__|__|__]	[__|__|__|__|__|__]

1. Final diagnoses: [_____]

☐ Probable Encephalitis

Diagnostic date: [__|__]/[__|__]/[__|__] (dd/mm/yy)

☐ Confirmed Encephalitis

Diagnostic date: [__|__]/[__|__]/[__|__] (dd/mm/yy)

2. Cause of Encephalitis: ○ Virus ○ Immunology (Autoimmune) ○ Both

Diagnostic date: [__|__]/[__|__]/[__|__] (dd/mm/yy)

- Infection syndromes:

3. Meningitis: ○ Yes ○ No
4. Bronchiolitis: ○ Yes ○ No
5. Pneumonia: ○ Yes ○ No
6. Urinary infection: ○ Yes ○ No
7. Peritonitis: ○ Yes ○ No
8. Gastroenteritis/Enterocolitis: ○ Yes ○ No
9. Cellulitis: ○ Yes ○ No
10. Dermal infection: ○ Yes ○ No
11. Central line related sepsis: ○ Yes ○ No
12. Sepsis: ○ Yes ○ No
13. Severe sepsis: ○ Yes ○ No
14. Septic shock: ○ Yes ○ No
15. Community-acquired infection: ○ Yes ○ No
16. Hospital-acquired infection: ○ Yes ○ No
17. HIV infection: ○ Yes ○ No if yes, specify: [_____]
18. Tuberculosis: ○ Yes ○ No if yes, specify: [_____]
19. TORCH ○ Yes ○ No if yes, specify: [_____]
20. Others ○ Yes ○ No if yes, specify: [_____]

- Associated conditions

21. Tumours/Teratoma: ○ Yes ○ No
22. Diabetes: ○ Yes ○ No
23. Hypertension: ○ Yes ○ No
24. Hepatic disease: ○ Yes ○ No if yes, specify: [_____]
25. Kidney disease: ○ Yes ○ No if yes, specify: [_____]
26. Congenital heart diseases: ○ Yes ○ No if yes, specify: [_____]

DIAGNOSIS **DIAG (2 / 2)**

27. Broncho-pulmonary dysplasia: ○ Yes ○ No
28. Pulmonary hypertension: ○ Yes ○ No
29. Congenital renal diseases: ○ Yes ○ No if yes, specify: [_____]
30. Neuromuscular diseases: ○ Yes ○ No if yes, specify: [_____]
31. Congenital GI anomalies: ○ Yes ○ No if yes, specify: [_____]
32. Systemic lupus erythematosus: ○ Yes ○ No
33. Other autoimmune diseases: ○ Yes ○ No if yes, specify: [_____]
34. Hypovolemic shock: ○ Yes ○ No
35. Anaphylactic shock: ○ Yes ○ No
36. Others: ○ Yes ○ No if yes, specify: [_____]

Completed by: _____ **[001]** Date: _____/____/_____ **[001]**

MANAGEMENT **MGMT (1 / 2)**

Study Identifier	Study Site Identifier	Subject Identifier	Subject Initials
[33EI]	**[001]**	[__|__|__]	[__|__|__|__|__|__]

1. Antibiotics ○ Yes ○ No

Antibiotics drug	Dose	Duration	As
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line
[_____]	[__|__|__] mg/kg/day	[__|__] days	[__|__] line

2. Antipyretics: ○ Yes ○ No

Duration of antipyretics: [__|__|__] days

3. Anti-convulsion: ○ Yes ○ No

Duration of anti-convulsion: [__|__|__] days

Diazepam: ○ Yes ○ No

Duration of diazepam: [__|__|__] days

Phenobarbital: ○ Yes ○ No

Duration of phenobarbital: [_____] days

Treament Name	Check	Number of doses	Start date	Duration
IV Immunoglobulin	○ Yes ○ No	[__|__] doses	__/__/__	[__|__|__] days
Methyl-prednisolone	○ Yes ○ No	[__|__] doses	__/__/__	[__|__|__] days
Mannitol 20%	○ Yes ○ No	[__|__] doses	__/__/__	[__|__|__] days
Sodium chloride 3%	○ Yes ○ No	[__|__] doses	__/__/__	[__|__|__] days
Haloperidol	○ Yes ○ No	[__|__] doses	__/__/__	[__|__|__] days

MANAGEMENT **MGMT (2 / 2)**

5. Respiratory support: ○ Yes ○ No

Start date of respiratory support: [__|__]/[__|__]/[__|__] (dd/mm/yy)

Duration of respiratory support: [__|__|__] days

Oxygen cannula: ○ Yes ○ No

Duration of oxygen cannula: [__|__|__] days

NCPAP: ○ Yes ○ No

Duration of NCPAP [__|__|__] days

Intubation/ventilator support: ○ Yes ○ No

Duration of intubation/ventilator support: [__|__|__] days

6. Shock management: ○ Yes ○ No

Start date of shock management: [__|__]/[__|__]/[__|__] (dd/mm/yy)

Duration of shock management: [__|__|__] days

Dopamine: ○ Yes ○ No

Dopamine highest dose: [__|__] (µg/kg/min)

Dobutamine: ○ Yes ○ No

Dobutamine highest dose: [__|__] (µg/kg/min)

Epinephrine: ○ Yes ○ No

Epinephrine highest dose: [__] . [__] (µg/kg/min)

Norepinephrine: ○ Yes ○ No

Norepinephrine highest dose: [__] . [__] (µg/kg/min)

7. Fluid infusion

Parenteral nutrition: ○ Yes ○ No

Start date of parenteral nutrition: [__|__]/[__|__]/[__|__] (dd/mm/yy)

Duration of parenteral nutrition: [__|__|__] days

Partial parenteral nutrition: ○ Yes ○ No

Duration of partial parenteral nutrition: [__|__|__] days

Total parenteral nutrition: ○ Yes ○ No

Duration of total parenteral nutrition: [__|__|__] days

Duration of central vascular catheters: [__|__|__] days

8. Other treatments

Blood products transfusion: ○ Yes ○ No

Antacid agents: ○ Yes ○ No

Invasive surgery/intervention: ○ Yes ○ No if yes, specify: [_____]

Tumour removal: ○ Yes ○ No

Date of tumour removal: [__|__]/[__|__]/[__|__] (dd/mm/yy)

Plasmapheresis: ○ Yes ○ No

Date of plasmapheresis: [__|__]/[__|__]/[__|__] (dd/mm/yy)

Completed by: _____ Date: _____/____/_____

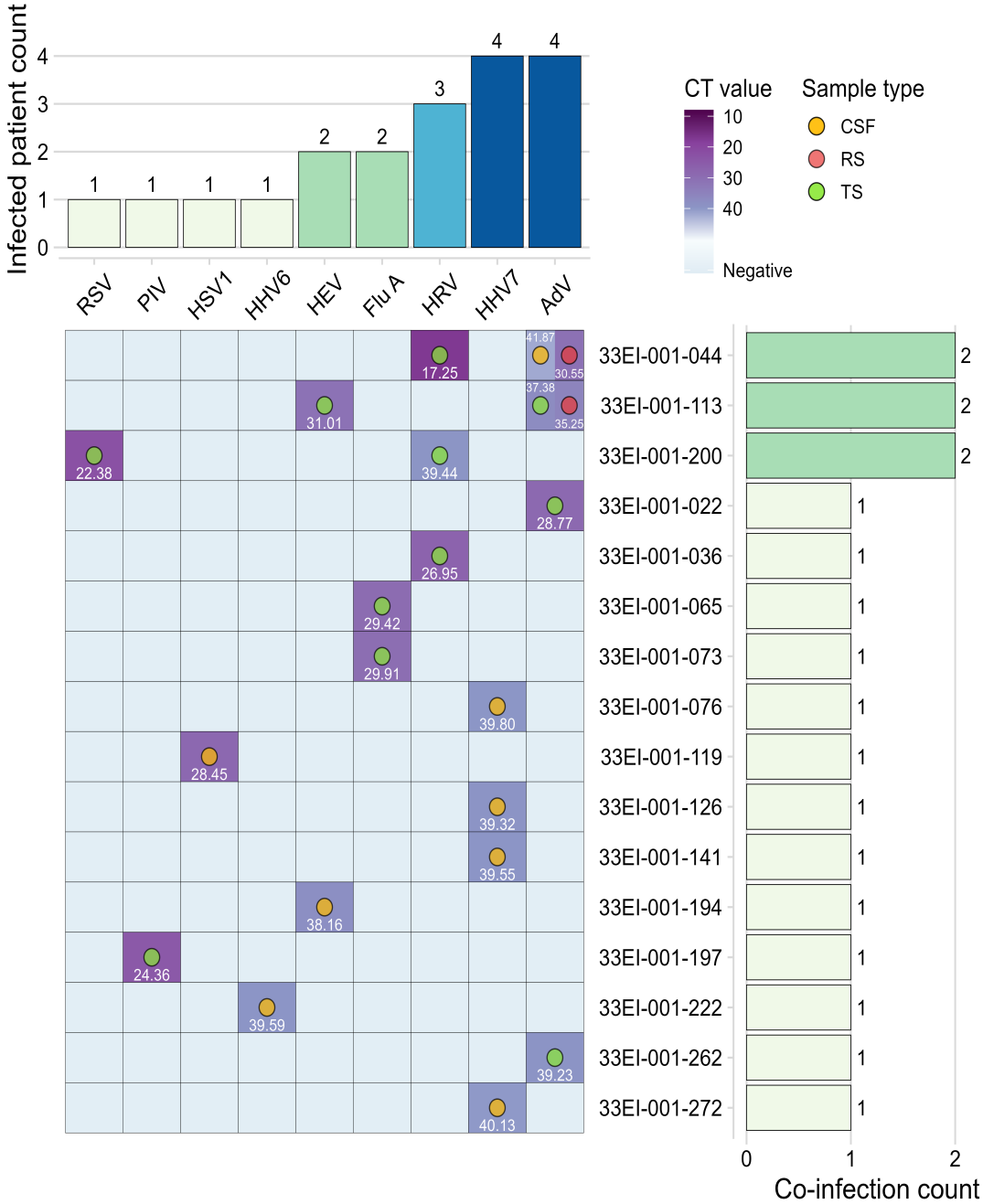
OUTCOME			FINAL (1 / 1)
Study Identifier **[33EI]**	Study Site Identifier **[001]**	Subject Identifier [__|__|__|__|__|__]	Subject Initials [__|__|__|__|__|__]

1. Mortality: ○ Survival ○ Died
2. Length of Stay: [__|__|__] days
3. Complications: ○ Yes ○ No
4. Sequelae: ○ Yes ○ No
5. Total cost of treatment: [_____] (VNĐ)

The modified Rankin scale (mRS) for children	**Score**
Score 0: No symptoms **Score 1: No significant disability despite symptoms** **Score 2: Slight disability** **Score 3: Moderate disability** **Score 4: Moderately severe disability** **Score 5: Severe disability** **Score 6: Death**	

Completed by: _____ Date: _____/____/_____

Appendix 4.1. Pathogens detected by additional multiplex PCR with Ct value, infected patient count, and co-infection count



Note: RSV: respiratory syncytial virus, PIV: parainfluenza virus, HSV1: Herpes simplex virus 1, HHV6: Human herpes virus 6, EV: enterovirus, FluA: influenza A virus, HRV: human rhinovirus, HHV7: human herpes virus 7, AdV: adenovirus, CSF: cerebrospinal fluid, RS: rectal swab, TS: throat swab.

Appendix 4.2. Clinical characteristics and outcomes of patients with other causes of viral encephalitis confirmed by additional multiplex PCR testing

Characteristics	AdV encephalitis (n = 1)	EV encephalitis (n = 1)
Demographic features		
Female	Yes	No
Male	No	Yes
Age (years)	14	5
Ho Chi Minh city	No	No
Other provinces	Yes	Yes
Clinical features		
Illness days before admission	6	4
Fever	No	Yes
Highest temperature during hospitalisation (^oC)	–	39.5
Duration of fever (days)	–	6
Paediatric GCS	6	12
Seizure	Yes	Yes
Neurologic deficits	Yes	No
Abnormal muscular tone	No	No
Psychiatric symptoms	No	Yes
Cognitive dysfunction	No	Yes
Language changes	No	No
Delirium	No	No
Abnormal movement	No	No
Dyskinesia	No	No
CSF		
WBC count (cells/mm^3)	1	30
Protein (g/L)	0.2	0.7
Glucose (mmol/L)	5.7	4.5
CSF / Plasma glucose	1.1	0.7
CSF Lactate (mmol/L)	1.6	2.1
MRI findings		
Cerebral cortex	No	No
Limbic system	No	No
Thalamus	No	No
Mid-brain	No	No
Cerebellum	No	No
Brain stem	No	Yes
EEG abnormality		
Delta brush	No	No
Slow waves	No	No
Spike waves	No	No
Beta waves	No	No
Brain dysfunction	Yes	No
Seizure	No	No
Fast activity	No	No
Slow baseline activity	No	No
Low voltage	No	No
Treatment		

Methylprednisolone	Yes	No
Immunoglobulin	No	No
Cyclophosphamide	No	No
Rituximab	No	No
Acyclovir	Yes	Yes
Mannitol	Yes	Yes
Sodium chloride 3%	No	No
Outcomes		
Length of hospital stay (days)	12	10
Mortality	Survival	Survival
Sequelae	No	No
Modified Rankin scale (mRS)	0	0

Note: Data are presented as n (%) or median (IQR).
AdV: adenovirus, EV: enterovirus.

Appendix 5.1. List of 96 FR26RV-Endoh and FR20RV primer sequences

Primer	Sequence of primer (5'-3')	Primer	Sequence of primer (5'-3')	Primer	Sequence of primer(5'-3')
FR20RV	GCCGGAGCTCTGCAGATATC	33	GCCGGAGCTCTGCAGATATCTAGTCG	66	GCCGGAGCTCTGCAGATATCTAACGC
1	GCCGGAGCTCTGCAGATATCGATATC	34	GCCGGAGCTCTGCAGATATCGTAGAC	67	GCCGGAGCTCTGCAGATATCGGTCAT
2	GCCGGAGCTCTGCAGATATCTAGTAT	35	GCCGGAGCTCTGCAGATATCCTATAG	68	GCCGGAGCTCTGCAGATATCCTCATA
3	GCCGGAGCTCTGCAGATATCTATAGT	36	GCCGGAGCTCTGCAGATATCTAGCTA	69	GCCGGAGCTCTGCAGATATCAATTTG
4	GCCGGAGCTCTGCAGATATCTATATA	37	GCCGGAGCTCTGCAGATATCACTACT	70	GCCGGAGCTCTGCAGATATCCTGGTA
5	GCCGGAGCTCTGCAGATATCATACTA	38	GCCGGAGCTCTGCAGATATCTAACGA	71	GCCGGAGCTCTGCAGATATCTTCATG
6	GCCGGAGCTCTGCAGATATCATATAT	39	GCCGGAGCTCTGCAGATATCCGACTA	72	GCCGGAGCTCTGCAGATATCGCGATA
7	GCCGGAGCTCTGCAGATATCGTGCAC	40	GCCGGAGCTCTGCAGATATCTACTAG	73	GCCGGAGCTCTGCAGATATCACTAAG
8	GCCGGAGCTCTGCAGATATCACTATA	41	GCCGGAGCTCTGCAGATATCAGTAGT	74	GCCGGAGCTCTGCAGATATCGCATAC
9	GCCGGAGCTCTGCAGATATCCGTAAT	42	GCCGGAGCTCTGCAGATATCGTTAAC	75	GCCGGAGCTCTGCAGATATCCAATAT
10	GCCGGAGCTCTGCAGATATCCTATAC	43	GCCGGAGCTCTGCAGATATCGTCTAC	76	GCCGGAGCTCTGCAGATATCACCGTA
11	GCCGGAGCTCTGCAGATATCTATACG	44	GCCGGAGCTCTGCAGATATCTACAAG	77	GCCGGAGCTCTGCAGATATCGTGCTA
12	GCCGGAGCTCTGCAGATATCTATGCG	45	GCCGGAGCTCTGCAGATATCTACCAG	78	GCCGGAGCTCTGCAGATATCACGCTA
13	GCCGGAGCTCTGCAGATATCGATACT	46	GCCGGAGCTCTGCAGATATCTGGATT	79	GCCGGAGCTCTGCAGATATCATGTCG
14	GCCGGAGCTCTGCAGATATCCG	47	GCCGGAGCTCTGCAGATATCT	80	GCCGGAGCTCTGCAGATA

	TATA		CGTTA		TCAGCTTA
15	GCCGGAGCTCTGCAGATATCGTATAG	48	GCCGGAGCTCTGCAGATATCATAGTA	81	GCCGGAGCTCTGCAGATATCCGACAT
16	GCCGGAGCTCTGCAGATATCCGGTTA	49	GCCGGAGCTCTGCAGATATCATAGTC	82	GCCGGAGCTCTGCAGATATCGCTATA
17	GCCGGAGCTCTGCAGATATCAATAGT	50	GCCGGAGCTCTGCAGATATCCTAGTA	83	GCCGGAGCTCTGCAGATATCGCTATG
18	GCCGGAGCTCTGCAGATATCCGCATA	51	GCCGGAGCTCTGCAGATATCGTACTA	84	GCCGGAGCTCTGCAGATATCTGTAAG
19	GCCGGAGCTCTGCAGATATCATTACG	52	GCCGGAGCTCTGCAGATATCTAAGTT	85	GCCGGAGCTCTGCAGATATCAACTTA
20	GCCGGAGCTCTGCAGATATCTTAACA	53	GCCGGAGCTCTGCAGATATCATATCC	86	GCCGGAGCTCTGCAGATATCATAACG
21	GCCGGAGCTCTGCAGATATCAGTATC	54	GCCGGAGCTCTGCAGATATCTCGATA	87	GCCGGAGCTCTGCAGATATCATGTTA
22	GCCGGAGCTCTGCAGATATCTGTTAA	55	GCCGGAGCTCTGCAGATATCGTACCA	88	GCCGGAGCTCTGCAGATATCTGGTAT
23	GCCGGAGCTCTGCAGATATCACTATT	56	GCCGGAGCTCTGCAGATATCGTATCA	89	GCCGGAGCTCTGCAGATATCTGCGTA
24	GCCGGAGCTCTGCAGATATCTAACCG	57	GCCGGAGCTCTGCAGATATCATACTC	90	GCCGGAGCTCTGCAGATATCTCGGATAT
25	GCCGGAGCTCTGCAGATATCCGATAT	58	GCCGGAGCTCTGCAGATATCACATTA	91	GCCGGAGCTCTGCAGATATCTCCATAGC
26	GCCGGAGCTCTGCAGATATCGTATAC	59	GCCGGAGCTCTGCAGATATCATATTG	92	GCCGGAGCTCTGCAGATATCTCCATACT
27	GCCGGAGCTCTGCAGATATCAATCCA	60	GCCGGAGCTCTGCAGATATCCGTCTA	93	GCCGGAGCTCTGCAGATATCTCCGGATA
28	GCCGGAGCTCT	61	GCCGGAGCTC	94	GCCGGAGCT

	GCAGATATCTAGCAC		TGCAGATATCCTTAGT		CTGCAGATATCTTACTA
29	GCCGGAGCTCTGCAGATATCATATCG	62	GCCGGAGCTCTGCAGATATCCTTACA	95	GCCGGAGCTCTGCAGATATCACTCGT
30	GCCGGAGCTCTGCAGATATCAATATT	63	GCCGGAGCTCTGCAGATATCTTATGC	96	GCCGGAGCTCTGCAGATATCTAAGGT
31	GCCGGAGCTCTGCAGATATCTATAGC	64	GCCGGAGCTCTGCAGATATCATACGC		
32	GCCGGAGCTCTGCAGATATCCTTGTA	65	GCCGGAGCTCTGCAGATATCCGCTTA		

Appendix 6.2. Monthly minimum wages in four regions in Vietnam

Region	Provinces	Minimum wages per month (US$)
I	Ho Chi Minh, Binh Duong, Dong Nai, Vung Tau, Can Tho	201
II	Hue, Tay Ninh	179
III	Binh Thuan, Binh Phuoc, Long An, Tien Giang, An Giang, Kien Giang, Ca Mau, Ben Tre, Bac Lieu, Lam Dong, Khanh Hoa, Hau Giang, Vinh Long, Quang Ngai	156
IV	Tra Vinh, Soc Trang, Dak Nong, Dak Lak, Dong Thap	140

Appendix 6.3. Evaluation of productivity losses

Scenarios*	Monthly income (US$)	Reported working days per month (days)	Daily value of a lost day used to monetize the productivity losses (US$)
Scenario 1 (n = 96): For caregivers that reported losing paid employment, productivity losses were evaluated based on their reported monthly salary. The daily value of a lost day was calculated based dividing the monthly salary by the reported number of working days per month	344 (236–430)	26 (24–26)	14 (10–17)
Scenario 2 (n = 68): For caregivers that reported losing unpaid work, productivity losses were evaluated based on the minimum wage. The daily value of a lost day was calculated based dividing the minimum wage by the reported number of working days per month	156 (156–201)	26 (22–28)	7 (6–8)

Note: Abbreviation: IQR, interquartile range.
*All data are presented as median (IQR)

Appendix 6.4. Questionnaire for the cost of illness analysis

Cost of Illness **COST**

Complete this form where relevant while patient is still in the hospital, and finalize when the patient comes back for follow up. If the patient does not make the follow up as appointed, reach the patient's relative by phone and finalize the form. In case follow up is not needed (e.g. convalescent blood was collected), reach the patient's relative by phone on day 5-7 after discharge and finalize the form.

1. Who is the main informant?
   ○Patient ○Sibling ○ Mother ○ Father
   ○ Aunt or uncle ○Grandparent ○ Other, specify: _____

2. Information on carers spent time assisting the patient **during the child's illness**

Who are carers?	**About the carers normal activities**	**How much time missed from work spent** assisting the patient **BEFORE** hospit alization	**How much time missed from work spent** assisting the patient **DURING** hospit alization	**How much time missed from work spent** assisting the patient **AFTER** Hospitalization
☐Name:_____ ☐Relationship with the patient: _____	**What does he/she do?** ○Housework (unpaid) ○Subsistence farming (unpaid) ○Attending school/university ○Paid employment, specify: _____ ○Other, specify: _____ ○For paid employment only -What is his/her monthly average income? _____ -What is the number of working days per month? _____	○ 2 hours ○ ½ day ○ 1 day ○ 2 days ○ 3 days ○ 4 days ○>5 days, SPECIFY: [__|__] days	○ 2 hours ○ ½ day ○ 1 day ○ 2 days ○ 3 days ○ 4 days ○>5 days, SPECIFY: [__|__] days	○ 2 hours ○ ½ day ○ 1 day ○ 2 days ○ 3 days ○ 4 days ○>5 days, SPECIFY: [__|__] days
☐Name:_____ ☐Relationship with the patient: _____	**What does he/she do?** ○Housework (unpaid) ○Subsistence farming (unpaid) ○Attending school/university ○Paid employment, specify: _____ ○Other, specify: _____ ○For paid employment only -What is his/her monthly average income? _____ -What is the number of working days per month?	○ 2 hours ○ ½ day ○ 1 day ○ 2 days ○ 3 days ○ 4 days ○>5 days, SPECIFY: [__|__] days	○ 2 hours ○ ½ day ○ 1 day ○ 2 days ○ 3 days ○ 4 days ○>5 days, SPECIFY: [__|__] days	○ 2 hours ○ ½ day ○ 1 day ○ 2 days ○ 3 days ○ 4 days ○>5 days, SPECIFY: [__|__] days

1

☐Name:__________ ☐Relationship with the patient: ______________	**What does he/she do?** ○Housework (unpaid) ○Subsistence farming (unpaid) ○Attending school/university ○Paid employment, specify: _________ ○Other, specify: ______________ ○For paid employment only -What is his/her monthly average income? ______________ -What is the number of working days per month? __________________	○ 2 hours ○ ½ day ○ 1 day ○ 2 days ○ 3 days ○ 4 days ○>5 days, SPECIFY: [__|__] days	○ 2 hours ○ ½ day ○ 1 day ○ 2 days ○ 3 days ○ 4 days ○>5 days, SPECIFY: [__|__] days	○ 2 hours ○ ½ day ○ 1 day ○ 2 days ○ 3 days ○ 4 days ○>5 days, SPECIFY: [__|__] days
☐Name:__________ ☐Relationship with the patient: ______________	**What does he/she do?** ○Housework (unpaid) ○Subsistence farming (unpaid) ○Attending school/university ○Paid employment, specify: _________ ○Other, specify: ______________ ○For paid employment only -What is his/her monthly average income? ______________ -What is the number of working days per month? __________________	○ 2 hours ○ ½ day ○ 1 day ○ 2 days ○ 3 days ○ 4 days ○>5 days, SPECIFY: [__|__] days	○ 2 hours ○ ½ day ○ 1 day ○ 2 days ○ 3 days ○ 4 days ○>5 days, SPECIFY: [__|__] days	○ 2 hours ○ ½ day ○ 1 day ○ 2 days ○ 3 days ○ 4 days ○>5 days, SPECIFY: [__|__] days
☐Name:__________ ☐Relationship with the patient: ______________	**What does he/she do?** ○Housework (unpaid) ○Subsistence farming (unpaid) ○Attending school/university ○Paid employment, specify: _________ ○Other, specify: ______________ ○For paid employment only -What is his/her monthly average income? ______________ -What is the number of working days per month? __________________	○ 2 hours ○ ½ day ○ 1 day ○ 2 days ○ 3 days ○ 4 days ○>5 days, SPECIFY: [__|__] days	○ 2 hours ○ ½ day ○ 1 day ○ 2 days ○ 3 days ○ 4 days ○>5 days, SPECIFY: [__|__] days	○ 2 hours ○ ½ day ○ 1 day ○ 2 days ○ 3 days ○ 4 days ○>5 days, SPECIFY: [__|__] days

3. What have the carers done to treat this illness of the child **BEFORE HOSPITALIZATION**, and how much have they spent?

Indicate any of these actions in the first column of the table below and complete the form with appropriate information: do nothing (1), went to a pharmacy (2), went to a district health facility (3), went to another clinic (4), went to a hospital (5), consulted traditional healer (6), other, specify (7)

Action?	**Action #** (Indicate which happened 1st, 2nd, 3rd …as appropriate)	**Treatment cost**	**Transport cost**	**Hours spent**

4. What have the carers done to treat this illness of the child **AFTER HOSPITALIZATION**, and how much have they spent?

Indicate any of these actions in the first column of the table below and complete the form with appropriate information: do nothing (1), went to a pharmacy (2), went to a district health facility (3), went to another clinic (4), went to a hospital (5), consulted traditional healer (6), other, specify (7)

Action?	**Action #** (Indicate which happened 1st, 2nd, 3rd …as appropriate)	**Treatment cost (VND)**	**Transport cost (VND)**	**Hours spent (VND)**

5. Cost of the child's illness?

1. Currency: ○VND ○ Other, specify: _____
2. Cost spent on transportation to and from the hospital by all household members in total during hospitalization: _____
3. Total cost of hospitalization and additional outpatient visits (collected through hospital record): _____
4. Any other expenses during hospitalization related to care of the patient: _____