Childhood encephalitis in the United Kingdom – epidemiology, trends in hospital admissions and the role of intravenous immunoglobulin



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Abstract

The burden from childhood encephalitis is significant, 30-60% of affected children develop long term disability despite treatment. Crucial to improving outcomes are a better understanding of the disease epidemiology, and the identification of alternative treatment strategies.

To contribute to ongoing work currently addressing these issues globally, I conducted a 30-year review of childhood encephalitis hospital admissions in England, and also a 10-year review of paediatric intensive care unit (PICU) admissions in England and Wales (E&W). Through these projects, I defined the incidence of all-cause childhood encephalitis in England, and severe encephalitis in E&W. I described hospital admission trends for childhood encephalitis in England, and evaluated how these had varied with introduction of the combined measles, mumps, rubella vaccine. Furthermore, I estimated the potential cost burden from encephalitis PICU admissions, and identified factors associated with mortality and length of stay on PICU in children with severe encephalitis. This thesis provides high quality data from a Cochrane systematic review on the role of intravenous immunoglobulin (IVIG) treatment in childhood encephalitis, and describes the initiation of the first ever randomised controlled trial (RCT) of IVIG all-cause childhood encephalitis (IgNITE).

The incidence for all-cause childhood encephalitis in England was between 2.91/100,000/year (95%CI 2.80-3.14) and 4.02/100,000/year (3.80-4.28), and the incidence of severe childhood encephalitis in E&W was 0.79/100,000/year (0.74-0.84). An increasing trend in encephalitis admissions was observed between 1999-2011, compared to the previous years. This increase was most marked in infants, and mostly seen in the 'encephalitis of unknown aetiology' group. Measles and mumps encephalitis

admissions reduced by 30-fold after the two dose MMR vaccination schedule was introduced, compared with the pre MMR period. The PICU encephalitis bed cost was ~£414,000/year. The Cochrane review revealed paucity of RCTs of IVIG in encephalitis. Although the findings indicated some benefit from IVIG, the quality of the evidence was very low. Accordingly, I initiated the first ever RCT of IVIG treatment in all-cause childhood encephalitis, which had recruited 18 participants from 21 sites. Data from an interim analysis of this cohort showed that 60% were admitted to PICU, and 89% required invasive ventilation. Also, 80% of affected children had persisting symptoms at hospital discharge, 67% made poor recovery at 4-8 weeks after hospital discharge while 42% and 30% made poor recovery at 6 and 12 months follow up, respectively.

The increasing trend in childhood encephalitis admissions in the context of the ongoing threat from emerging and reemerging pathogens, indicates the need for continued disease surveillance. The findings of the systematic review rationalise the need for the IgNiTE study. The results of the interim analysis of data from the IgNiTE trial indicate the need to identify strategies to improve outcomes in children with encephalitis. It is anticipated that the IgNiTE trial would yield results that could alter the way that children with encephalitis are managed.

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Finally, I would like to dedicate this thesis to the memory of my brother, Clement Nnamdi, who would have been very proud.

Statement of responsibility

This thesis is based on a literature review, two epidemiological studies, a systematic review, and a multi-centre randomised controlled trial.

i. Literature review (Chapter 1)

The introduction literature review Chapter is my own work and has not been published elsewhere.

ii. Epidemiological studies

Encephalitis hospital admission trends in England (Chapter 2)

I generated the idea for, and initiated this project. I produced the list of the International Classification for Diseases Codes for encephalitis that were used in the study. The dataset was provided by Prof. Michael Goldacre and Mr Raph Goldcare. The data analyses, excluding the Poisson regression analyses, were performed by myself. Alecia Nickless performed the Poisson regression analyses, provided the graphs for these and verified the results of my analyses. Most of the work presented in Chapter 2 were published in The Lancet Infectious Diseases Journal in April 2017. I wrote the manuscript for the publication and prepared all the revisions that were required prior to publication. Dr Manish Sadarangani, Alecia Nickless, Prof. Michael Goldacre, Mr Raph Goldacre and Prof. Andrew Pollard were co-authors on the publication and contributed to the scientific and critical review of the manuscript. A further analysis of the data was undertaken by myself for this thesis. These new data are marked with a footnote, and have not been published elsewhere.

Encephalitis PICU admissions study (Chapter 3)

I generated the idea for this project. The dataset was provided by the Paediatric Intensive Care Unit Network. All the analyses were performed by myself and these (with the exception of the logistic regression analysis) were verified by Alecia Nickless who also performed the linear regression analysis.

iii. Systematic review (Chapter 4)

The idea to conduct this work came from Professor Pollard following which I identified an appropriate Cochrane Group to register the review. I designed the review protocol which was published in November 2014. The search strategy was designed by Nia Roberts of the Bodleian Library in Oxford. Search of the main databases was performed by the Cochrane Multiple Sclerosis and Rare Diseases of the CNS Group's trials search coordinator. Dr Natalie Martin and myself searched two additional databases for relevant articles.

I designed the screening template used for the review and both levels of article screening were performed by myself and Dr Natalie Martin. I designed the data extraction form. I extracted relevant data from the included studies and Dr Natalie Martin verified the extracted data. I wrote the results manuscript including the various revisions that were required prior to publication.

The protocol and publication of the review findings were co-authored by Dr Natalie Martin, Dr Michael Absoud and Professor Pollard who provided critical and scientific review of both the protocol and manuscript. All the data presented in this thesis Chapter were published in the Cochrane database of systematic reviews in October 2017.

iv. Immunoglobulin in the Treatment of Encephalitis (IgNiTE, Chapter 5)

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3 Abbreviations

ADEM	Acute demyelinating encephalomyelitis
ANOVA	Analysis of Variance
APC	Annual Percentage Change
APE	Acute Post-measles Encephalitis
AR	Admission rate
CEP	California Encephalitis Project
CI	Confidence Interval
CIDP	Chronic Inflammatory Demyelinating Polyradiculopathy
CNS	Central Nervous System
CSF	Cerebrospinal fluid
CT	Computed Tomography
CTP	Clinical Trials Pharmacy
DOIV	Duration of Invasive ventilation
DRE	Drug Resistant Epilepsy
EEG	Electroencephalogram
EudraCT	European Clinical Trials Database
EV	Enterovirus
GCP	Good Clinical Practice
GCS	Glasgow Coma Score
GMFCS	Gross Motor Function Classification System
GOSE-Peds	Glasgow outcome Score Extended -Pediatric version
HES	Hospital Episode Statistics
HFMD	Hand Foot and Mouth Disease
HIPE	Hospital Inpatient Enquiry
HPeV	Human Paraechovirus
HSCIC	Health and Social Care Information Centre
HSE	Herpes Simplex Encephalitis
HSV	Herpes Simplex Virus
ICD	International Classification of Diseases
ICH	International Conference on Harmonisation
ICU	Intensive Care Unit
IFN	Interferon
IgNiTE	Immunoglobulin in the Treatment of Encephalitis
IL	Interleukin
IMM	Immune mediated
IMP	Investigational Medicinal Product
IQR	Interquartile Range
IR	Incidence Rate
IVIG	Intravenous Immunoglobulin
JE	Japanese Encephalitis
LOHS	Length of Hospital Stay
LOS	Liverpool Outcome Score
LP	Lumbar Puncture
MACS	Manual Ability Classification System

MIBE	Measles inclusion Body Encephalitis
MMR	Measles, Mumps and Rubella
MRI	Magnetic Resonance Imaging
NHS	National Health System
NMDAR	N-Methyl-D-Aspartate Receptor
OCB	Oligoclonal Band
OR	Odds ratio
ORLS	Oxford Record Linkage Study
PCR	Polymerase Chain Reaction
PICANet	Paediatric Intensive Care Network
PICU	Paediatric Intensive Care Unit
PIM2	Paediatric Index of Mortality Version 2
PME	Primary Measles Encephalitis
RCT	Randomised Controlled Trial
SAE	Serious Adverse Event
SIV	Site Initiation Visit
SSPE	Subacute Sclerosing Panencephalitis
UHCE	Unit of Health-Care Epidemiology
UNS	Unspecified
USA	United States of America
VGKC	Voltage gated Potassium Channel
VZV	Varicella Zoster Virus
WCC	White Cell Count
WNV	West Nile Virus

1. Childhood encephalitis - an overview of the literature

Encephalitis is a serious neurological condition that occurs due to inflammation of the brain. Encephalitis manifests as a clinical syndrome characterised by encephalopathy, behavioral changes, fever, seizure and focal neurological deficits. The brain inflammation in encephalitis can either be isolated or can occur concurrently with inflammation of other parts of the central nervous system (CNS) including the meninges (meningoencephalitis) and spinal cord (encephalomyelitis).

The link between encephalopathy and acute infection or inflammation is prehistoric. In their work, Hippocrates and Galen observed that inflammation could be accompanied by delirium or 'phrenitis'.[1,2] Later on in the 17th century, Sydenham described a syndrome of coma accompanying fever (*febris comatose*), which in more recent times would be termed encephalitis.[3] Over the years, there has been a lot of progress made in terms of understanding the aetiology, biological mechanisms, epidemiology, and treatment of encephalitis.

1.1 Types of encephalitis

Encephalitis can occur either as a result of an infection (infectious encephalitis), or can be due to an immune process (autoimmune encephalitis and acute demyelinating encephalomyelitis). Across paediatric studies, a proven or suggested aetiological diagnosis is reported in 40-60% of all cases.[4-7] However despite extensive investigations, a specific cause is not identified in 40-60% of cases.[5,8-12]

1.1.1 Infectious encephalitis

Infection is the most commonly identified cause of encephalitis, and over 100 causative pathogens have been identified.[13] In a 2-year study of 203 adults and children with encephalitis in England, an infectious cause was identified in 40% of all cases.[14] Viruses are the most frequently identified pathogens and are reported to account for 20% [15,16] to 40% [9,17,18] of all cases across paediatric studies, although a higher rate of 65% was reported in one prospective study of 40 children conducted in Bangkok.[19]

In studies conducted in England[14] and the United States,[10,20,21] the leading definitive infectious causes of encephalitis are herpes simplex virus (HSV), varicella zoster virus (VZV), and enteroviruses. Enterovirus-71 particularly predominates in India and China.[6,22] In a 2 year prospective multi-centre study of 175 children in Finland, VZV, respiratory viruses and enteroviruses were the most commonly identified pathogens,[8] whereas measles and mumps were the most frequently identified agents in a Japanese study conducted between 1984 and 1990.[5] For the most part of the period covered in the Japanese study, only the single measles and rubella vaccines were being used, although rubella vaccination was not universal. Also, the Japanese study[5] was conducted at a time that coincided with a period of low measles and rubella vaccination uptake in the country, and included epidemic years. These factors could explain the relatively high prevalence of measles and rubella encephalitis in the study.

Arboviruses play a significant role in disease causation in the United States and Southeast Asia.[19,21] There is evolving evidence of an increasing number of viruses that can cause encephalitis.[23] While this might be due to improved diagnostics, a genuine rise in the number of pathogens causing disease needs to be considered.

1.1.1.1 Viral encephalitis

Common viral pathogens causing sporadic disease in the United Kingdom (UK)

HSV encephalitis (HSE)

Although first suggested to have a role in encephalitis by the Mathewson Commission in 1926, [24] it was not until 1941 that the first convincing case of HSE was reported following the identification of intranuclear inclusion bodies in the brain tissue of a 4-week-old with encephalitis taken at autopsy.[25] HSV is considered to be the most common cause of non-epidemic encephalitis. In a recent American study, the reported annual hospitalisation rate of HSE in children was 2.4 (standard deviation: ± 0.3) cases per million children and 10.3 (±2.2) cases/million in neonates.[26] Across paediatric encephalitis studies, HSV accounts for 2-10% of all cases,[4,9,11,12,15,17,27,28] although a higher prevalence of 20% of all cases was reported in a single centre French study.[29] In studies of all ages in England, HSV accounted for 55% of all viral encephalitis cases where a cause was identified,[30] and 19% of all encephalitis cases.[14] Both HSV-1 and HSV- 2 can cause encephalitis. Whereas >90% of HSE in children are due to HSV-1, the main causative pathogen in the neonatal period is HSV- 2.[31] The reported case fatality from HSE in children is 1.2%,[26] increasing to 7-15% in neonates. [26,32]

Enteroviral encephalitis (EVE)

Following the first reports of EVE in the 1950's, EVs have become increasingly identified as an important cause of encephalitis, and several of the over 100 serotypes have been implicated in encephalitis. Characteristically causing hand, foot and mouth disease in children, the reported prevalence of encephalitis among EV hospitalised patients is 13-17%.[33,34] In paediatric studies, EV accounts for 4-10% of all encephalitis cases,[9,15]

increasing to 20% in studies conducted in encephalitis endemic areas including in Kuwait, China and France.[6,18,29,35] In outbreak situations, the reported incidence is higher, Kumar *et al* detected EV ribonucleic acid (RNA) in 41% (37/90) cerebrospinal fluid (CSF) samples obtained during an outbreak in India.[36] In an American encephalitis study of all ages, EVE was either confirmed or suspected in 73/1571 (4.6%) patients, with a case fatality rate of 5.5%.[37] An Australian study of 61 children with EVE reported a case fatality of 7% during an outbreak of EV-71.[38] Variation in the distribution of EVs across different geographical regions has been observed. EV-71, a notoriously aggressive serotype which causes brainstem encephalitis in 60% of affected patients[39] is common in Southeast Asia and was responsible for the highly fatal rhomboencephalitis during a large epidemic in Taiwan.[40] In a prospective study conducted in India,[36] Coxsackie B virus was identified in over 50% of positive CSF samples obtained from children during an EV epidemic. Echovirus type 9 and type 30 were identified from 83% and 17% respectively of 103 paediatric encephalitis samples in a Kuwaiti study.[35]

Human Paraechovirus (HPeV) encephalitis

HPeVs are increasingly implicated in encephalitis.[41] First identified in 1956, HPeVs were originally known as echovirus 22 (HPeV-1) and echovirus 23 (HPeV-2) but were reclassified by phylogenetic analysis as a new genus.[42] There are currently 16 genotypes (HPeV1-16) for which complete genome sequencing is available.[43] HPeVs cause a spectrum of diseases including meningitis, encephalitis and acute flaccid paralysis, of which encephalitis is the most severe. HPeV3, discovered in 1999, is particularly neurotropic and is often isolated from the cerebrospinal fluid (CSF) of infants presenting with fever and possible sepsis. In an Australian study, HPeV accounted for 10% of 133 children (≤ 14 years) with suspected encephalitis.[44] Studies examining HPeV in CSF

samples of children with suspected CNS disease report a HPeV prevalence of 2-7%,[45-50] with most identification occurring in infants <3 months. One study of 149 children reported a case fatality of 6% case fatality from HPeV infection.[51]

Varicella zoster virus (VZV) encephalitis

In natural state, most cases of VZV infections occur in children <5 years.[52,53] VZV is a leading cause of infectious encephalitis, with an estimated incidence of 1.8 per 10,000 cases of varicella zoster infection.[54] Encephalitis occurs in 18-23% of children with severe complicated varicella infection. [55-58] Across paediatric studies, VZV accounted for 3-10% of all encephalitis cases,[5,7,9,15] although higher rates of approximately 30% were reported in Finland [8,11] and France.[29] In studies of all ages in England, VZV accounted for 13% of all viral encephalitis cases where a cause was identified,[30] and 5% of all encephalitis cases.[14] A Canadian study of 84 children reported a VZV encephalitis mortality rate of 18%.[57]

Measles encephalitis

Encephalitis is the most frequent neurological complication of measles infection and four disease presentations can occur – (i) primary measles encephalitis (PME), (ii) acute post-measles encephalitis (APE), (iii) measles inclusion body encephalitis (MIBE) and (iv) subacute sclerosing panencephalitis (SSPE).[59] Whereas PME and MIBE involve ongoing infection, APE and SSPE do not. APE, the most frequent neurological presentation, occurs as a demyelinating disease, usually 2-30 days post infection.[60] In children, the reported mortality from APE is 5%.[61] Overall, SSPE occurs in 1 in 25000 measles infections. The incidence in children is higher, with a reported risk in this group of approximately 1 in 5500.[62] Overall, measles infections, and associated complications, have reduced with the introduction of vaccination, although measles outbreaks have been

reported, particularly affecting previously unvaccinated individuals and those with incomplete vaccination history. In the UK, two measles outbreaks occurred in 2013 in the North West of England and in Swansea, affecting 376 and 664 individuals respectively.[63,64]

Mumps encephalitis

Like measles infections, the number of case reports of mumps infection have reduced drastically with vaccination. However, mumps outbreaks continue to occur and have recently been reported in the UK, [65,66] and the United States.[67,68] Waning immunity and genetic differences between the vaccine strain and wild type circulating virus have been suggested as reasons behind these outbreaks. Mumps encephalitis is estimated to occur in 1-2 per 10 000 cases of mumps infection,[69] increasing to 0.5-10% in outbreak situations,[70] and a male preponderance has been reported.[71,72] Mumps encephalitis is usually benign, death or major complications are uncommon, with a case-fatality rate of around 1.0%.[69]

A global perspective on viral encephalitis with emphasis on Japanese and tick-borne encephalitis

Arboviral encephalitis

Of the over 500-catalogued arboviruses, approximately 100 are known to cause disease in humans. Although arboviral infections are typically mild and asymptomatic, severe disease including encephalitis can occur. Both sporadic and epidemic cases of arboviral encephalitis have been described. Most arboviruses that cause encephalitis are arthropod borne, but non-arthropod-borne viral zoonotic pathogens can affect the CNS. These include lymphocytic choriomeningitis virus (transmitted by rodents), rabies virus (transmitted by terrestrial mammals or bats), and Nipah virus (transmitted by bats or

pigs). Arboviruses are present worldwide but have a predilection for the tropics due to the warm climate which allows for all year round transmission. Several arboviruses are known to cause encephalitis. Some of these occur globally e.g. Japanese encephalitis, Murray Valley, and West Nile virus, whereas others are typically found in certain regions e.g. Eastern equine, Western equine, St Louis, La Crosse in the United States.

Following its detection in the Western Hemisphere in 1999, West Nile virus (WNV) is the leading cause of domestically acquired neuroinvasive arboviral disease in the United States. [73]

Japanese encephalitis (JE)

Numerically speaking, JE is considered the most important global cause of encephalitis, affecting an estimated 67,900 people and causing approximately 15,000 deaths annually.[74] JE is the leading cause of encephalitis in Asia where it affects 20,000-50,000 cases each year.[75] It is the most common cause of preventable encephalitis in Asia, large parts of the Western Pacific, including the northern parts of Australia.[76] The reported risk of encephalitis in those infected by the JE virus is 1%.

First identified in 1871 in Japan,[77] JE is now endemic in 32 areas within 24 Asian and West Pacific countries, [74,78,79] Seventy-five percent of the estimated total number of cases occur in children (0-14 years), 81% in areas with developing or well established vaccination programs, and 18% in areas with little or no JE vaccination programmes.[74] Geographical differences in the incidence of JE in children have been reported (Table 1.1), possibly reflecting different surveillance methods and also age differences in the populations studied. A World Health Organisation (WHO) systematic review estimates an annual global JE incidence of 1.8 per 100,000 cases, increasing to 5.4 per 100,000 in children overall, [74] and 10.6/100,000 in children (<15 years) living in endemic areas with

no vaccination programme.[74] JE is endemic with seasonal distribution in parts of China, the Russian Federation's south-east, and South and South-East Asia.[80] In endemic countries, JE is primarily a disease of children since adults acquire immunity through previous infection.[76]

Following the first account of an outbreak of JE in Japan in 1924 in which 6000 cases were affected,[81] JE is now implicated in several major epidemics. More recently was the 2012 outbreak in Odisha, India, which occurred 20 years after the first outbreak, and affected 94 people.[82] Another was the 2014 outbreak in West Bengal, India in which the JEV was isolated from 134 of 398 (49%) listed acute encephalitis cases.[83]

The reported overall case fatality of JE ranges from 20-30% and 30-50% of survivors develop sequelae.[74] Although lower percentages of 19% and 15% for mortality and morbidity were reported in a randomised controlled trial (RCT) of JE in children.[84]

No cure exists for JE and the mainstay of prevention is vaccination. The first JE vaccine introduced in 1930 was withdrawn due to safety concerns. There are currently four different types of JE vaccines that are available: inactivated mouse brain-derived vaccines (first licensed in Japan in 1954), inactivated Vero cell vaccines (first licensed in China in 1998, Japan/USA/Europe in 2009), live attenuated SA 14-14-2 vaccines (first licensed in China in 1988), and a live recombinant (chimeric) vaccine (first licensed in Australia in 2010). Present JE vaccines are derived from the G3 JE virus, one of the five known genotypes (G1-G5). The most widely used JE vaccine IC51 (IXIARO in the US and Europe, JESPECT in Australia and New Zealand), is an inactivated vaccine made in Vero cells, with demonstrable safety and efficacy, and is licensed from 2 months of age in non-endemic settings. However, a major drawback with JE vaccines is the need for multiple doses which may not be achievable within short notice for travelers. Additionally, there

are concerns that the currently available vaccines derived from the G3 JE virus may not be protective against emerging JEV strains.[85]

Other potential prevention strategies for JE including chemical control of vector populations, and the vaccination of pigs have been suggested.[77]

Table 1.1 Reported country specific incidence for Japanese encephalitis, adapted from Wang et al 2015.[79]

Country	Existed of JE	Surveillance and JE age characteristics	Estimated JE incidence
Bangladesh	1977	Hospital based (4 sites/2 years) 22 patients, 2/3 < 15 years	0.6-2.7/100,000
Cambodia	1965	Hospital based (6 sites/2 years) 92% <12 years	11.1/100,000 in children < 15 years
India	1950	Hospital based, mainly children	15/100,000 in 5-9 years
Indonesia	1974	95% <15 years	8.2/100,000 in <10 year olds
Malaysia	1952	National, mainly children	4.3/100,000 <12 years old
Nepal	1978	Hospital based, mainly children	1.3/100,000

Key: JE=Japanese encephalitis

Tick borne encephalitis (TBE)

The first description of TBE like disease dates as far back as the 18th century. It was not until 1931 that the disease entity was described in Austria.[86] Six years later, the causative pathogen was identified in Russia. Among the neurotropic flaviviruses, the frequency of morbidity with TBE is second only to that from JE. TBE is a potentially fatal neurological infection, affecting people in Europe and Asia, with up to 3000-6000 treated hospital every year in Europe.[87] While an increase in incidence has been observed in Europe and Russia, perhaps due to an expanding tick population, some countries with an effective vaccination programme like Austria have seen a decline in the number of reported cases.[88] TBE is the most common viral tick borne disease in Europe, causing thousands of cases of neuroinvasive illness in humans across Europe and Asia, and is a growing public health concern.[89]

Emerging viral aetiologies

Encephalitis is considered a 'sentinel condition' for new and emerging infections. Although over 100 causes have been identified, there is mounting evidence of an increasing number of viruses that can cause disease in humans (Table 1.2). Mechanisms that have been suggested to propagate emerging viruses include changes in vector populations, in human association with reservoir hosts, and the appearance of new viral variants that are more efficiently transmitted. Additionally, acquisition of increased neurovirulence by a previously non-neurotropic virus can facilitate its entry to the CNS. Emergence of new viruses through these associations is exemplified by the introduction of West Nile Virus (WNV) into North America from the Middle East,[90,91] the spread of the JE virus into India, Nepal and northern Australia,[92] the outbreaks of Nipah virus

encephalitis in Bangladesh in 2001 and 2003[93] and the 2002 case report of the death of a bat conservationist in Scotland, UK due to the European bat lyssavirus.[94] Furthermore, are the isolation of West Nile Virus from a human sample in Serbia that clustered closely with a specific lineage previously identified in Greece,[95] and the identification of cases of Poswan virus encephalitis in Massachusetts and New Hampshire regions of the United States.[96]

Emerging viruses can cause encephalitis epidemics, with high fatality rates. One large encephalitis outbreak affecting children in Southern India that was associated with the Chandipura virus resulted in a case fatality of 52%.[97] Similarly, the Nipah Virus encephalitis outbreak that occurred in Malaysia in 1999, resulted in a case fatality of 40%.[98] The potential for these emerging viruses to cause large epidemics with high mortality and morbidity is a threat to global health security, poses significant health and economic burden, and highlights the crucial need for more effective surveillance systems, both at a national, regional and international level.

Table 1.2: Emerging viruses implicated in encephalitis (adapted from Granerod 2010) [99]

Infectious agent	First emerged	Subsequent spread
Nipah virus	1999 - Malaysia	2001 and 2003 - Bangladesh
Hendra virus	1994 - Brisbane, Australia	Queensland and New South Wales
Avian influenza virus A	2004 - Vietnam	
Australian bat lyssavirus	1996 - Australia	
Tick borne encephalitis	Czech Republic Estonia, Lithuania, Russia and Slovenia, Germany, Poland, Switzerland, Sweden, Finland, Slovakia, Hungary	Denmark, France, Greece, Italy, Norway and Turkey
Murray Valley encephalitis	1999 – Western hemisphere	Re-emerged as epidemics in the Murray-Darling River basin. Reappearance in central Australia and western New South Wales in 2000 -2001
West Nile Virus	Early 20th century- Australia's east coast	Across North America and as far south as Argentina
West Nile Virus (Kunjin Clade)	1960 - Northern Queensland, Australia	Across tropical northern Queensland, the Northern Territory and Western Australia

1.1.1.2 Bacterial encephalitis - the role of Mycoplasma pneumoniae

An association between *M. pneumoniae,* and CNS disease was first described in the 1950's,[100] although there is uncertainty regarding a direct role in disease causation, mainly because identification of live bacteria in the CSF is difficult. *M. pneumoniae* is frequently implicated as a cause of a spectrum of neurological diseases, of which encephalitis is the most common, occurring in 67% of 27 patients with serologically confirmed *M.pneumoniae* infection.[101] Several studies have reported a neurological presentation in 3-7% of patients with serological evidence of *M. pneumoniae,* [101-103] and positive *M. pneumoniae* serology in 2% of patients presenting with a neurological syndrome.[103] *M. pneumoniae* is increasingly being implicated in encephalitis and reports of identification of *M. pneumoniae* in brain tissue or CSF of children with encephalitis/meningoencephalitis have been consistent.[104-110] Notably, in the California encephalitis project (CEP) that was established in 1998 to study the epidemiology and aetiology of encephalitis, *M.pneumoniae* was the most commonly identified pathogen,[10] although clinician referral bias to the CEP might have influenced the study findings. In studies of acute encephalitis in children, *M. pneumoniae* accounted for 1-10% of all cases, [8,102-104,111] with a reported case fatality of 7-9%.[112,113] In one Finnish study of 462 children with encephalitis, the risk of death or severe damage in affected patients with *M.pneumoniae* encephalitis was 7 fold higher than for other aetiologies, being second only to HSE.[113] The mechanisms that result in the neurological presentations of *M. pneumoniae* are not fully understood. An underlying immune mediated mechanism consequent to the development of cross-reactive antibodies to the brain and other structures in the CNS following primary infection has been suggested.[114]

1.1.2 Immune mediated encephalitis

The immune forms of encephalitis include acute demyelinating encephalomyelitis (ADEM) and autoimmune encephalitis (AIE). ADEM is an acute demyelinating condition characterised by multiple white matter lesions on magnetic resonance imaging (MRI). ADEM can occur sporadically, but can follow an infection, or more rarely, vaccination. An association between ADEM and childhood exanthems was first recognised in the 18th century.[115] Studies in children report a preceding history of vaccination in 4-15% of cases[116-122] and of infection in 25-70% of cases, with a mean latency period of 4 to 13 days.[117-120,123,124]

The overall estimated incidence of vaccine associated ADEM is 0.1-0.2/100,000 vaccinated individuals,[125] although the reported incidence varies vaccine type. For ADEM associated with measles and human papilloma virus (HPV) vaccines, the reported incidence is 0.1/100,000[126] and 0.05/100,000[127] respectively. HPV vaccine associated ADEM is considered common following booster vaccination than after primary vaccination.[128,129] Despite reports of ADEM following vaccination, with the exception of the definitive association with older formulations of the rabies vaccine that are no longer in routine use,[130] direct causality between vaccination and ADEM has not been demonstrated in case control studies.

ADEM occurs frequently in prepubertal children, with a mean age of onset of 5 to 8 years.[116-118,121-124,131,132] The reported incidence of ADEM across paediatric studies is 0.1-0.6 per 100,000 children per year.[120,122,131-135] With increasing identification of ADEM cases due to the more widespread and routine use of magnetic resonance imaging (MRI), the incidence may be higher. Although generally thought to affect both sexes equally, a male predominance has been reported in several paediatric studies.[119,124,136-138] The clinical course of ADEM can either be monophasic or multiphasic. Multiphasic ADEM

is defined as two episodes consistent with ADEM occurring three months apart, in which the second episode can involve new or a re-emergence of prior neurological signs or MRI findings, but is not followed by any subsequent events.[139] Paediatric ADEM is typically monophasic, 90% of 84 children enrolled to a prospective single centre study conducted in Argentina showed a monophasic course.[124] Relapsing disease which has been described in 5-30% of cases in paediatric ADEM studies[75,118,138,140] poses a diagnostic challenge for clinicians given the difficulty sometimes in distinguishing this diagnosis from multiple sclerosis.

The pathogenesis of ADEM is not fully elucidated but it is postulated that myelin autoantigens share antigenic determinants with those of the infectious pathogen.[141] This is demonstrated by a 10 times higher frequency of T cell reactivity to myelin basic protein seen in patients with ADEM compared with non-ADEM encephalitis patients or normal controls.[142] Childhood ADEM is considered to be a relatively benign condition.[124] A study of 48 children with ADEM seen at a single centre in England reported 100% survival and full recovery in 57% of cases during a mean follow up period of 5.8 years.[117] Two further studies of 48 children in Kuwait[143] and 84 children in Argentina[124] report 76% and 89% complete recovery respectively when patients were followed up between 12 and 24 months after the illness[143] or at a mean follow up period of 6.6 years.[124] However, acute haemorrhagic leucoencephalitis, a rare and fulminant form of the disease can occur.[144,145]

AIE is characterised by the presence of autoantibodies that bind to neuronal synaptic receptors and ion-channel related proteins. These autoantibodies may be directed against intracellular, cell surface, or extracellular synaptic antigens, and could be paraneoplastic (i.e. directed against an occult tumor), or non-paraneoplastic.[146] Other triggers for the autoimmune process include infection or vaccination. However, in the majority of cases,

no trigger is identified.[146] Anti-N-methyl-D-Aspartate receptor encephalitis, a well described important subtype of AIE, [147,148] is a leading cause of AIE in children and adolescents. First described in 2005 in four young women with ovarian teratoma who presented with prominent psychiatric symptoms, memory loss, reduced consciousness and central hypoventilation,[87] the target antigen was soon discovered 2 years later.[149] Anti-NMDAR encephalitis is characterised by the presence of IgG antibodies directed against the extracellular domain of the GluN1 (also known as NR1) subunit of the N-Methyl-D-aspartic acid receptor (NMDAR), leading to internalisation and surface under expression of the receptor.[150-152]

In the UK, the reported incidence of paediatric anti-NMDAR encephalitis is 0.85 per million per year.[153] Anti-NMDAR encephalitis accounted for 4% of all encephalitis cases in a multicenter population based study of all ages in England,[14] with 40% of cases occurring in those ≤18 years. [20,154,155] In the CEP cohort, anti-NMDAR encephalitis was the leading diagnosis, identified >4 times as frequently as other viral aetiologies including HSV-1, VZV and West Nile virus.[20]

Another form of AIE that occurs in children is that due to antibodies to the voltage gated potassium channel (VGKC), typically presenting as limbic encephalitis with medial temporal lobe involvement on neuroimaging.[156-159]

In an retrospective single centre cohort study of 164 Australian children, in total, immune mediated/autoantibody associated encephalitis accounted for 34% of cases of which 21% were due to ADEM, and 6% and 4% due to anti-NMDAR and VGKC encephalitis respectively.[160] In an England study of all ages, an immune mediated cause was identified in 21% of 203 patients, of which 11% were due to ADEM, while 4% and 3% were due to anti-NMDAR and VGKC encephalitis respectively.[14]

1.2 Epidemiology of childhood encephalitis

The epidemiology of encephalitis is constantly changing. Although some pathogens like HSV, VZV and EV occur more globally, some regional differences are observed with others. Japanese encephalitis is common in Asia, tick borne encephalitis in Eastern and Northern Europe and Eastern Russia and Flaviviruses in Northern America. In a French prospective study,[161] bacteria including *Mycobacterium tuberculosis* and *Listeria monocytogenes* were the most frequently identified pathogens after HSV and VZV. Measles, mumps, rubella and varicella encephalitis are prevalent in areas without a routine vaccination programme against these pathogens or where the vaccine coverage is low. Tick borne encephalitis was the most frequently identified pathogen in a paediatric study in Sweden,[9] enterovirus in studies conducted in China,[6,18] Israel [162] and the United States,[163] mycoplasma in Toronto,[4] immune mediated/antibody associated encephalitis in Australia,[160,164] and mumps in Turkey.[17]

Predisposing factors

Exposure to insect and animal bites and travel to endemic areas are risk factors for arbovirus infections. *Listeria monocytogenes*, *Toxoplasma gondii* and *Cryptococcus neoformans* infections are common in the immunocompromised. Measles, mumps, and rubella encephalitis are more likely in children unvaccinated against these organisms. Ingestion of unpasteurised milk carries a high risk for infection with *L. monocytogenes* and *T. gondii.* A genetic predisposition to NMDAR encephalitis has been suggested, in their retrospective case series of 16 children with anti-NMDAR encephalitis, Jones *et al* observed a higher incidence and more severe disease in those of Māori and/or Pacific

Island ancestry.[165] Deficiency of intracellular proteins including toll like receptor 3 and UNC-93B is associated with an increased risk of HSE.[166-168] A genetic epidemiologic survey of childhood HSE found a high frequency (14%) of consanguinity in families of affected patients indicating a Mendelian predisposition to HSE.[169] HSE is a trigger for brain autoimmunity and high levels of anti-NMDAR antibodies have been detected in patients with relapsing HSE.[170-172] Similarly, anti-NMDAR antibodies were detected in the CSF of three children with JE relapse.[173] Despite these findings, a mediator of susceptibility is not identified in majority of encephalitis cases. These data suggest the need for further studies to improve understanding of both pathogen neurovirulence and host susceptibility.

Age and sex distribution

Encephalitis affects all age groups although the incidence is higher in infants and in the elderly.[8,30,174-177] A male preponderance is reported across several paediatric studies of all-encephalitis in Pakistan, China, Japan, Sweden, Turkey, England, Indonesia and the USA.[5,6,8,12,17,22,174,178,179] In one study, mumps and varicella encephalitis were more common in boys while adenovirus and *M. pneumonia* encephalitis were more common in girls.[180] A female predominance is reported for mycoplasma encephalitis,[181] anti-NMDAR encephalitis, [20,182,183] and HPeV3 encephalitis.[44]

Seasonality

Encephalitis occurs all year round but a seasonal pattern has been described, with some differences across studies. In a 20-year survey of 405 children from Helsinki, most encephalitis cases occurred between September and January, while the lowest number of cases presented between May and August.[184] In a Japanese study of 256 patients, an

increase in encephalitis cases was seen in late spring which is typically March to May,[5] whereas in a Canadian study, most cases were seen in winter months (November to February).[4] Most cases in an Australian study presented during the Australian winter season (June to August).[160] All tick-borne encephalitis cases in a Swedish study presented between May and August while all respiratory syncytial virus and influenza associated encephalitis admissions presented between November and February.[9] Contrary to the finding in the Swedish study, all 13 cases of influenza associated encephalitis in an Australian study occurred between May and October.[185] Although varicella infections are reported to be predominant in the spring and winter in temperate climates, Koskiniemi *et al* did not observe seasonality with varicella encephalitis admissions in their cohort.[180] Seasonality was not observed for *M. pneumoniae* encephalitis in studies conducted in Canada,[4] and Finland.[111,180] In a Canadian encephalitis study of mixed ages, an increased prevalence in arbovirus and unknown encephalitides was observed in summer and autumn.[186] Data from a Chinese study of enteroviral encephalitis suggested increased prevalence in summer and autumn whereas ADEM cases presented more commonly in winter and spring in studies conducted in Turkey,[136] Kuwait,[143] and the United States.[75]

Incidence of childhood encephalitis

There are several published reports on the incidence of encephalitis and these are summarised in Table 1.3. From the solely paediatric studies, the overall reported incidence of childhood encephalitis ranged from 3.3 to 10.5/100,000 children, [5,28,187] being highest overall in studies conducted in Finland.[8,11,180] The variation in the reported incidence in these studies possibly reflects geographical differences, and heterogeneity in the methods used in identifying cases. For example, in the Swedish study,[28] a stringent case definition was applied whereas the criteria used in two the Finnish studies[8,180] were

less strict. Also, while cases were identified either retrospectively using hospital discharge data in the Swedish study[28] or prospectively in the Finnish studies,[180,184] identification of cases in the Japan study[5] was solely based on paediatrician notification which is likely to be less specific. Thus, the lower incidence in the Japanese study of 3.3/100,000 child years could reflect an under reporting of cases. Furthermore, whereas the Japanese study[5] was conducted across multiple centres and is therefore likely to be more representative of the entire population, the studies in Finland[8] and Sweden[28] were conducted in a single centre and may not be as representative. Of note, only patients originating from the Helsinki area were included in one of the Finland studies.[180]

Epidemiologic studies comprising data from both adults and children have been conducted in England, France, USA, Canada and Italy and are summarised in Table 1.3. From these studies, the overall reported incidence for all-cause encephalitis across all ages ranged between 2.6 and 8.7/100,000 population. One consistent finding across these studies is a much higher incidence reported for infants.

To date, only two studies in England have investigated the incidence of encephalitis. One was a 10-year study of 6414 adults and children with viral encephalitis,[30] the other was a 2-year study of 203 adults and children with all-cause encephalitis.[174] Based on these studies, the incidence of viral encephalitis across all ages in England was estimated to be 1.5/100,000 population, and 2.8/100,000 child years in children,[30] while the incidence of all-cause encephalitis in England was estimated to be at best 5.23/100,000 population.[174] The incidence of all-cause childhood encephalitis in England is not known and cannot be derived from these studies since data for children in the studies of all-cause encephalitis are not presented separately.

Table 1.3 Summary of main studies reporting on the incidence of encephalitis

First author and year of study	Country	Type of study	Type of encephalitis/age	Reported incidence
Paediatric only studies				
Rantala H 1989[11]	Finland	Retrospective (1973 to 1987)	All encephalitis <16 years	8.8/100,000 child years (95% CI: 6.7-10.1)
Koskiniemi M 1991[180]	Finland	Retrospective (1968 to 1987)	All encephalitis (1 month to 16 years)	16.7/100,000 child years
Ishikawa T 1993[5]	Japan	Retrospective (1984 to 1990)	All encephalitis (<15 years)	3.3/100,000 child years
Koskiniemi M 1997[8]	Finland	Prospective (1993 to 1994)	All encephalitis (1 month to 15 years)	10.5/100,000 child years 18.4/100,000 child years for infants
Galanakis E 2009[188]	Greece	Prospective (2005 to 2007)	All encephalitis (1 month to 15 years)	4.5/100,000 child years
Wickström R 2017[28]	Sweden	Retrospective (1970 to 2009)	All encephalitis (1 month to 17 years)	6.4-8.7/100,000 child years
Britton P 2016[16]	Australia	Retrospective (2002 to 2012)	All encephalitis ≤ 14 years	3.8/100,000 child years (95%CI 3.6-4.0) [a] 5.0/100,000 (95% CI 4.6-5.4) [b]
Mixed population (adult and paediatric) studies				
Nicolosi A 1986 & Beghi E 1986[189]	United States	Retrospective (1950 to 1981)	Viral encephalitis	7.4/100,000 person years (95%CI: 6.3-8.5) 22.5/100,000 person years in infants
Khestsuriani N 2002[21]	United States	Retrospective (1988 to 1997)	All encephalitis	7.3/100,000 population
Davison KL 2003[30]	England	Retrospective (1989 to 1998)	Viral encephalitis	1.5/100,000 population 2.8/100,000 child years 8.7/100,000 for infants
Huppatz C 2009[190]	Australia	Retrospective (1990 to 2007)	All encephalitis	5.2/100,000 population (range: 4.2-6.7)
Barbadoro P 2012[177]	Italy	Retrospective (1999-2005)	All encephalitis	5.9/100,000 population 10.1/100,000 population in infants
Bernard S 2013[191]	France	Retrospective (2007)	All encephalitis	2.6/100,000 population
Granerod J 2013[174]	England	Mixed -prospective and retrospective (2005 to 2009)	All encephalitis	2.7-8.7/100,000/year, best estimate of 5.23/100,000 population
Kulkarni MA 2013[186]	Canada	Retrospective (1994 to 2008)	All encephalitis	5.4/100,000 population 10.3/100,000 population in infants
George BP 2014[176]	United States	Retrospective (2000 to 2010)	All encephalitis	7.6/100,000 population 13.5/100,000 in infants
Vora N 2014[192]	United States	Retrospective (1998 to 2010)	All encephalitis	6.9/100,000 population (95% CI: 6.8-7.1)

Key: [a] includes all cases identified by the primary diagnostic field only [b] includes all cases identified by any diagnostic field

1.3 Pathogenesis of acute viral encephalitis

The pathogenesis of encephalitis is not well understood and varies with aetiology. Viruses enter the body through different routes including the olfactory neurons (HSV), respiratory tract (mumps, measles), gastrointestinal tract (enteroviruses), or through a mosquito or tick bite (arboviruses). After entry into the host, it is supposed that the virus must gain access to the CNS to cause disease, although isolation of the suspected virus from the CNS is unusual for some viral aetiologies such as influenza, possibly indicating a parainfectious phenomenon.

Invasion of the CNS by the virus occurs through two main routes - an intraneuronal route (e.g. HSV, VZV, and rabies), or through haematogenous spread (e.g. EV, HPeV, and arboviruses). Viral entry to the CNS is facilitated by an increase in the permeability of the blood brain barrier that occurs during the infection. Once access is gained into the CNS, these viruses use cellular machinery for their replication and cause neuronal damage. It has been suggested that the neuronal damage that occurs can sometimes be a consequence of an unchecked inflammatory response of the host to the virus.

Some viruses show predilection for certain areas of the brain - the primary target for arboviruses is the cortical grey matter, brainstem and thalamic nuclei whereas HSV shows a predilection for the temporal lobes, orbital frontal lobes cortex and limbic structures, characteristically cause haemorrhagic and necrotising lesions in these areas.

1.4 Encephalitis diagnosis

Case definitions

No single clinical feature clearly differentiates encephalitis from other causes of encephalopathy. The World Health Organization (WHO) gives a broad definition of a clinical case of Acute Encephalitis Syndrome (AES) as any person presenting with acute onset of fever and a change in mental status (including signs and symptoms such as confusion, disorientation, coma, or inability to talk) and/ or new onset of seizures (excluding simple febrile seizures).[193] To enable some consistency and to facilitate epidemiological studies and clinical research into encephalitis, the International Encephalitis Consortium published a standardised case definition for encephalitis in 2013 (Table 1.4).[194]

Table 1.4 The International Encephalitis Consortium case definition adapted from Venkatesan et al[194]

Major Criterion (required in all cases) Altered mental state defined as decreased or altered level of consciousness, lethargy or personality change lasting ≥24 h with no alternative cause identified. Minor Criteria (2 required for possible encephalitis; ≥3 required for probable or confirmed encephalitis) • Documented fever ≥38°C (100.4°F) within the 72 h before or after presentation • Generalized or partial seizures not fully attributable to a pre-existing seizure disorder • New onset of focal neurologic findings • Cerebrospinal fluid (CSF) white cell count (WCC) ≥5/cubic mm • Abnormality of brain parenchyma on neuroimaging suggestive of encephalitis that is either new or appears acute in onset • Abnormality on electroencephalography that is consistent with encephalitis and not attributable to another cause

Key: WCC=white cell count

Similarly, the International Paediatric Multiple Sclerosis Study Group published diagnostic criteria for ADEM in 2007,[195] which was revised in 2013 (Table 1.5).[139] Case definition guideline for AIE is also available (Table 1.6).[196]

Table 1.5 International Paediatric Multiple Sclerosis Study Group criteria for ADEM. Adapted from Krupp et al [139]

- A first polyfocal, clinical CNS event with presumed inflammatory demyelinating cause
- Encephalopathy that cannot be explained by fever
- No new clinical and MRI findings emerge three months or more after the onset
- Brain MRI is abnormal during the acute (three month) phase.
- Typically, on brain MRI:
  - Diffuse, poorly demarcated, large (>1–2 cm) lesions involving predominantly the cerebral white matter
  - T1 hypointense lesions in the white matter are rare
  - Deep grey matter lesions (e.g. thalamus or basal ganglia) can be present

Key: ADEM-acute demyelinating encephalomyelitis, CNS=central nervous system, MRI=magnetic resonance imaging.

Table 1.6 Diagnostic criteria for possible AIE. Adapted from Graus et al [196]

Diagnosis can be made when all three of the following criteria have been met:

- Subacute onset (rapid progression of less than 3 months) of working memory deficits (short-term memory loss), altered mental status[&], or psychiatric symptoms 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*
- Reasonable exclusion of alternative causes

Key: [&]Altered mental status defined as decreased or altered level of consciousness, lethargy, or personality change. *Brain MRI hyperintense signal on T2-weighted fluid-attenuated inversion recovery sequences highly restricted to one or both medial temporal lobes (limbic encephalitis), or in multifocal areas involving grey matter, white matter, or both compatible with demyelination or inflammation, CNS=central nervous system, CSF=cerebrospinal fluid, MRI=magnetic resonance imaging.

Clinical features

The clinical manifestation of encephalitis can be variable and typically reflects the area of brain involvement. Clinical patterns are described for some aetiologies. A child with EV encephalitis characteristically presents with altered mental state, which may range from lethargy, irritability or mild disorientation to outright coma. A unique biphasic presentation of myoclonus, tremor, and ataxia that is typically preceded by hand-foot-mouth disease or herpangia, is seen with some enteroviruses particularly EV-71 and coxackievirus A16. HSE classically presents as fever, reduced consciousness, visual defects, dysphasia, focal motor seizures and hemiparesis. Whereas over 90% of adults

with HSE characteristically present with these symptoms, the clinical presentation in children can be atypical. Behavioral change and neuropsychiatric features are commonly seen in patients with anti-NMDAR encephalitis. Neurological features including movement disorders, cognitive regression, hypoventilation and autonomic features can indicate AIE.

Supportive evidence for an encephalitis diagnosis

Evidence of brain inflammation either on neuroimaging or CSF analysis are indicative of encephalitis. The CSF is abnormal in >90% of cases, typically consisting of a lymphocytic pleocytosis, mildly elevated protein, and normal glucose,[197] although the CSF can be normal early on in the illness. This is exemplified by the findings of a Canadian study in which CSF pleocytosis was absent in 41 % of children at the time of presentation,[4] and another French study of children with HSE in which CSF pleocytosis was absent in 20% of cases at the time of admission.[198] CSF pleocytosis is usually absent in HPeV encephalitis.[44]

Neuroimaging is essential in the evaluation of patients with suspected encephalitis. Computed tomography (CT) scan of the brain which is more readily available in some settings, is useful in excluding alternative diagnoses with similar presentation to encephalitis such as an intracranial space occupying lesion. A CT scan can also helpful in determining when it is safe to perform a lumbar puncture (LP). Brain MRI is the neuroimaging modality of choice and is more sensitive that a CT scan. MRI abnormality is reported in 20-50% of patients with anti NMDAR encephalitis,[155,182,199,200] and in the majority of ADEM cases with several studies reporting abnormality in all cases who had an MRI.[75,117,132] MRI findings can sometimes indicate a specific encephalitis diagnosis. Temporal lobe involvement is typically seen with HSV encephalitis. Multifocal, usually bilateral, but asymmetric and large hyperintense lesions, involving peripheral white and

gray matter demyelination is a classic finding in ADEM. Large vessel vasculitis can occur with VZV encephalitis.

An electroencephalogram (EEG) is particularly useful in demonstrating brain involvement in the early stages of encephalitis, especially in children for whom the diagnosis of a psychiatric or non-organic cause is suspected. An abnormal EEG is reported in 90-100% of patients with NMDAR encephalitis[151,155,182,199] and in 40-100% of children with ADEM[75,117,121,124,137] Diffuse arrhythmic and/or rhythmic delta slowing of the background activity is a usual finding in encephalitis. EEG findings can also suggest an aetiological diagnosis, periodic lateralising epileptiform discharges predominantly between 5 and 10 days of the illness and focal abnormalities can be seen with HSE. A unique 'extreme delta brush' pattern seen in a third of adult patients with NMDAR encephalitis,[201,202] is rarely seen in children,[182,203] although has been described in two cases been reported in two paediatric cases. [155,204]

Aetiological diagnosis

Identifying the specific cause in patients with encephalitis is an ongoing challenge. Knowledge of the aetiological diagnosis is essential to inform appropriate treatment. Traditionally, the diagnosis of infectious encephalitis relies on isolation of a pathogenic organism from the brain or CSF and this is considered robust evidence of direct disease causation. Brain biopsy is rarely performed and is not indicated in the initial management of immunocompetent children with suspected viral encephalitis in children. Brain biopsy may play a role in patients with encephalitis of unknown aetiology who deteriorate despite aciclovir treatment.

Viral culture

For several decades, viral culture was included in the standard protocol for diagnosing viral infections of the CNS but is now rarely done in routine microbiological laboratories. Viral cultures are labor intensive, have slow turnaround times, and lack enough sensitivity to make a significant impact on the acute management of a patient with suspected encephalitis. In addition, several factors including sample age and transport time can affect the yield. Also, some viruses require specialised culture systems that are may not be routinely available in all hospitals and /or require a lot of expertise. Further limitations are the reduced breadth of viruses that can be detected at one time, and the lack of standardisation. However, unlike for PCR testing, prior knowledge of the likely causative pathogen is not required, enabling isolation of a wide range of viruses. Viral culture may still have some role, particularly in situations where nucleic acid amplification tests are not available for the identification of rare but cultivable viruses including influenza, parainfluenza, measles and mumps.

Molecular methods

Based on the findings of two studies demonstrating high specificity and sensitivity of polymerase chain reaction (PCR) assay in biopsy proven HSE patients,[205,206] PCR testing of CSF is now the gold standard for virus identification in patients with suspected encephalitis. PCR has been used to detect HSV since the 1990s and over time, has been extended to other viruses. The clinical relevance of PCR testing was evaluated in 2233 consecutive samples obtained from 2162 patients in a laboratory in Oxford, UK. It was shown in that study that use of three primer sets sufficiently detected 95% of viruses known to cause CNS disease in the UK.[207] Additionally, the authors demonstrated that clinically diagnosed viral CNS disease was 88 times more likely in patients with positive

CSF PCR than those with a negative result. The use of PCR testing in HSE has been well studied.[206,208,209] The reported sensitivity and specificity of PCR testing in HSE are 96% and 99% respectively when CSF is tested between 48 hours and 10 days from symptom onset.[206,210] However, in early disease, the false negative rate of PCR testing can be as high as 10-30%.[198,211,212] In the UK, first line testing usually targets HSV-1 and 2, EV and VZV while extended testing, looking for less common pathogens is guided by history and known risk factors.[213] The advantages of PCR testing when compared with previous traditional methods like viral culture include improved diagnostic accuracy, more timely results, ability to detect multiple viruses in one test, and reduced cost.

Serological tests

Antibodies against viruses known to cause encephalitis can be detected in serum and CSF and are indicative of an aetiological diagnosis. Seroconversion of immunoglobulin M (IgM) or IgG, or a fourfold rise in antibody levels between an acute and convalescent sample, are supportive of an encephalitis diagnosis. Detection of higher levels of virus specific IgM antibodies in CSF than in serum is indicative of local production of antibody in response to an infection and can suggest the likely causative agent. Serology is the most useful assay for most arboviruses and is preferable to PCR since the peak viral load occurs before the onset of symptoms.[214] This is demonstrated in a study of West Nile virus neuroinvasive disease where sensitivity of CSF WNV (IgM) was higher compared with CSF PCR.[215] Seropositivity increases in the first week of the illness therefore repeat serological testing can be useful particularly where the initial results are negative but clinical suspicion is high. However, the requirement for antibody measurement in a convalescent sample to establish a diagnosis limits the usefulness of serological tests in

early diagnosis. Notably, IgM antibodies can persist for a long time following acute infection, and their presence does not necessarily indicate current infection.

Autoantibody testing

Detection of anti-neuronal antibodies in either serum and/or CSF is the mainstay for making a diagnosis of antibody-mediated encephalitis. The sensitivity of anti-NMDAR antibody testing is higher in CSF than serum. In one study, parallel identification of NMDAR antibodies in serum was lacking for 14% of patients with detectable antibodies in CSF, possibly due to the frequent intrathecal synthesis of antibodies in anti-NMDAR encephalitis, whereas the CSF was positive in all patients with a positive serum NMDAR antibody test.[216,217] The autoantibody test can be negative in patients with AIE due to a variety of possible reasons including antigen denaturation during tissue fixation, a small amount of Ab, differences between human and mouse epitopes, and the presence of T-cell-dominant AIE. [218]

Impact of current diagnostic methods on aetiological diagnosis

Increasing use of molecular testing has led to a seven-fold increase in laboratory reports of viral meningoencephalitis in E&W. Kadambari *et al* showed rapidly increasing diagnosis rates across all age groups and viruses tested including HSV, EV, VZV. [219] Wolthers *et al* demonstrated a 31% increase in viral detection with the use of HPeV specific PCR testing in children (< 5 years) presenting with CNS disease.[45] Similarly, Dupius and colleagues showed a 1.7-fold increase in the percentage of virus positive samples obtained from patients with suspected encephalitis or meningitis.[220] Identification of patients with ADEM and AIE is increasing with and the widespread use of MRI scans and the inclusion of autoantibody testing to the arsenal of diagnostic tools

for patients with suspected or confirmed encephalitis. Understanding the impact of these diagnostic advances is necessary to understand the burden of encephalitis, monitor and evaluate changes in incidence, particularly for specific forms of encephalitis and to inform future research and clinical practices.

Newer diagnostic methods

Failure to identify the specific cause in encephalitis patients impairs institution of appropriate treatment and is associated with increased mortality and morbidity. The compelling need to improve aetiological diagnosis in patients with encephalitis drives the impetus for ongoing research. Debatably, the most important recent aetiological development in the field of encephalitis is the identification of antibody-mediated forms of encephalitis. In the CEP, the frequency of NMDAR antibody encephalitis far surpassed that of individual viral aetiologies. However, despite extensive testing, including autoantibody testing, aetiological diagnosis remains a challenge and a specific cause remains unknown in approximately 40-60% of encephalitis cases.[5,8-12,14]

In the last decade, high throughput next generation sequencing (NGS) has emerged as a powerful tool to enhance understanding of the host pathogen interaction, and to unpick a large number of viral genomes using metagenomics.[221,222] The use of NGS also offers some promise in the identification of new and emerging infectious agents, [223-225] and has the potential to revolutionise the diagnosis of encephalitis.[226,227] Yet, the utility of this diagnostic approach especially in the current setting where specific therapies are lacking for majority of the infectious aetiologies is yet to be elucidated. In addition, the current turnaround times hamper their usefulness for diagnosis of encephalitis in the acute setting. Identification and characterisation of differentially expressed genes is an evolving methodology aimed at improving diagnosis in patients with a range of infectious

diseases,[228-230] although still an experimental exercise in the field of encephalitis.[231,232] Another suggested potentially useful strategy is the use of peptide libraries,[233] and also CSF cytokine profiling,[234,235] to identify the most likely antigenic targets for antibodies identified in the CSF of patients with encephalitis.

1.5 Prevention of encephalitis - impact of routine vaccination

One of the greatest medical achievements in the past century is the development of effective vaccines against a variety of infectious agents. The introduction of various vaccination programs globally has resulted in remarkable changes in the incidence of infectious diseases. Varicella associated diseases are less prevalent following the introduction of varicella vaccination programmes in the United States,[236] Taiwan,[237] and Germany,[58] and may account for the low number of varicella cases in the CEP.[10] Studies in Canada[57] and Australia[16] have demonstrated a lower incidence of VZV encephalitis following introduction of the varicella vaccine. Particularly, Science *et al* showed that the incidence of VZV related CNS complications were 2.8 times lower in the period after full vaccine coverage than the period preceding this.[57]

A reduction in the number of measles, mumps and rubella encephalitis cases following introduction of routine vaccination against these pathogens has been demonstrated particularly in countries like Finland,[8,11] and the Czech Republic.[238] While the effect of routine mumps vaccination on mumps associated complications including orchitis, meningitis and pancreatitis has been demonstrated in E&W,[239] there are no data specifically on the impact of vaccination on mumps, measles and rubella associated encephalitis hospitalisation rates in England. This information will contribute to a better understanding of the epidemiology of childhood encephalitis in England.

1.6 Treatment of encephalitis

The treatment of encephalitis should be guided by the aetiology however, a specific cause is not known in majority of cases at the time of presentation. Empiric treatment, usually broad-spectrum antimicrobials and antiviral agents that target common pathogens predominating in a particular region are instituted pending results of investigations, while taking into cognizance predisposing factors such as history of travel or immune compromise. In the UK, a national guideline was published in 2012, which provides evidence based recommendation on the diagnosis and treatment of children suspected to have viral encephalitis.[213] The only treatment that has shown efficacy in randomised controlled trials is aciclovir. Aciclovir is effective against herpes viruses especially HSV and is the current treatment of choice for HSE. In a randomised controlled trial (RCT) of 127 patients with HSE, the proportion of deaths in patients treated with aciclovir was 19% compared with 50% in those treated with vidarabine (p=0.04) and at 12 months follow up, 56% of patients treated with aciclovir had none or mild sequelae compared with 13% in the vidarabine group (p-0.002).[240] Another RCT also demonstrated efficacy with aciclovir treatment in HSE.[241] Since delay in commencing treatment is associated with poor outcomes,[242] aciclovir is usually started empirically in patients suspected to have encephalitis. However, mortality from HSE remains high at 30%, despite aciclovir treatment. The German trial assessing the efficacy of aciclovir and corticosteroids in the treatment of HSE is ongoing.[243]

Empiric antibiotic treatment with azithromycin or doxycycline is considered in *M. pneumoniae* encephalitis although there are no RCTs of antibiotic use for mycoplasma encephalitis. Oseltamivir is used in the treatment of influenza virus encephalitis although a definite beneficial effect in encephalitis has not been demonstrated in a RCT setting.

Pleconaril has been trialed in infants with EV meningitis[244] and neonates with EV sepsis,[245] but there are no data for encephalitis.

Based on the presumption of an autoimmune basis for the disease, first line treatment for ADEM comprises of an initial course of high dose intravenous methylprednisolone followed by a subsequent tapering course of oral steroids.[246] While there are no randomised controlled trials establishing efficacy, spontaneous improvement with corticosteroid therapy has been reported in ADEM studies.[247] Intravenous immunoglobulin (IVIG) and plasma exchange are considered in refractory cases of ADEM. IVIG, corticosteroid therapy and plasma exchange either given alone or in combination are used as first line immunotherapy for NMDAR encephalitis. Second line therapy includes other immunomodulatory treatment including cyclophosphamide and rituximab.

1.7 The aftermath of encephalitis

Outcomes for children affected by encephalitis have been described in several published studies. Across several paediatric encephalitis studies, the reported mortality rate is 2 - 10%, [16,17,113,248,249] A much higher mortality rate of 16% was reported in a study of children with viral encephalitis conducted in Pakistan[22] and might reflect differences in access to medical care and in the aetiological pathogens implicated in encephalitis in these areas. Individual studies report an overall long-term morbidity for 30-60%.[17,160,163,211,249-252] Persisting symptoms include physical, psychological, cognitive, behavioral and social impairments. In an Australian study of all-cause encephalitis comprising 164 children, an abnormal outcome (Liverpool Outcome score of 1-4) was observed in 49% of cases, of which 8% had severe disability, when assessed at a median follow up period of 5.8 years.[160] In the same study, the most common abnormal

outcomes by category were learning disability (28%), behavioural problems (24%), epilepsy (18%) and speech (17%).[160] In a 10-year Israeli of 46 children with all-cause encephalitis, 33% of affected children had focal motor deficit at discharge while 15% showed varying levels of cognitive impairment.[162] In the same study, after a mean follow up period of 5.8 years, 50% of affected children had persisting symptoms, including behavioural problems (52%), recurrent headache (22%), and problems with sleep (19%).[162] Additionally, a significantly higher prevalence of attention deficit/hyperactive disorder (ADHD) (50%) and learning disability (20%) occurred in affected children compared to the general population (5-10% and 10% respectively), and lower intelligence scores were observed even in those that appeared to have made full recovery at discharge.[162] Of 71 children with acute encephalitis in a Swedish study, 54% had persisting symptoms and even children who appeared to have made full recovery at discharge had persisting symptoms at follow up.[252] In a Turkish study of 36 children, mental and/or focal neurological deficits occurred in 53% of cases.[17] In a prospective Malaysian study of 118 children with JE, 8% died during the acute phase of the illness, while 60% exhibited neurological sequelae at hospital discharge – severe (31%), moderate (26%) and mild (3%).[253] In the same study, 38% of 34 patients who had apparent full recovery at discharge had varying degrees of neurological sequelae at follow up. Of the 48 patients who were of school age, 13% never returned to school due to severe physical disabilities from the illness, while 38% of the remaining 42 children had marked deterioration in school performance which resulted in discontinuation of schooling in 6 (38%) of the children. [253] In an Indian study of JE, 10% of 39 children had parkinsonian features at discharge and about 30% had residual symptoms at 14 month follow up.[254] Ten of eighteen children (56%) in a Greek study had neurological deficit at discharge while 29% of those assessed at a median follow up period of 4 years

still had mild to moderate deficits.[255] In a North Indian study of 168 adults and children admitted to intensive care, the mortality rate was 26% and 14% of survivors had some disability at 3 month follow up.[256] Rasmanchi and colleagues reported neurological sequelae including learning difficulties, developmental delay, behavioural problems and focal neurological deficit in 49/99 children with presumed encephalitis at a mean follow up period of 29 months. [257]

A meta-analysis comprising 890 children with infectious encephalitis from 15 studies showed that 10-20% of children with had abnormal behaviour, motor and cognitive impairment at follow up 1 to 12 years later while a third of patients had developmental delay.[258] A higher rate of ADHD is reported for children with EV-71 encephalitis than matched control subjects.[259] Following HSE, 40-65% of affected children develop seizures.[211] In an Australian study of 147 children followed up for a median duration of 7 years, post-encephalitic epilepsy and drug-resistant epilepsy were reported in 31 (21 %) and 15 (10 %) children respectively.[260] Notably, neurological sequelae do occur even in affected children who appear to have made full recovery at discharge.[162,252]

Can outcomes for children with encephalitis be predicted?

Attempts have been made to identify predictors of outcomes in children with encephalitis. An association between age and outcomes has been reported although the findings are conflicting - Rautonen and Kennedy reported an inverse association,[113,261] while Bhutto and Klein found no association between age and outcomes.[178] Aetiological diagnosis is a strong indicator of outcome. Higher rates of neurological sequelae are reported for children with HSE than for other aetiologies.[162,249] In a systematic review of long term outcomes of infectious encephalitis, the proportion of children affected by HSV encephalitis who had long term sequelae was 1.6 times higher than for those with other

infective causes.[258] In a Finnish study of 462 children with encephalitis, the risk of death or severe damage in children with HSV and Mycoplasma encephalitis were respectively 11 and 7-fold higher than the risk of death for other children in the study.[113] The findings of a single center Australian study showed that children with HSV encephalitis and encephalitis of unknown aetiology had worse outcomes while outcomes were best for those with ADEM.[160] The authors of a prospective cohort study in England of all-cause encephalitis survivors in all age groups, reported that for patients < 15 years, the worst quality of life was in patients in the unknown aetiology group.[262] A study of encephalitis across all age groups in England reported worse a higher case fatality of 30% in patients with Mycobacterium when compared to other identified infectious aetiologies[14] In the same study, patients with antibody associated encephalitis had the worst outcome with death or disability occurring in 56% of cases.[14]

A worse outcome is associated with a low Glasgow coma score on admission.[113,163,178,263] Du Bray *et al* showed that patients with a score of ≥7 had nearly 12 times greater odds of recovery at 1-year post discharge than patients with lower scores.[163] However, the authors of one study noted that the GCS was only useful in predicting poor outcomes when profoundly depressed.[178]

Several studies have shown an association between abnormal neuroimaging and poor outcomes.[178,251,252] Wang *et al* found that poor outcomes are more likely only in children with focal parenchymal hyper-intensity on MRI.[249] Pillai *et al* [160] showed increased odds of 2.5 with diffusion restriction on MRI whereas Klein *et al*[178] indicated any abnormality on neuroimaging to be predictive of a poor outcome.

Seizures at presentation is associated with poor outcomes. Wang *et al* reported that children with encephalitis who presented with multiple seizures or status epilepticus had a higher incidence of neurological sequelae than those who did not.[249] Bhutto *et al*

demonstrated that the odds of having a poor outcome increased 4 times with a history of seizures for more than 3 days.[22] In a single cohort study of 164 patients followed up for a mean duration of 5.8 years, the odds of having an abnormal long-term outcome was 3.8 times higher in patients who presented with in status epilepticus.[160] Early seizures during hospitalisation has been suggested as a strong predictor of post encephalitis epilepsy.[264-267] Pillai *et al* reported increased odds of developing drug resistant epilepsy (DRE) in affected children with the occurrence of any seizures (4 times), focal seizures or visual disturbance (6 times), status epilepticus (11 times), MRI abnormality involving the hippocampus/amygdala (5 times), MRI galadonium enhancement (4 times), and intensive care admission (5 times).[260] The association between drug resistant epilepsy and status epilepticus, focal seizures and EEG epileptiform discharges had also been reported in several other paediatric studies.[252,268-270] In the same study by Pillai and colleagues the proportion of participants with DRE varied according to aetiology and was 33% (3/9), 20% (8/40) and 11% (1/9) for HSE, encephalitis of unknown aetiology, and mycoplasma encephalitis respectively, none of the patients with ADEM (n=32), anti-NMDAR encephalitis (n-9) or EV encephalitis (n=18) developed DRE.[160]

Another consistent finding across studies reporting on outcomes of encephalitis in children is the association between abnormal neurological examination on admission to hospital and poor outcomes.[162,178] Klein and colleagues presented that children with focal neurological signs or an abnormal neurological examination on admission had respectively 16 and 3 times higher odds of having an abnormal neurologic examination at hospital discharge.[178]

In a Taiwan study of non-polio EV CNS infection comprising 333 EV culture positive cases of which 44 (13.2%) had a diagnosis of encephalitis, an unfavorable outcome (death or neurological sequelae) was associated with younger age (<4 years), high peak

leukocyte counts (>13 000/mm^3), seizures, skin rash, myoclonic jerks, lower CSF viral yield rate, and EV 71 infection.[33]

In an observational study of 577 adults and children with a diagnosis of anti-NMDAR encephalitis, predictors of good outcome were early treatment (OR 0·62, CI 0·50-0·76) and lack of ICU admission (OR 0.12, CI 0·06-0·22).[216]

Knowledge of what prognostic factors are important in encephalitis provides valuable insight to the biology and natural history of the disease, provides a platform for treatment strategies to be optimised and is useful for the design, conduct and analysis of clinical trials.

1.8 Financial and economic considerations

In England, the cost burden of encephalitis is estimated at £40 million per year which is likely to be an underestimate since additional costs such as those incurred from admission to intensive care were not considered in the cost analyses.[174] A national hospital discharge survey in the United States estimated the average cost of an encephalitis associated hospitalisation to be $28,000 giving an average annual national cost of $650 million for 230,000 hospital days during the study period.[21] The economic and functional impact of encephalitis is demonstrated in a study by Griffiths and colleagues who assessed children with acute encephalitis at 5-12 months after discharge from 2 Nepali hospitals.[271] Based on their findings, the median total 'out of pocket' cost to a family with an affected child is 5.4 times the median family monthly income while the median 'out of pocket' cost to the family of an affected child with severe or moderate functional impairment is 10 times the family income. Eighty-one percent of parents with an affected child miss work for a median duration of 15 days with a loss in income of US$38 (range 0-192). [271]

1.9 Strategies to improve outcomes from encephalitis

The poor outcomes despite treatment and the financial and economic burden due to encephalitis indicate the need to identify strategies to improve outcomes. Such strategies would include approaches aimed at optimising the timely aetiological diagnosis in patients with encephalitis to allow early institution of appropriate therapy, identifying what prognostic factors determine outcomes, and the use of alternative non-specific treatment such as immunoglobulin, which possess immunomodulatory properties and might improve outcomes, regardless of aetiology. Another strategy is the development of vaccines that could be used in prevention of the main pathogens implicated in encephalitis. Reports of aciclovir resistance are increasing indicating the need for continued surveillance.

1.10 Alternative treatment options for encephalitis

Immunoglobulin is a blood product consisting of pooled polyclonal IgG from thousands of blood donors. The first report of its use in treating infectious disease dates back to the 19th century when von Behring and Kitasato observed that immune sera could be used to treat diphtheria and tetanus.[272] This breakthrough and the subsequent demonstration that patients with Bruton's disease, an inherited severe form of aggamaglobulinaemia characterised by low blood antibody levels, responded well to treatment with human serum globulin, fostered the routine use of immunoglobulin both as replacement therapy and prophylactic treatment in patients with primary or acquired antibody deficiency disorders.

Immunoglobulin has potent anti-inflammatory and immunomodulatory activity. In view of the knowledge that inflammation plays an important role in determining morbidity

from encephalitis, intravenous immunoglobulin (IVIG) has been suggested as a possible treatment option for encephalitis.

1.10.1 IVIG evidence

There are several reports that allude to a beneficial effect from IVIG when used to treat patients with different forms of encephalitis.

1.10.1.1 IVIG in infectious encephalitis

Various case reports describing improvement within days following IVIG treatment in children with *M. pneumoniae* encephalitis have been published.[273-275] There are also reports of successful treatment with IVIG in immunocompromised patients with echovirus meningoencephalitis,[276] and in children with Japanese encephalitis.[277] However, Misbah *et al* reported a single case of chronic enteroviral meningoencephalitis in an 11-year-old who succumbed to the illness despite two courses of immunoglobulin treatment.[278] In a clinical observation of an outbreak of brainstem encephalitis in Catalonia, all but 3 of 34 children who received IVIG showed a good clinical course and had no significant sequelae.[279] Bhatt *et al* studied 83 children with acute viral encephalitis complicated by myocarditis and demonstrated a borderline significant lower rate of mortality and an improvement in ejection fraction in the those treated with IVIG compared with those treated with standard care alone.[280] In their retrospective studies, Ooi and Chang *et al* suggested a benefit of IVIG in patients with AES if treatment was instituted early.[253,281] However, an earlier Cincinnati review of 25 children and adults with measles encephalitis failed to show a beneficial effect from gamma globulin treatment.[282]

1.10.1.2 IVIG in AIE

In a retrospective case series of 20 children with anti-NMDAR encephalitis who received immunomodulatory treatment including IVIG, an 85% recovery rate was reported in a median follow up time of 17.5 months.[155] A 2-year study of 11 children with anti - NMDAR encephalitis conducted in North India showed that 58% of patients showed significant response to steroids and immunoglobulin. [283] In a prospective Indian study of 15 patients aged 2-64 years with AIE, 67% of patients who were treated with IVIG, steroids or both had no further seizures or clinical relapse years at follow up.[284] To date, the largest available evidence suggesting a positive effect of IVIG in anti-NMDAR encephalitis is from a Philadelphia multi-institutional observational cohort study in which 577 patients (211 children) were enrolled.[216] Of the 472 patients who underwent first-line immunotherapy (which included any combination of steroids, IVIG and plasmapheresis) or tumor removal, 53% had symptom improvement within 4 weeks. Good recovery i.e. mRS of 0-2 was observed in 221 patients at 3 months and in 97% of these at 24 months. Conversely, a case series of 10 Taiwanese children with limbic encephalitis showed that all enrolled patient had neuropsychiatric symptoms and 90% developed refractory epilepsy at a mean follow up of 5.6 years, despite receiving high dose corticosteroid or IVIG treatment.[285] Similarly, a retrospective case series of 10 children with limbic encephalitis, a form of AIE, failed to show benefit from IVIG treatment.[286]

1.10.1.3 IVIG in ADEM

A positive benefit from IVIG in patients with severe ADEM is suggested in several single case reports and case series.[287-291]

In a retrospective case series of 15 children with ADEM admitted to a single institution in Turkey, all 3 children who received IVIG following poor response to high dose methylprednisolone, recovered from neurological deficits.[292] A similar finding was reported in a second Turkish study of 15 children.[136] One study in Japan showed rapid recovery of consciousness and complete clinical improvement in 3 children with ADEM treated with IVIG.[293] Pradhan and colleagues reported on 4 patients with steroid refractory ADEM and also reported quick recovery following IVIG treatment.[294] A prospective observational study of 18 children with ADEM indicated that outcomes were better in children who received IVIG in the first week than those who did so in the second week of the illness.[295] The authors of a single centre Israeli study of 16 children with ADEM suggested that treatment with IVIG given separately or combined with high dose methylprednisolone may be effective in severe ADEM. [296]

An Australian study demonstrated an improvement in mRS scores in 11 children with infectious encephalitis, 8 children with anti-NMDAR encephalitis and 16 children with other immune mediated encephalitis.[203] In the same study, the proportion of children treated with IVIG who had a good outcome at follow up (mean duration of 52 months) increased from baseline by 88% and 11% for anti-NMDAR and other immune mediated encephalitis respectively.

Is the available evidence sufficient to change clinical practice?

The evidence from studies of IVIG in ADEM led to the recommendation for its routine use for the treatment of ADEM in Canada[297] and Finland.[298] Despite the mounting evidence indicating a beneficial role of IVIG, there is uncertainty among pediatricians in the UK regarding its use in the treatment of children with encephalitis. IVIG is used more readily in patients with confirmed or suspected autoantibody encephalitis and ADEM whereas in patients with an infectious form of encephalitis, clinicians would consider its use as a last resort for patients who are refractory to the usual standard of care. For example, a prospective surveillance study of children with NMDAR encephalitis in the UK identified that 9 of 31 children (29%) received IVIG treatment.[182] Anecdotal evidence from the UK suggests that in AIE, IVIG treatment is invariably used several weeks into the illness possibly due to the inevitable delays with obtaining results of autoantibody tests and/or those of more extensive tests conducted to exclude an infectious cause.

While IVIG is licensed for use in the treatment of other conditions like chronic inflammatory demyelinating polyradiculopathy (CIDP) that bear a similar pathogenesis to encephalitis, it is not a licensed for use in encephalitis. Nevertheless, encephalitis is a frequent indication for IVIG use in clinical practice. In a retrospective cohort study of children with encephalitis conducted in the USA, 17% received IVIG treatment.[299] The authors observed an increasing use of IVIG during the study period and suggested that this could be due to an increase in the identification of AIE cases. Similarly, Feasby *et al* saw a 115% increase in IVIG use over a 9-year period and this was mostly attributable to off-label use, [297] although the authors did not specifically report on usage in encephalitis patients. A retrospective chart review of 196 patients receiving IVIG treatment in a major Australian paediatric hospital during a 14-year period revealed that encephalitis was the

most common CNS indication for IVIG use followed by inflammatory CNS disorders in 24% and 15% of children respectively.[300]

Circumstantial evidence suggests an increasing use of IVIG as first line treatment in children with confirmed or suspected AIE earlier in the illness than was previously observed. This is likely reflective of the quicker turnaround times for autoantibody testing results, evidence from the studies like those presented above, knowledge of an immunological basis in AIE, and an increasing willingness by clinicians to use IVIG in encephalitis.

There are limitations in the studies described above that indicate benefit from IVIG treatment which affect generalisability of the findings and impact on translation of the results to routine clinical care. In particular, most of the evidence from IVIG use in infectious encephalitis are from single case reports which can be are flawed by the risk of reporting bias. The largest evidence of IVIG in infectious encephalitis is from a prospective study of children with viral encephalitis complicated by myocarditis conducted in India.[280] However, this was a non-randomised study in which the study investigators were aware of treatment allocation. Another limitation of this study is the small sample size of 83 patients. In addition, this study was conducted in a developing setting (India), which raises the question about generalisability of the findings to developed settings. The evidence from the studies of AIE, particularly that by Titulaer and colleagues [216] are suggestive of a role for IVIG in AIE. Nonetheless, most of the studies of IVIG in AIE are small sized retrospective case series and there are no randomised controlled trials of IVIG in patients with AIE or ADEM. Moreover, in the study by Titulaer *et al,*[216] a subgroup analysis to explore the effect of IVIG was not performed. All patients in the study received other immunomodulatory treatments in

combination with IVIG, which makes it impossible to determine the individual contribution of IVIG.

The rising use of IVIG in patients with encephalitis in the absence of robust evidence is not without cost implications. In a previously described single centre study of IVIG use in Australia,[300] the total cost of IVIG was US$ 2,595,907 (median $3538/patient, range $544-260,766). In the UK, IVIG costs £18-22 per gram, equivalent to £2100 to £3080 per 2g/kg for a 70kg patient.[301] Given the cost implications from IVIG use and the crucial need to improve outcomes from encephalitis, robust evidence of efficacy is imperative.

IVIG use in the UK

Guidelines to regulate IVIG use are in existence in several countries including Australia, North America, Europe and the UK. In the UK, a Demand Management Programme for IVIG was set up as a strategy to ensure consistent and evidence based use of IVIG. As part of this movement, clinical guidelines for IVIG use were developed and first implemented in 2008. According to these guidelines, ADEM and AIE are grey or weak indications indicating that the evidence supporting IVIG use in these conditions is lacking.[302] Thus, for ADEM and AIE, IVIG is not currently routinely commissioned by the National Health Service (NHS) England and its use in patients with these conditions is considered on a case-by-case basis.

1.10.2 How might IVIG work in encephalitis

Several potential mechanisms through which IVIG may exert a beneficial effect have been suggested. The most relevant actions of IVIG include inhibition of complement binding and prevention of membrane attack complex formation, neutralisation of certain pathogenic cytokines, regulation of autoantibodies or cytokines by anti-idiotypic or anticytokine antibodies, blockade of Fc gamma receptors on macrophages and

modulation of T-cell function and antigen recognition. Although these mechanisms are mutually non-exclusive, it has been suggested that only one of these is likely to predominate in a given disease condition. Reports on potential mechanisms in encephalitis are sparse. The first report on IVIG protection against HSE was by Erlich and colleagues, who demonstrated that IVIG was protective against death, and the protective effects were dose and time dependent.[303] Subsequent to this, through studying mouse models of HSE, a range of mechanisms of action of IVIG have been suggested including (i) alteration of the composition of leucocytes infiltrating the CNS, with more T cell infiltration, (ii) modulation of the activation of macrophages and microglia in the CNS, (iii) modulation of macrophage degranulation, (iv) alteration of Fc receptor expression on macrophages and monocytes, (iv) induction of regulatory CD4+T cells that control hyperinflammatory responses, and (v) prolongation of the integrity of the blood brain barrier. [304]

Another possible mechanism in encephalitis is an anti-inflammatory effect through modulation of cytokine production. Ramakrishna *et al* demonstrated in their in vitro study that the IVIG effect that was observed in a mouse model of HSE was modulated through IL-10.[304] Wang *et al* showed that in patients with EV-76 associated brainstem encephalitis complicated by pulmonary oedema, levels of interferon (IFN)-c, interleukin (IL)-6, IL-8, IL-10, and IL-13 levels significantly decreased in patients with pulmonary edema after IVIG administration.[305] Wang and colleagues also showed that plasma levels of IL-6 and IL-8 were significantly decreased after administration of IVIG in those patients with autonomic nervous system dysregulation. Rayamajhi *et al* showed an abundance of IL-4 and IL-6 in patients treated with IVIG than those treated with placebo, but the difference in cytokine levels before and after treatment was only significant for IL-4.[277]

Another suggested mechanism of action of IVIG in encephalitis is through the provision of neutralising antibodies. Although an earlier in vitro study suggested that IVIG protection against HSE was independent of HSV-1-neutralising antibodies,[304] an antibody neutralising effect has been demonstrated in other forms of infectious encephalitis. In their in vitro work, Konishi *et al* demonstrated that neutralising antibody prevented virus dissemination from the periphery to the CNS, and that antibody-mediated mechanisms of IVIG protection were more efficient than cytotoxic T cell responses.[306] In animal studies of WNV encephalitis, administration of IVIG containing anti-WNV specific antibody during the viraemic phase and before viral entry to the CNS resulted in a 100% survival rate.[307,308] Also, mortality was delayed for up to five days after infection in IVIG treated mice compared with control mice.[309,310] In their pilot RCT of IVIG in JE, Rayamajhi *et al* showed significantly higher levels of neutralising antibodies in children who were treated with IVIG compared with those treated with placebo. The authors observed that post treatment neutralising antibody levels were associated with JEV antibody positivity, being much higher, and significantly than pre-treatment levels in JEV antibody positive children than antibody negative children. Nonetheless, the predominating mechanism underlying the potential beneficial effect of IVIG in encephalitis is yet to be fully understood and is an area of ongoing research.

1.11 Aims of thesis

Within the broad context of childhood encephalitis presented above, this thesis aims to define the epidemiology of childhood encephalitis in England, to systematically appraise the role of IVIG in encephalitis and to establish the first ever randomised controlled trial of IVIG in childhood encephalitis.

The specific aims that this thesis will address are:

- To define the incidence of all-cause childhood encephalitis in England
- To describe a cohort of children with severe encephalitis in England and Wales (E&W)
- To determine the incidence of severe childhood encephalitis in E&W
- To investigate which factors are associated with outcomes for children with severe encephalitis
- To observe how trends in hospital admissions for childhood encephalitis in children in England have been influenced by routine use of the combined measles, mumps and rubella vaccine
- To conduct a systematic review of the evidence on IVIG in encephalitis
- To initiate and co-ordinate a multi-centre RCT of IVIG in childhood encephalitis

2. Hospital admission rates for childhood encephalitis in England, impact of improved diagnostics and vaccination

Encephalitis is a significant public health problem, due to the associated high rates of mortality and morbidity. The increasing identification of pathogens with the potential to cause encephalitis outbreaks that result in devastating outcomes makes encephalitis a public health priority, and indicates the fundamental need to fully understand its epidemiology. In order to do this, several parameters need to be closely examined including an appreciation of the incidence, prevalence and disease patterns. Crucial to understanding the epidemiology of encephalitis is the observation of long term trends in hospital admissions. Long term trend studies are particularly useful to evaluate the impact of public health interventions such as the introduction of vaccination programmes, which are less likely to be sufficiently characterised by studies conducted over a few years.

The epidemiology of all-cause childhood encephalitis in England is poorly understood. The two previous epidemiologic studies conducted in England have either involved only patients with a specific form of encephalitis e.g. viral encephalitis,[30] or have presented aggregate data from adults and children with all-cause encephalitis.[174] Although epidemiologic studies in children have been conducted worldwide as highlighted in Section 1.2 of this thesis, variation in the methods used to identify encephalitis cases in those studies, and the geographical differences in disease patterns limit the extent to which the data can be extrapolated to the paediatric population in England. With the provision of healthcare being high on the political agenda and the need for continued research to identify strategies to improve outcomes for children with encephalitis, it is now more important than ever to fully understand the epidemiology of childhood encephalitis in England. Studies investigating this will not only provide benchmark data against which comparisons can be made in the future, but will also aid further

understanding of disease patterns, delineate the burden of encephalitis on the NHS in England, inform on provision of services, and ultimately aid priority setting for future research into the prevention and treatment of encephalitis.

There have been improvements in the field of medicine in the past few decades. In particular, the introduction of routine vaccination against viruses that are implicated in encephalitis such as measles and mumps. The combined measles, mumps, rubella (MMR) vaccine was introduced in the England in the 1980's, initially as a single vaccine, then as single and two dose combined MMR vaccination schedules in 1988 and 1996 respectively. PCR testing for virus identification was introduced in the 1990's,[207] and is now the gold standard for identifying pathogens implicated in encephalitis, particularly HSV, VZV and EV. Furthermore, is the widened availability and more routine use of MRI in the investigation of patients with suspected neurological disease, and the development of a consensus definition for ADEM in 2007.[195] The effect that these improvements may have on the epidemiology of childhood encephalitis in England has not been studied.

In this study, I aimed to define the incidence of all-cause childhood encephalitis in England, to describe long-term trends in hospital admissions, and to evaluate how these have varied with the aforementioned innovations in the field.

Some of the work described here were published in the Lancet Infectious Diseases Journal[1] and are related to the accepted manuscript, as per Elsevier copyright agreement (2014) for personal use in a thesis. All new data are indicated by a footnote.

[1] Iro M.A *et al*. 30-year trends in admission rates for encephalitis in children in England and effect of improved diagnostics and measles-mumps-rubella-vaccination: a population based observational study. TLID 2017 April; 17(4): 422-430

2.1. Methods

Anonymised data[2] on encephalitis admissions (1979-2011) for individuals <20 years in England were obtained from two main national sources: (i) Hospital In-Patient Enquiry (HIPE, 1979-1985) and (ii) Hospital Episode Statistics (HES, 1990-2011). The HES data were available as individual-level records for each episode of care, from the Health and Social Care Information Centre (HSCIC) and its predecessor organisations. HIPE contained a random 10% sample of every NHS hospital admission in England, whereas HES was a complete dataset (100%). National data were not collected between 1986 and 1989. Therefore, to evaluate admission trends during this period, and to corroborate the findings of this study, data were also obtained from the Oxford Record Linkage Study (ORLS), a fully linked regional dataset from all NHS hospital admissions in the former Oxford NHS Regional Health Authority area. ORLS data were collected independently of HIPE and HES until 1998, after which the data are a subset of linked HES. The HSCIC was unable to provide HES data for 2012-2015. To account for out of hospital deaths, which are unlikely to feature in HES, data on any deaths from encephalitis that occurred without a corresponding HES admission record were obtained from the Office for National Statistics.[311] Admission records collected nationally between 1979 and 1998 were not linked. They only became linkable nationally from 1999 and this was done by exact matching on encrypted NHS numbers, and encrypted HES patient identification numbers. Match rates were considered to be very high and missing values for data items relevant to this study were very low.

[2] All data were provided by Raph and Michael Goldacre at the Unit of Health-Care Epidemiology, Nuffield Department of Population Health, University of Oxford

2.1.1. Definitions

An encephalitis admission was identified by the occurrence of an International Classification of Diseases (ICD) code for encephalitis in any diagnostic position in the hospital record. A list of codes used in this study is shown in Table 2.1.

A hospital episode is a period of continuous care under one hospital Consultant during an admission and there may be multiple episodes for one person in one admission period. Episode-based admission rates (ARs) were derived from all hospital episodes, regardless of the number of these per person, hereafter termed the "hospital AR". Availability of linkage data made it possible to also calculate person-based rates. The person-based (first record for each person) rate counts each person once within a calendar year and is the best available proxy for incidence using HES data, hereafter termed the "incidence rate" (IR). 'Encephalitis of unknown aetiology' was defined by subtracting specific encephalitis diagnosis ICD codes[3] (including ADEM) from 'all-cause encephalitis' codes.

[3] Specific diagnosis codes were defined as any code within ICD9 001-049.8, 050-138, 323.0-323.8 or ICD10 A00-A85, A87-B94.0, B95-B98, G04.0-G04.8, G36.1 (See Table 2.1)

Table 2.1 List of encephalitis codes by ICD period

	ICD-9 (1979-1994)	ICD-10 (1995-2011)
INFECTIOUS ENECPHALITIS		
Mumps encephalitis	072.2*	B26.2*
Meningococcal encephalitis	036.1*	A39.8
Herpes viral encephalitis	054.3*	B00.4*
TB encephalitis	013.6	A17.82
Listeria encephalitis	-	A32.1*
Cytomegalovirus encephalitis	078.5	B25.8
Rubella encephalitis	056.01	B06.01
HHV-6 encephalitis	058.21	B10.01
Other human herpes virus encephalitis	058.29	B10.09
Mosquito borne viral encephalitis	062.0-063.9*	A83-A84.9*
Congenital syphilitic encephalitis	090.41	A50.42
Late syphilitic encephalitis	094.81	A52.14
Toxoplasma encephalitis	130.0	B58.2*
Zoster encephalitis	053.10	B02.0*
Varicella encephalitis	-	B01.1*
Adenovirus encephalitis	049.8	A85.1*
Enteroviral encephalitis	049.8	A85.0*
Other specified viral encephalitis/ Infections of the central nervous system	049.8	A85.8*
Unspecified viral encephalitis	049.9*	A86*
IMMUNE MEDIATED ENCEPHALITIS		
ADEM	323.61	G04.0*
Encephalitis, myelitis and encephalomyelitis	323.0*	
POST INFECTIOUS		
Post varicella	052	B01.11*
Post measles	055.0*	B05.0*
Subacute sclerosing panencephalitis	046.2	A81.1*
Progressive multifocal leucoencephalopathy	046.3	A81.2*
Acute haemorrhagic leucoencephalitis	-	G36.1

Key: * indicates codes used in this study. All ICD 10 codes from G04.0-G05.9 and A85.0-A86.9 were included. ADEM =acute demyelinating encephalomyelitis.

2.1.2. Data analysis

For these data presented here, HIPE numbers are unscaled but the associated rates are scaled to the equivalent of 100%. Annual hospital ARs and their 95% confidence intervals (CIs) were calculated using mid-year population estimates from the Office for National Statistics. Summary age standardised admission rates and their 95% CIs were calculated from age-specific rates using the direct method of standardisation and the European standard population. Hospital ARs were calculated using data from 1979 to 2011 while person-based (incidence) rates were calculated using linked data from 1999 to 2011. Hospital ARs were compared across five different age groups (<1, 1-4, 5-9, 10-14, 15-19 years) using the one-way Kruskal-Wallis ANOVA test. The Mann-Whitney *U* test was used to test for differences in ARs between the two ICD periods covered in this study (ICD 9 and ICD 10). Annual percentage change (APC) was calculated by Poisson regression, where the APC = $(\exp(\beta) - 1) \times 100$ and β represented the model coefficient for year and an additional indicator variable was included to separate the two ICD periods[1]. To evaluate the impact of MMR vaccination, hospital ARs for measles and mumps encephalitis were compared across three periods: (i) 1979 to 1985: before the introduction of the single dose combined MMR schedule, (ii) 1990 to 1995: after introduction of the single-dose schedule in 1988, and (iii) 1997 to 2011: following introduction of a two-dose MMR schedule in 1996. To evaluate the impact of PCR testing on encephalitis admissions, hospital ARs for herpes viral encephalitis, encephalitis of unknown aetiology, and unspecified viral encephalitis were compared between two periods: before the introduction of PCR testing (1979 to 1989), and after its introduction

[1] Poisson regression analyses were performed by Alecia Nickless who also verified results of the analyses performed for the publication.

(1991 to 2011). The impact of PCR testing on enteroviral encephalitis hospital ARs could not be studied due to the absence of a sufficiently specific code for EV encephalitis in the ICD 9 period, which corresponds to the period before PCR testing was introduced. To avoid overestimation due to multiple episodes occurring for one person, person-based data were used to determine the percentage of all encephalitis admissions that were due to the individual aetiologies considered in this study. The median cumulative lengths of stay and their interquartile ranges were also calculated. Data were analysed using SAS (V.9.2, SAS Institute Inc, Cary, NC, USA), R (http://www.R-project.com), SPSS (V21) and Graph Pad 6.0, GraphPad Software, La Jolla California USA.

2.1.3. Data presentation

The numbers of encephalitis hospital episodes, persons admitted, hospital ARs and IRs are presented by age group category, gender, and aetiology or specific diagnosis (e.g. ADEM). Episode based admission rates were used to report on admission trends throughout the study period and to study the impact of vaccination and PCR testing, while incidence rates are presented only for the period between 1999 and 2011.

Admission rate trends and average hospital AR for encephalitis diagnoses with sufficiently specific codes in both coding periods and unlikely to have been affected by the transition in ICD codes during the study period (ADEM, herpes, measles, mumps), are reported as continuous data. The average hospital ARs for these were calculated using data from the entire period (1979-2011). Admission trends for all-cause encephalitis, encephalitis of unknown aetiology, and unspecified viral encephalitis are reported separately for the two ICD coding periods, to account for the transition in codes. Encephalitis diagnoses with sufficiently specific codes only in one of the ICD code periods (e.g. VZV, enterovirus) are presented for that period only.

2.2. Funding

This study had no specific funding. The National Institute for Health Research funded (grant RNC/035/02) works undertaken by the Unit of Health-Care Epidemiology (UHCE), Oxford in designing and building its multi-purpose, multi-study HIPE and HES datasets.

2.3. Ethical approval

Ethical approval for studying the record linkage datasets was obtained from the Central and South Bristol Multi-Centre Research Ethics Committee (04/Q2006/176).

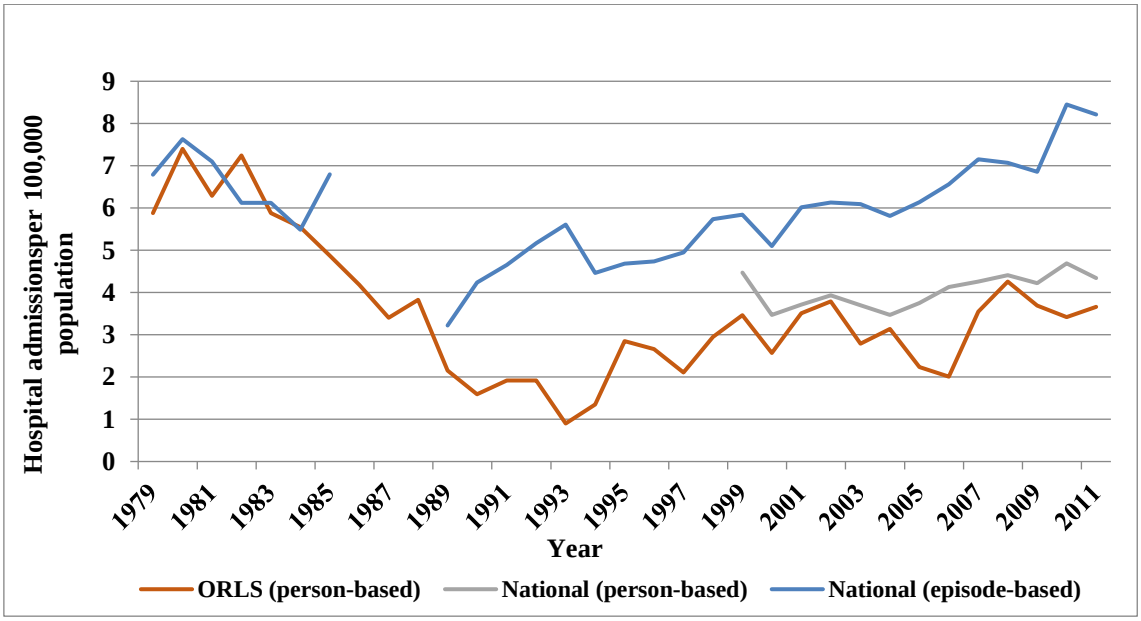
2.4. Results

2.4.1. All-cause encephalitis

In the entire study period (1979-2011), 16571 encephalitis hospital episodes occurred (males=8546, 52%). Hospital ARs varied annually (Figure 2.1) and differed between age groups ($p<0.0001$), being highest in infants (Figure 2.2). The average hospital AR (1979-2011) was 5.97/100,000/year (95%CI 5.52-6.41). Overall, hospital ARs declined during 1979-1985 (Figure 2.1), ranging (lowest to highest) from 5.49-7.63/100,000/year (average: 6.58/100,000/year; 95%CI 5.92-7.24) although an increase was observed from 6.30/100,000/year (1982) to 10.16/100,000/year (1983) for people aged 5-9 years, and from 5.05/100,000 (1983) to 16.76/100,000/year (1984) for those <1 year. The decline observed between 1979 and 1985 was sustained through the mid-1980s (ORLS data, Figure 2.1). Thereafter (1989-1994), hospitals ARs were 3.22-8.45/100,000/year (average: 5.78/100,000/year; 5.24-6.32).

Summary statistics for episode and person based rates are presented by age group and gender in Tables 2.2 and 2.3.

Figure 2.1 Hospital admission rates for all-cause encephalitis (0-19 years) in England



Key: Episode-based admission rate (AR) includes all episodes (from admission to discharge with multiple counting of people with multiple episodes), regardless of number of admissions. Person-based AR is based on the number of people admitted, and counts each person once (i.e. first known record for each person), allocating him or her to the year of the first-recorded admission. ORLS: Oxford Record Linkage Study.

Figure 2.2 Hospital admission rates for all-cause encephalitis in England by age group

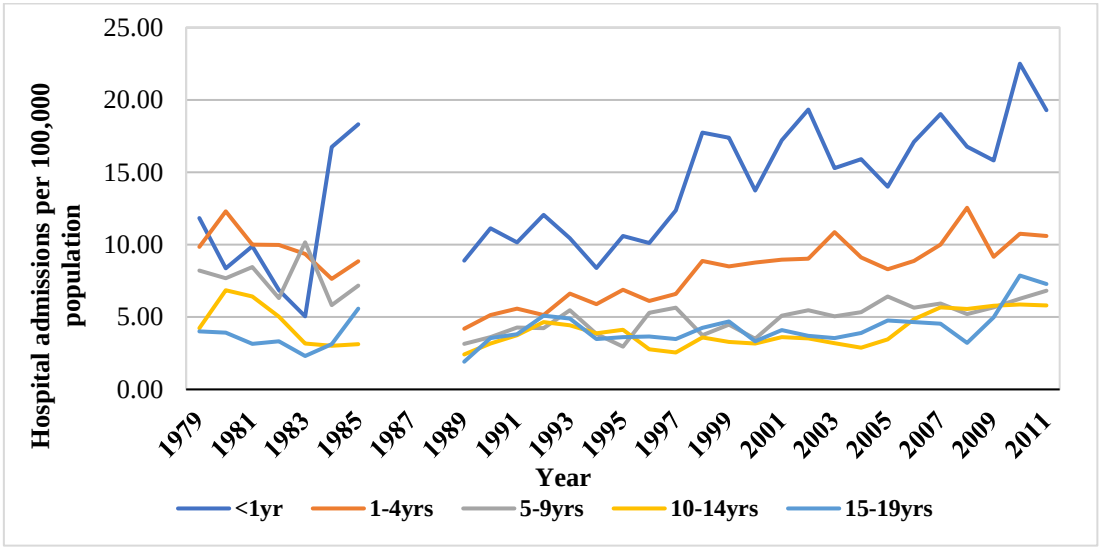


Table 2.2 Episode and person based counts and rates for all-cause encephalitis in England by age group [1]

Age group	Episode based count (1979-2011) n= 16571	Episode based rates per 100,000 population per year (1979-2011)	Person based count (1999-2011) n=6287	Person based rates per 100,000 population per year (1999-2011)
<1	2130 (12.9)	13.8 (12.1-15.4)	923 (14.7)	11.4 (10.4-12.5)
1-4	4736 (28.6)	8.5 (7.7-9.3)	1966 (31.3)	6.3 (5.9-6.6)
5-9	3604 (21.7)	5.6 (4.9-6.2)	1248 (19.9)	3.2 (3.0-3.4)
10-14	2931 (17.7)	4.1 (3.7-4.6)	1014 (16.1)	2.5 (2.3-2.7)
15-19	3170 (19.1)	4.1 (3.7-4.6)	1136 (18.1)	2.7 (2.4-3.0)

Table 2.3 Episode and person based counts and rates for all-cause encephalitis in England by gender [1]

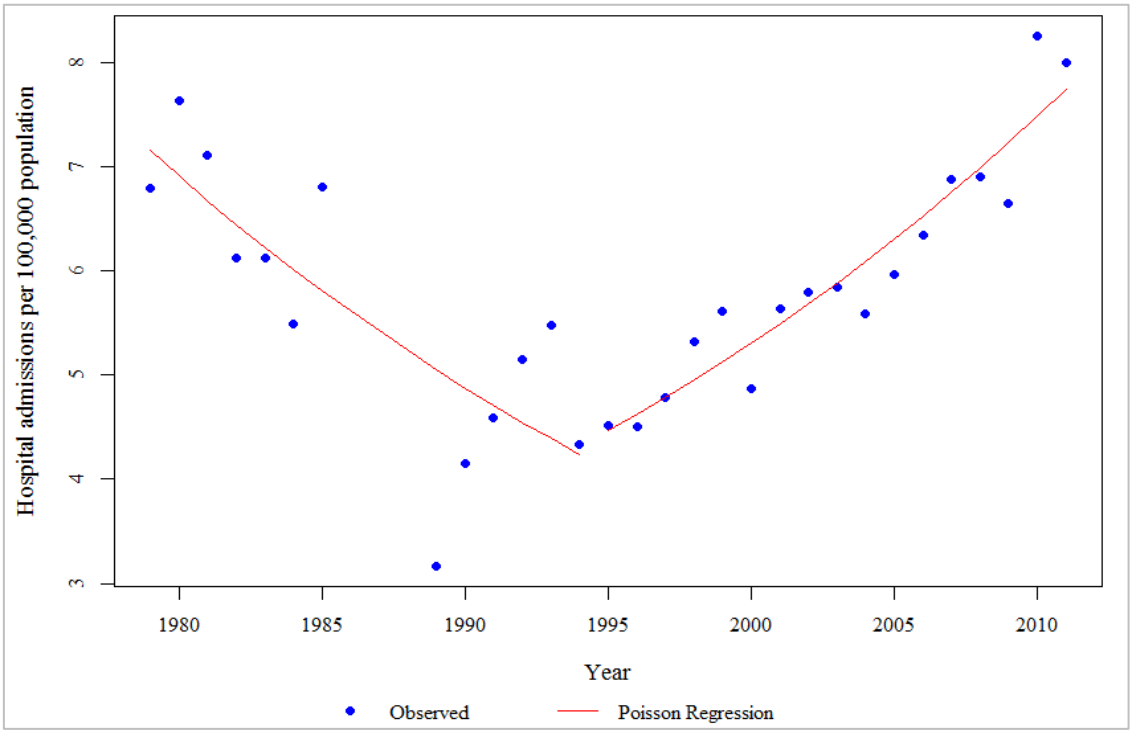
Males				
Age group	Episode based count (1979-2011) n= 8546[a]	Episode based rates per 100,000 population (1979-2011)	Person based count (1999-2011) n=3300[a]	Person based rates per 100,000 population per year (1999-2011)
<1	1177 (13.8)	14.1 (11.6-16.7)	528 (16.0)	12.8 (11.3-14.2)
1-4	2413 (28.2)	8.6 (7.7-9.4)	993 (30.0)	6.2 (5.7-6.7)
5-9	2012 (23.5)	6.3 (5.5-7.2)	691 (21.0)	3.5 (3.1-3.8)
10-14	1549 (18.1)	4.3 (3.8-4.9)	540 (16.4)	2.6 (2.2-3.0)
15-19	1395 (16.3)	3.8 (3.7-4.2)	548 (16.6)	2.6 (2.3-2.8)
Overall (**male**)	8546 (51.6)	6.2 (5.6-6.7)	3300 (52.5)	4.1 (3.9-4.4)
Females				
Age group	Episode based count (1979-2011) n= 7990[a]	Episode based rates per 100,000 population (1979-2011)	People based counts (1999-2011) n=2980[a]	Person based rates per 100,000 population per year (1999-2011)
<1	951 (11.9)	13.3 (11.6-15.0)	395 (13.3)	10.0 (8.8-11.3)
1-4	2313 (28.9)	8.3 (7.4-9.3)	969 (32.5)	6.3 (5.8-6.8)
5-9	1585 (19.8)	4.8 (4.0-5.5)	555 (18.6)	2.9 (2.7-3.1)
10-14	1376 (17.2)	3.9 (3.4-4.5)	474 (15.9)	2.4 (2.1-2.6)
15-19	1765 (22.1)	4.5 (3.8-5.2)	587 (19.7)	2.9 (2.5-3.2)
Overall (**female**)	7990 (48.2)	5.7 (5.2-6.3)	2980 (47.4)	3.9 (3.7-4.2)

Key: Episode-based counts and admission rate (AR) are based on all episodes (from admission to discharge with multiple counting of people with multiple episodes), regardless of number of admissions. Person-based counts and AR are based on the number of people admitted, with each person counted once (i.e. first known record for each person), allocating him or her to the year of the first-recorded admission. a=data on gender were not available for 35 episodes (1979-2011) and for 7 people (1999-2011)

[1] New data

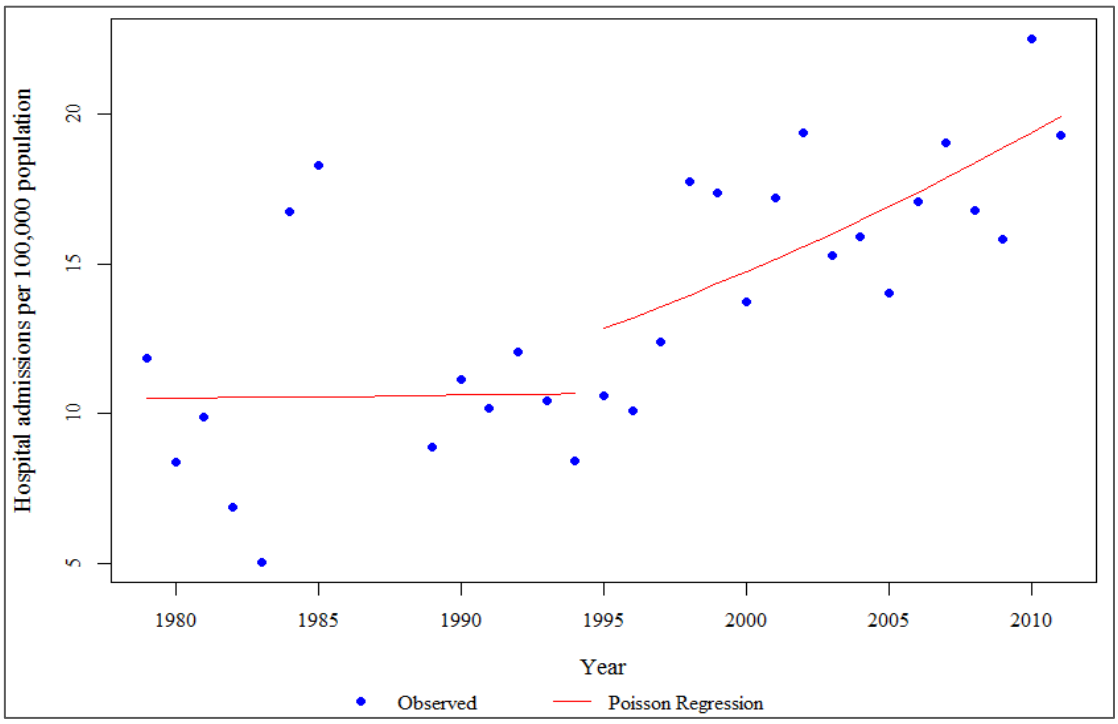
In the ICD 9 coding period (1979 to 1994), there were 3892 episodes (males=2108, 54%), which gave an average hospital AR of 5.65/100,000/year (95%CI 4.87-6.42). An overall decline in hospital ARs was observed in this period (ICD9 period; APC =-3.30%; -2.88% to -3.66%; p<0.0001) (Figure 2.3). In the ICD10 coding period (1995 to 2011), 12679 encephalitis hospital episodes occurred (average hospital AR 6.21/100,000/year; 95%CI 5.65-6.77). Hospital ARs increased (APC=3.30%; 2.75%-3.85%; p<0.0001) (Figure 2.3) from 4.68/100,000 (95%CI 4.30-5.07) in 1995 to 8.21/100,000 (7.71-8.71) in 2011, representing a 1.8-fold increase during this period. The increase was wholly in the 0-4 years age group, was most marked in infants (APC=2.79%; 2.05%-3.54%; p <0.0001) (Figure 2.4), and mostly seen in the 'encephalitis of unknown aetiology' group, but was also observed for ADEM and 'other encephalitis (Figure 2.5).

Figure 2.3 Poisson regression model for all-encephalitis admissions (0-19 years) in England [2]



[2] Poisson regression graph provided by Alecia Nickless

Figure 2.4 Poisson regression model for all-cause encephalitis admissions (<1 year olds) in England [2]

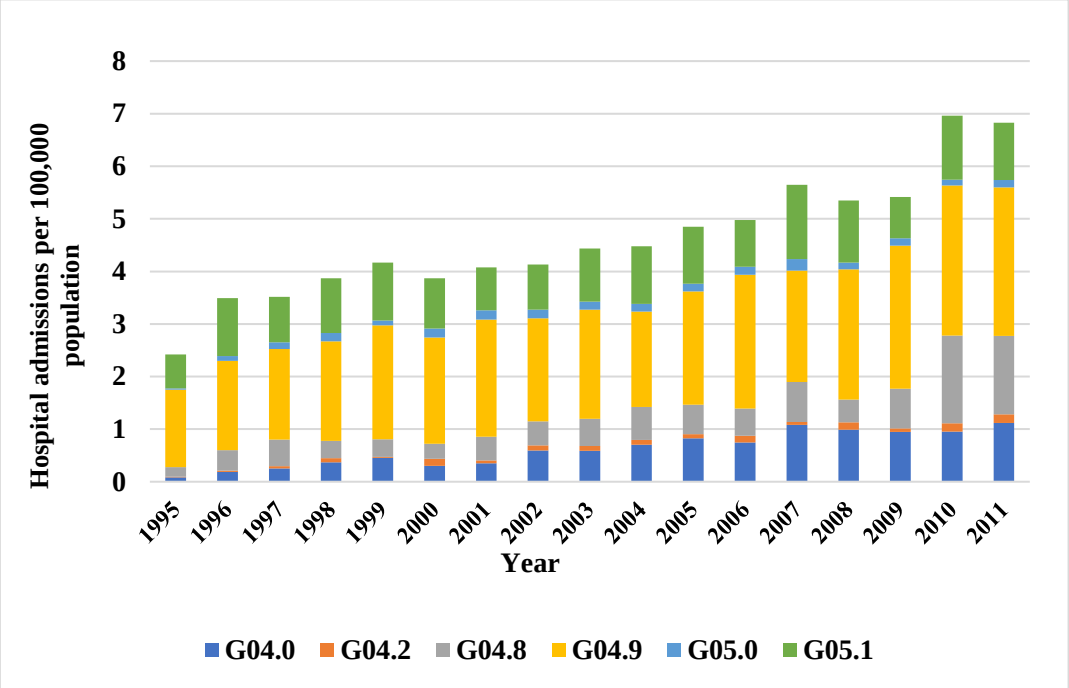


From 1999-2011, when admissions data became linkable, there were 10254 encephalitis admissions for 6287 different individuals (0-19 years). In this period, IRs ranged between 3.47/100,000/year (95%CI 3.13-3.81) and 4.69/100,000/year (4.31-5.08), giving an average IR of 4.02/100,000/year (3.80-4.28). A sustained increase in IRs was observed between 1999 and 2011 (APC=3.41%; 2.81%-4.01%; p<0.0001) (Figure 2.6). Excluding person-based admissions with a cumulative length of hospital stay <5 days (n=1439), the upward trend persisted (Figure 2.6) with IRs between 2.54/100,000/year (2.25-2.83) and 3.45/100,000/year (3.12-3.78), giving a recalculated average IR of 2.91/100,000/year (2.80-3.14). There were 122 death certificates mentioning encephalitis, without a corresponding HES record for encephalitis, identified between 1999 and 2011. These 122

[2] Poisson regression graph provided by Alecia Nickless

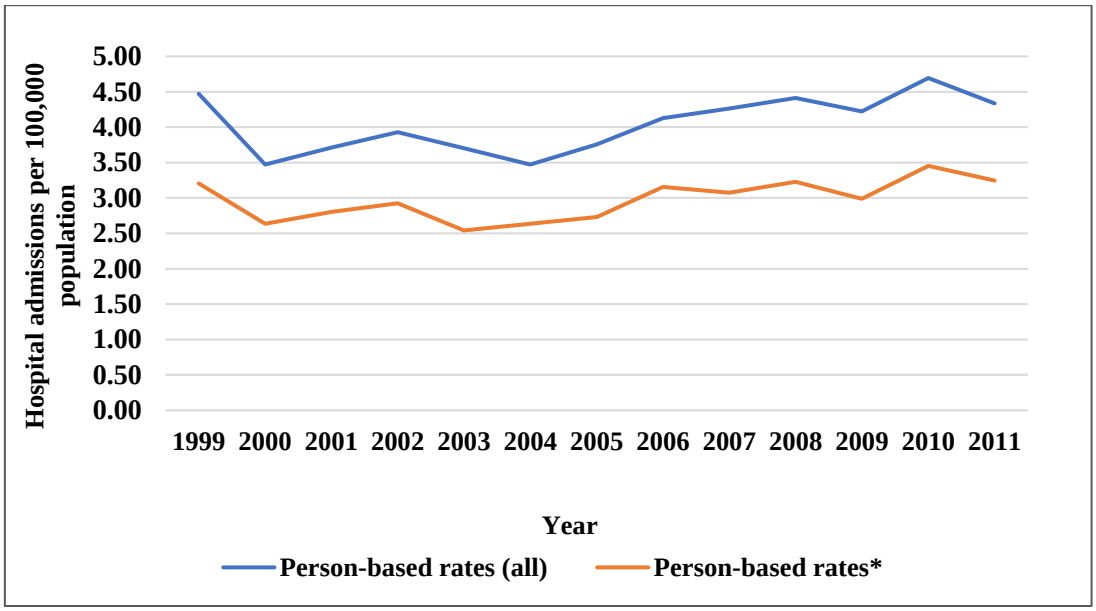
deaths add 1.9% to the 6286 people admitted with encephalitis between 1999 and 2011, with no up or downward trend over time.

Figure 2.5 Hospital admission rates (1995-2011) by diagnosis category for people aged 0-19 years in England [1]



Key: G04.0 - acute disseminated encephalomyelitis; G04.2 - bacterial meninoncephalitis and meningomtelitis, not classified elsewhere; G04.8 - Other encephalitis, myelitis and encephalomyelitis; G04.9 - Encephalitis, myelitis and encephalomyelitis, unspecified; G05.0 - Encephalitis, myelitis and encephalomyelitis in bacterial diseases classified elsewhere; G05.1 - Encephalitis, myelitis and encephalomyelitis in viral diseases classified elsewhere.

Figure 2.6 Person based rates for all encephalitis for people aged 0-19 years in England



Key: Person-based rate = incidence rate. * Excludes admissions with short cumulative length of hospital stay (<5 days), including deaths.

2.4.2. Vaccine preventable aetiologies

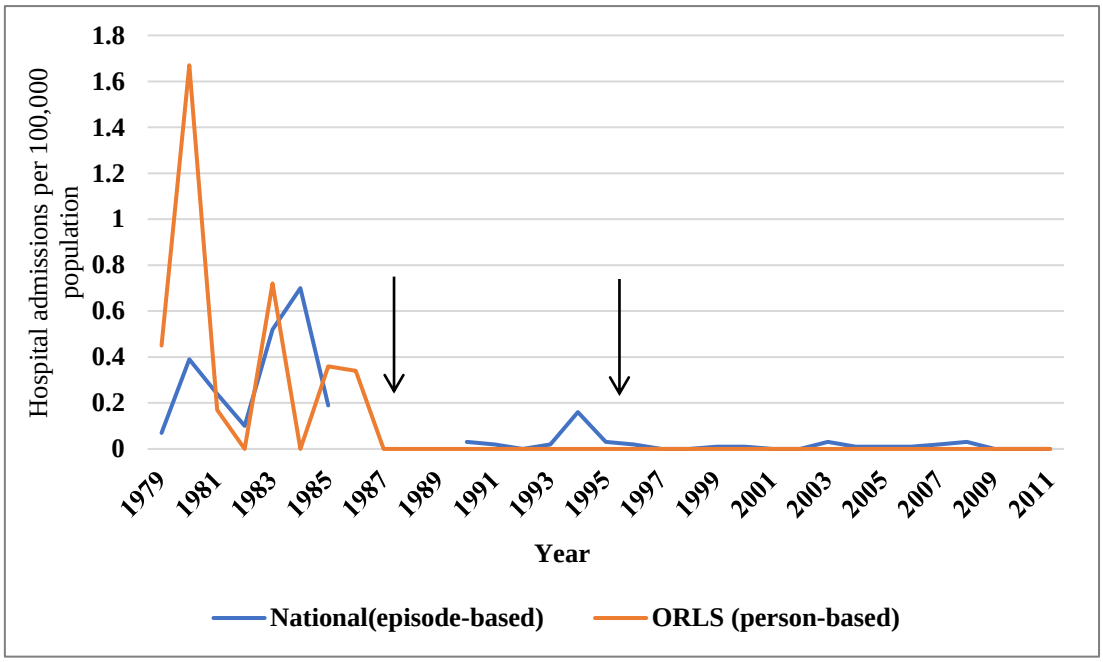
Measles encephalitis

In the study period before the single-dose MMR was introduced (1979-1985), measles encephalitis admissions peaked every 3 years (Figure 2.7), with an average hospital AR of 0.32/100,000/year (95%CI 0.10-0.53) in this period. Thereafter (1990-1995), the average hospital AR fell 8-fold (0.04/100,000/year; 0.00 to 0.10).

Following introduction of the 2-dose MMR vaccination schedule in 1996, the average hospital AR (1997-2011) was 0.009/100,000/year (95%CI 0.003-0.01), corresponding to a 35-fold and 4-fold decrease when compared with before and after the introduction of a single-dose MMR schedule. The post-MMR decrease in hospital ARs was observed across all age groups (Figure 2.8). Between 1999 and 2011 there were 15 measles encephalitis admissions for 9 individual people, giving an incidence rate of measles

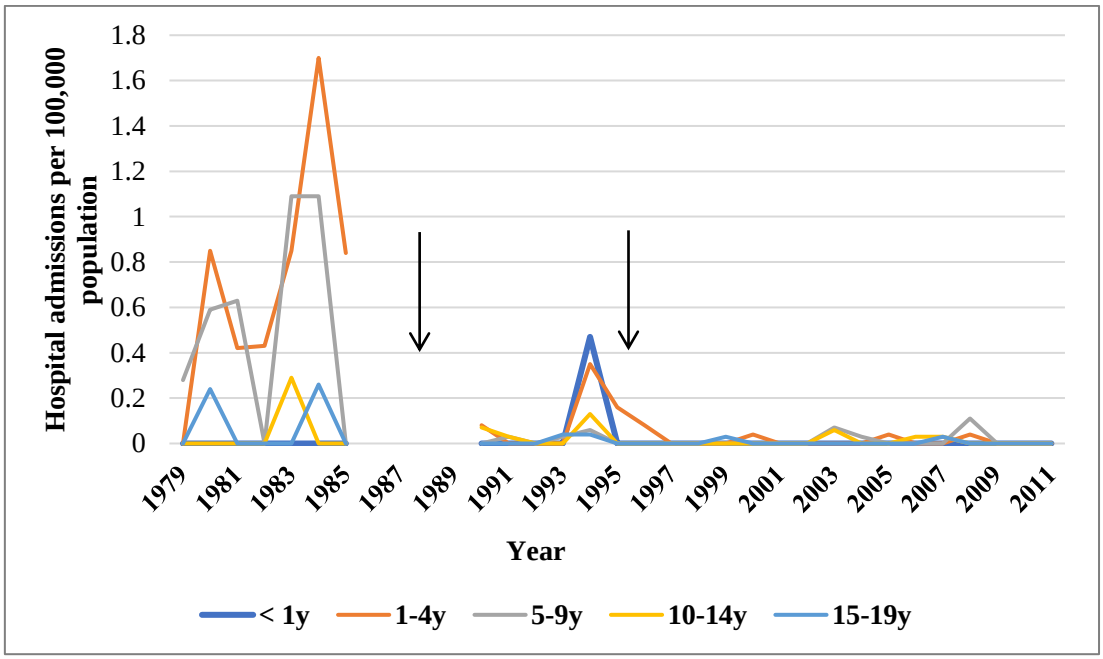
encephalitis for people aged 0-19 years in England (1999-2011) of 0.01/100,000/year (95%CI 0.001-0.12).

Figure 2.7 Hospital admissions for measles encephalitis (0-19 years) in England



Key: Arrows show introduction of the single and 2 dose MMR vaccination schedules in 1988 and 1996 respectively.

Figure 2.8 Hospital admission rates for measles encephalitis (0-19 years) in England by age group

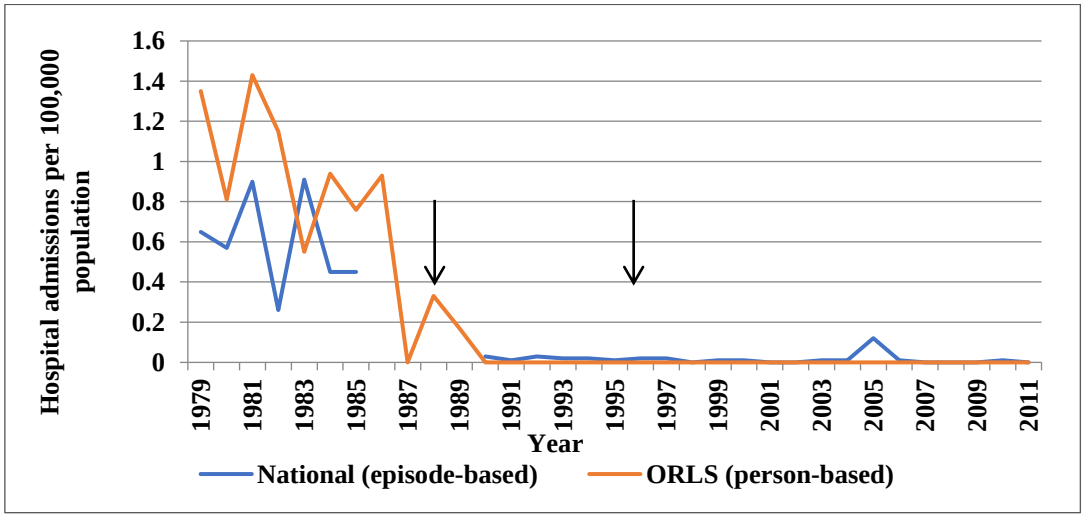


Key: Arrows show introduction of the single and 2 dose MMR vaccination schedules in 1988 and 1996 respectively.

Mumps encephalitis

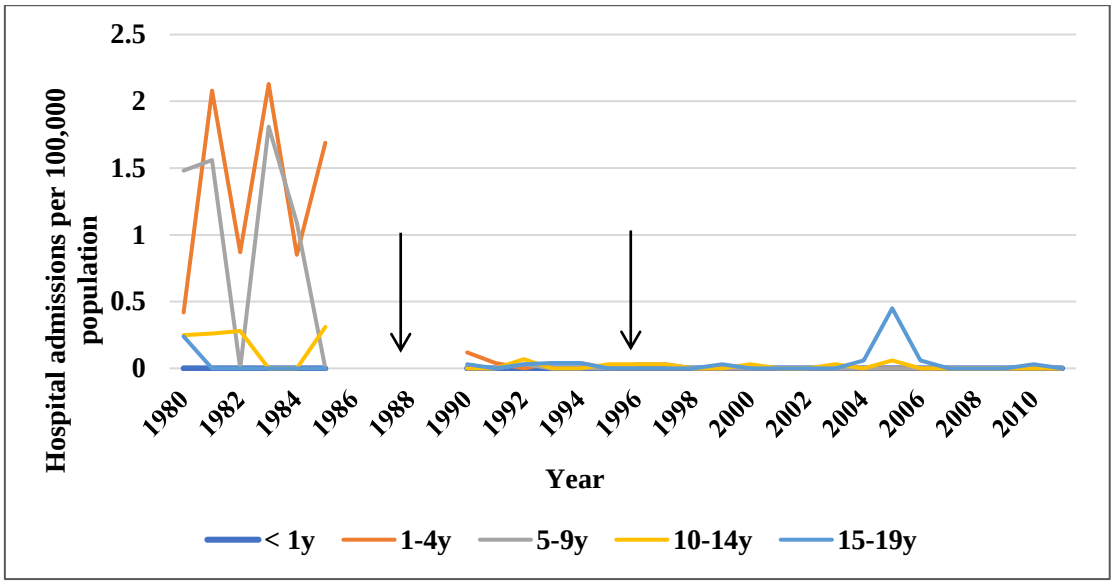
Hospital ARs for mumps encephalitis fell from a pre-single-dose MMR high of 0.91/100,000/year in 1983 to a post-single-dose MMR low of almost zero in 1991 (Figure 2.9). The average hospital ARs for mumps encephalitis differed between the vaccination periods: 0.60/100,000/year (95%CI 0.38-0.82) [1979-1985]; 0.02/100,000/year (0.01-0.03) [1990-1995] and 0.01/100,000/year (0.00-0.03) [1997-2011]; p<0.0001. Compared with the pre-single-dose MMR period, this corresponds to 30- and 60-fold declines in average hospital ARs following the introduction of a single-dose and two-dose MMR vaccine respectively. The post MMR decline was observed across all age groups (Figure 2.10). A 12-fold rise in mumps admissions occurred between 2004 and 2005, attributable to an increase in the 15-19 years age group. Between 1999 and 2011, there were 18 different people admitted with mumps encephalitis, which gives an incidence rate of 0.01/100,000/year (95% CI 0.00-0.02).

Figure 2.9 Hospital admission rates for mumps encephalitis (0-19 years) in England



Key: ORLS=Oxford Record Linkage Study. Arrows show introduction of the single and 2 dose MMR vaccination schedules in 1988 and 1996 respectively.

Figure 2.10 Hospital admission rates for mumps encephalitis (0-19 years) in England by age group



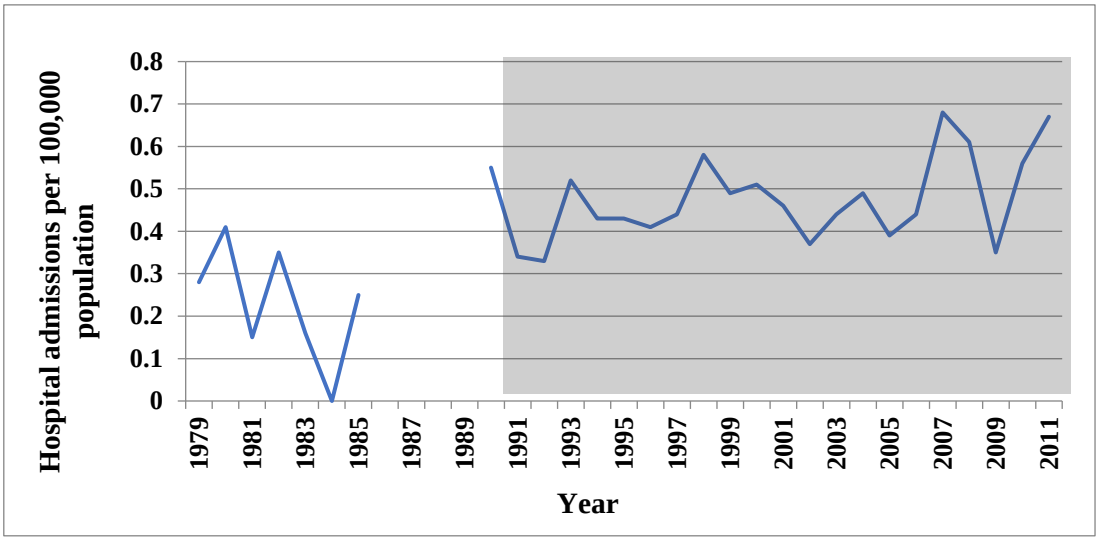
Key: Arrows show introduction of the single and 2 dose MMR vaccination schedules in 1988 and 1996 respectively.

2.4.3. Hospital admission rate trends and introduction of PCR testing

Herpes viral encephalitis

Pre-PCR (1979-1985), hospital ARs for herpes viral encephalitis ranged (lowest to highest) from 0.15-0.41/100,000/year with an average AR in this period of 0.23/100,000/year (95%CI 0.10-0.36). In the period following the introduction of PCR testing (1991-2011), hospital ARs for herpes viral encephalitis ranged from 0.33 to 0.68/100,000/year (average: 0.47/100,000/year; 0.43-0.52) (Figure 2.11), corresponding to a 2-fold increase ($p<0.0001$). Between 1999 and 2011, there were 755 herpes viral encephalitis episodes for 452 different people, resulting in an incidence rate of 0.30/100,000/year (0.26-0.33) in this period.

Figure 2.11 Hospital admission rates for herpes viral encephalitis (0-19 years) in England

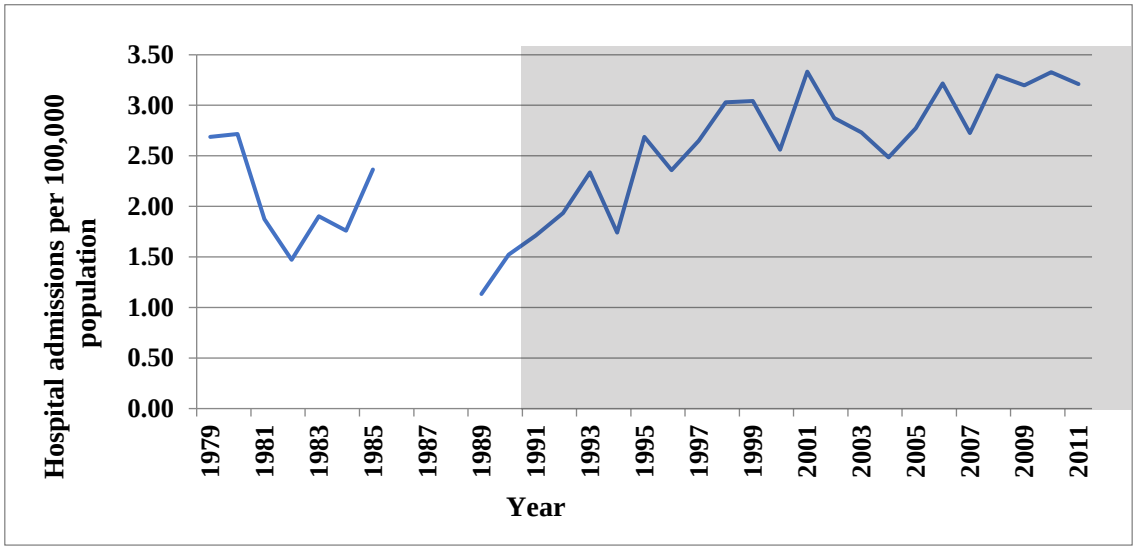


Key: Shaded area shows period following the introduction of PCR testing in 1990

Encephalitis of unknown aetiology

In the period prior to the introduction of PCR testing (1979-1994), the average hospital AR for encephalitis of unknown aetiology was 1.93/100,000/year (1.65-2.22) [1979-1994] and 2.91/100,000/year (2.75-3.08) [1995-2011] (Figure 2.12). The average hospital AR was 1.99/100,000/year (1.51-2.46) pre-PCR (1979-1989) and 2.73/100,000/year (2.50-2.95) thereafter (1991-2011); p=0.0073. Between 1999 and 2011, there were 3270 individual encephalitis admissions for which the aetiology was unknown, giving an incidence rate (1999-2011) of 2.03/100,000/year (1.89-2.17) for encephalitis of unknown aetiology, in this period.

Figure 2.12 Hospital admission rates for encephalitis of unknown aetiology (0-19 years) in England



Key: Shaded area shows period following the introduction of PCR testing in 1990

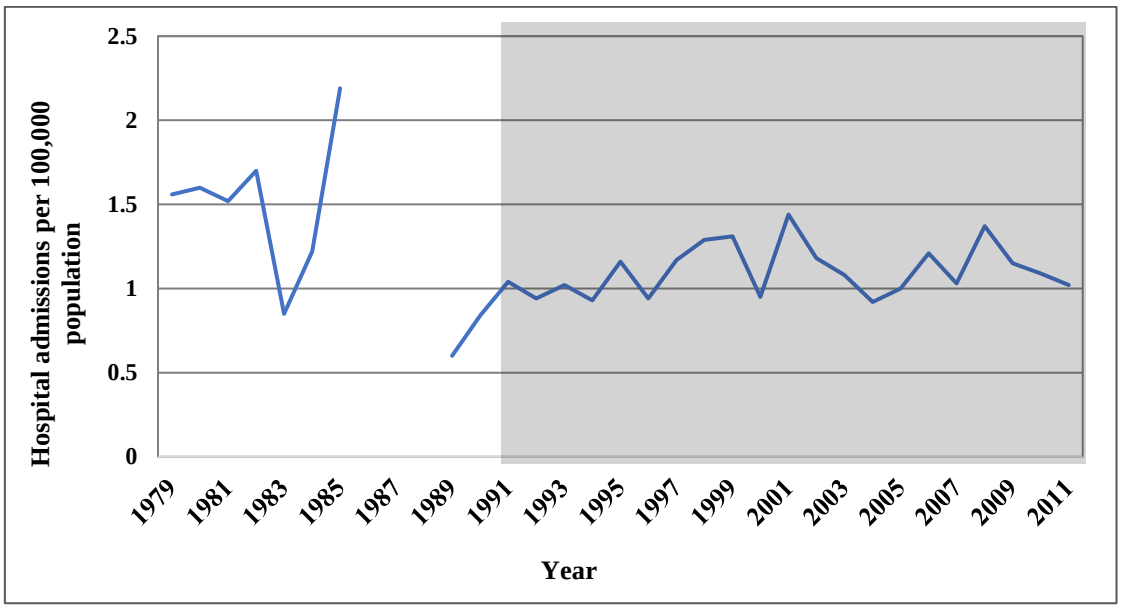
Unspecified viral encephalitis [1]

Hospital ARs for admissions codes as unspecified viral encephalitis ranged between 0.6/100,000/year (95%CI 0.46-0.74) to 2.19/100,000/year (1.35-3.02) with an average hospital AR (1979-2011) of 1.12/100,000/year (95%CI 1.06-1.30) (Fig 2.13). A peak in admissions were observed in 1985 and corresponded to a 1.8 times increase from the previous year. Hospital ARs for unspecified viral encephalitis were highest for the <1-year age group (average AR, 95%CI: 3.00/100,000/year, 2.28-3.7) than for the 1-4y (1.67, 1.48-1.86), 5-9y (1.00, 0.82-1.09), 10-14y (0.84, 0.68-1.01), and 15-19y (0.88, 0.74-1.01) age groups, p=<0.0001. The average AR was 1.04/100,000/year (95%CI 0.49-1.59) pre-PCR and 1.11/100,000/year (1.04-1.12) post PCR; p=0.7651. The average AR was 1.23/100,000/year (95%CI 0.96-1.50) in the ICD9 period (1979-1994) and 1.14/100,000/year (1.01-1.22) in the ICD10 period (1995-2011); p=0.9918. Between

[1] New data

1999 and 2011, there were 1766 episodes for 1503 different people, diving an incidence rate of 0.93/100,000/year (95%CI 0.89-1.01)

Figure 2.13 Episode based admission rates for unspecified viral encephalitis (0-19 years) in England [1]



2.4.4. Specific diagnoses

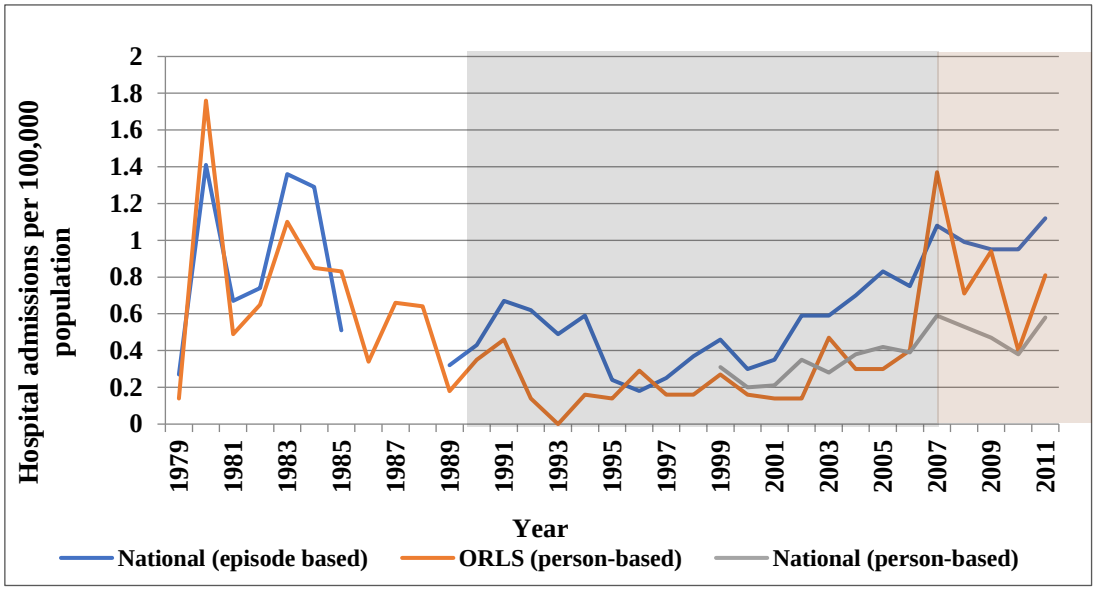
ADEM

Hospital ARs for ADEM (1979-2011) ranged between 0.18/100,000/year (95%CI 0.11-0.26) and 1.41/100,000/year (0.73-2.09), peaking in 1980, 1983 and 1984 (Figure 2.14). ADEM admissions (1979-1985) were mostly in the 1-9 year age group with minimal contribution from the other age groups (Figure 2.15-2.17). Admissions in the 1-9-year age group fell in the early 1990's and were lower than they were in the 1980's. Hospital ARs for ADEM (1995-2011) varied annually, showing an increasing trend in the 1-19 year age group. Admissions in infants were virtually non-existent prior to the 1990's, rising thereafter, with 3-yearly peaks. Hospital ARs for ADEM (1979-1994) decreased

[1] New data

(APC= -3.78%; -2.69 to -4.86; p<0.0001) and thereafter rose steadily (APC=10.55%; 8.76-12.37; p <0.0001) from 0.24/100,000/year (0.15-0.32) in 1995 to 1.12 /100,000/year (0.93-1.30) in 2011(Figure 2.18). Between 1999 and 2011, there were 1174 ADEM episodes for 613 different people, giving an incidence rate for ADEM (1999-2011) of 0.39/100,000/year (95%CI 0.32-0.47).

Figure 2.14 Hospital admission rates for ADEM (0-19 years) in England



Key: Grey shaded area indicates period of widespread use of MRI (1990-2011). Light orange shaded area indicates period when consensus definition guideline for ADEM came into effect (2007-2011)

Figure 2.15 Episode based admission rates for ADEM in children <1 year old in England

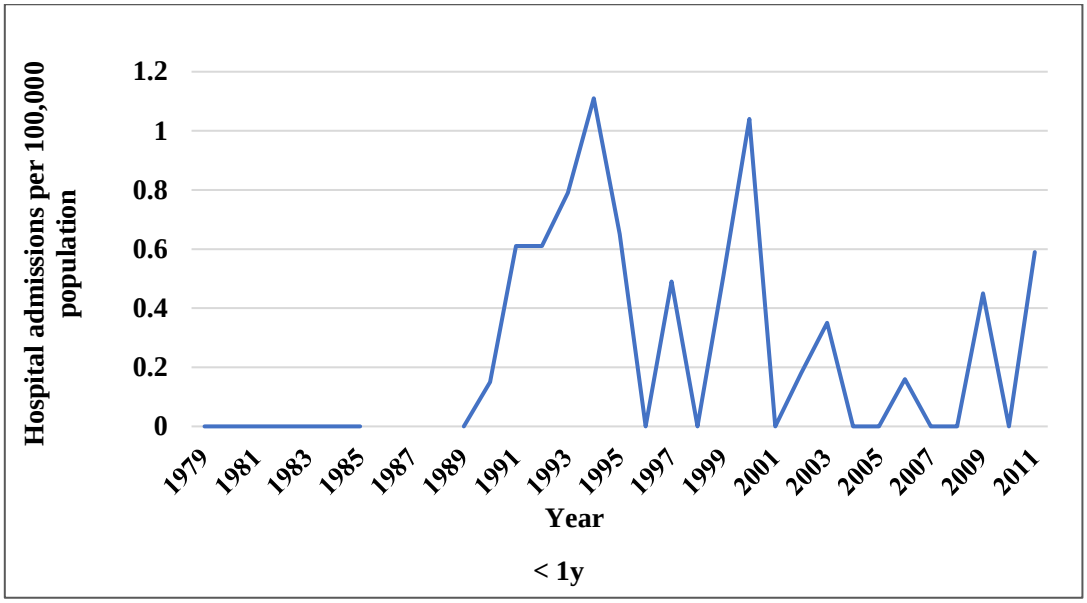


Figure 2.16 Episode based admission rates for ADEM (1-9 year olds) in England

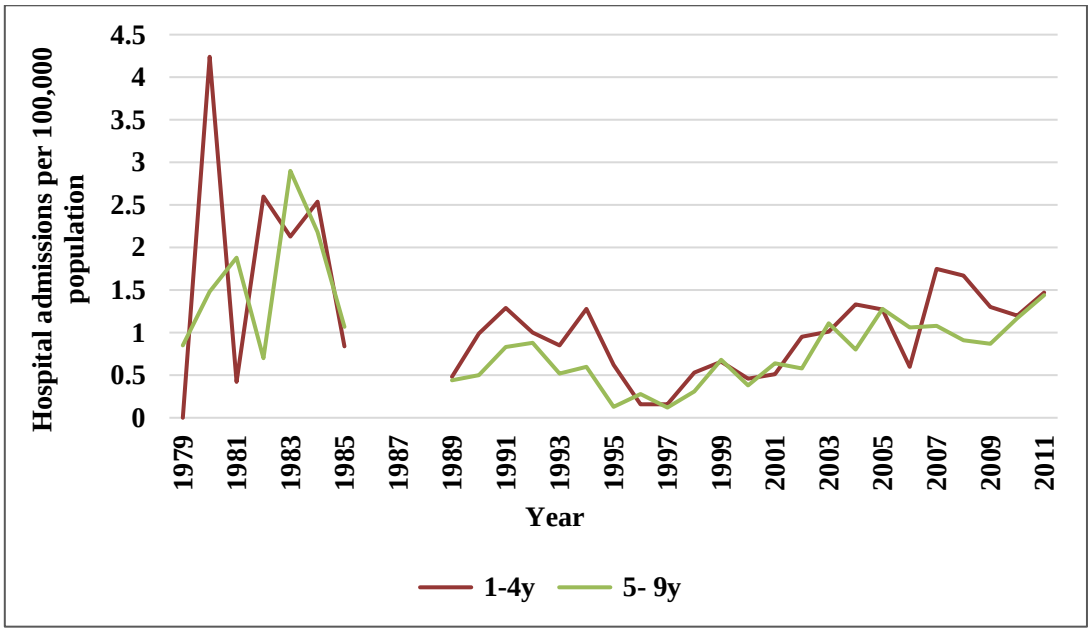


Figure 2.17 Episode based admission rates for ADEM (10-19 year olds) in England

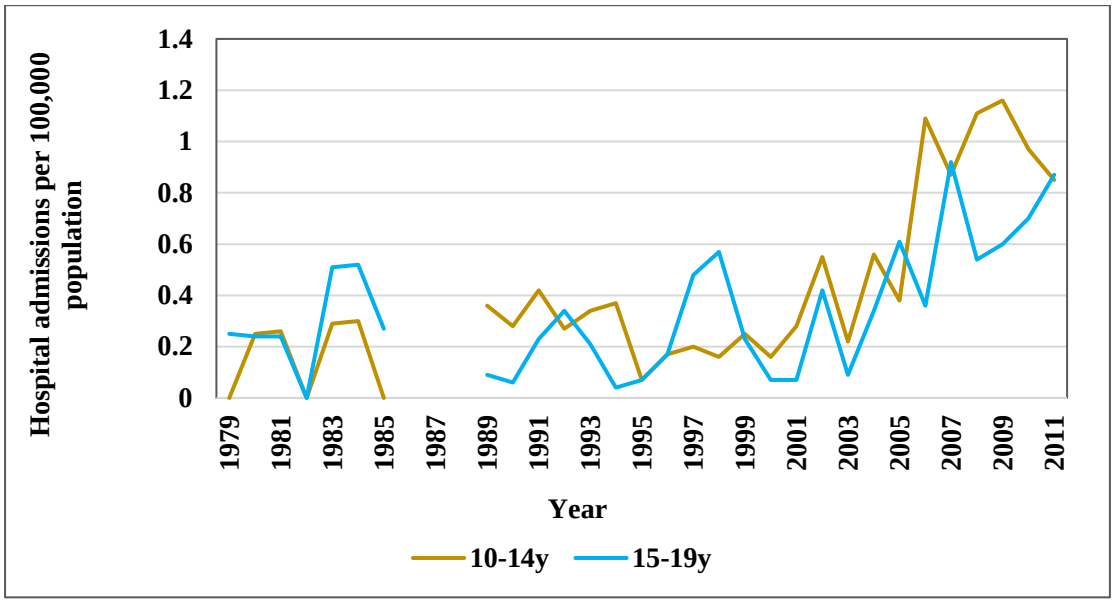
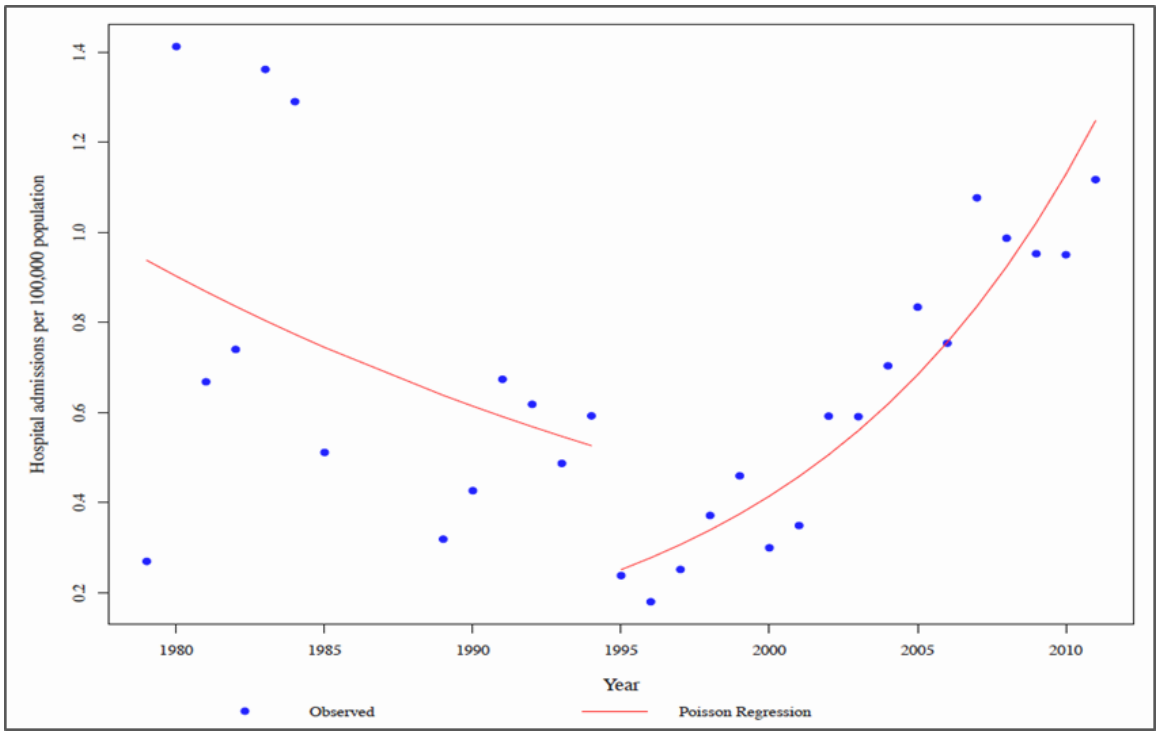


Figure 2.18 Poisson regression model for ADEM admissions (0-19 years) in England [2]

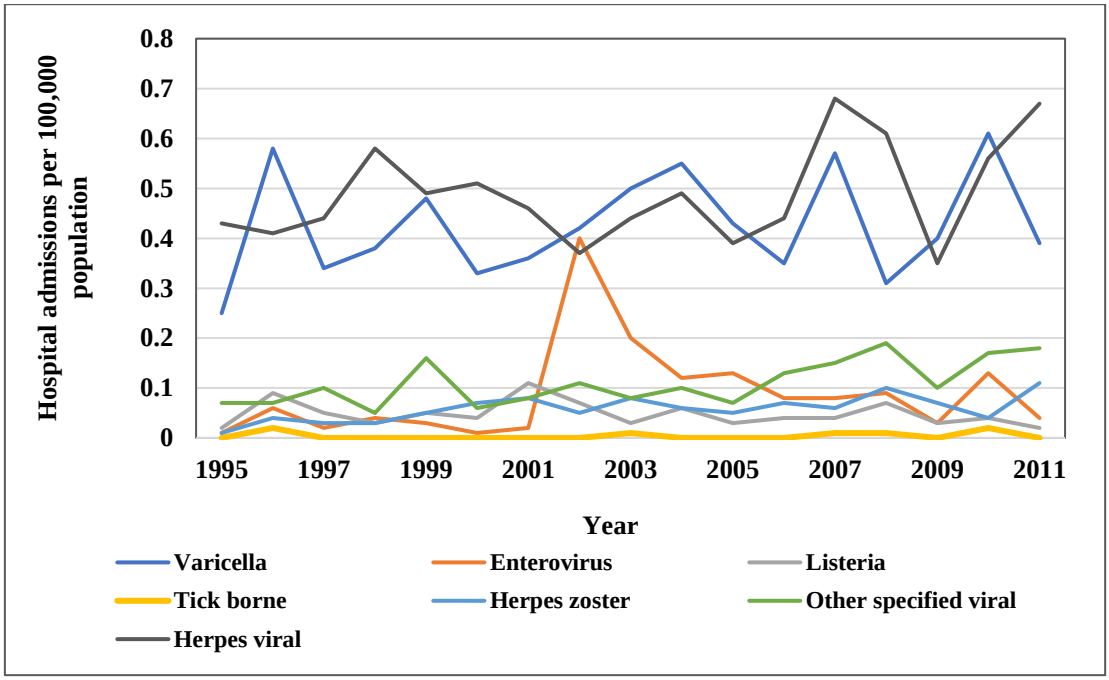


[2] Poisson regression graph provided by Alecia Nickless

2.4.5. Specific infectious aetiology groups

Summary hospital admission trends for specific infectious aetiologies are shown in Figure 2.19. The incidence rates of these specific aetiologies and their contribution to the total number of person admissions from 1999-2011 are summarised in Tables 2.4 and 2.5.

Figure 2.19 Hospital admission rates in England (0-19 years) due to infectious aetiologies [3]



[3] Only data for varicella and enterovirus were presented in the publication

Table 2.4 Person based (incidence) rates for the different encephalitis diagnoses by age group and gender [1]

	Varicella encephalitis	Herpes viral encephalitis	EV encephalitis	Encephalitis of unknown aetiology	ADEM	Herpes zoster encephalitis	Other specified viral encephalitis	Unspecified viral encephalitis
<1 year	0.23 (0.05-0.39)	2.26 (1.85-2.68)	0.45 (0.24-0.67)	5.02 (4.45-5.58)	0.18 (0.03-0.33)	0.21 (0.12-0.30)	0.43 (0.09-0.76)	2.04 (1.64-2.43)
1-4 years	1.01 (0.93-1.19)	0.30 (0.22-0.38)	0.08 (0.04-0.11)	3.13 (2.86-3.41)	0.67 (0.49-0.85)	0.09 (0.05-1.3)	0.10 (0.06- 0.14)	1.47 (1.26-1.68)
5-9 years	0.40 (0.33-0.47)	0.15 (0.11-0.19)	0.02 (0.01-0.04)	1.67 (1.55-1.80)	0.47 (0.39-0.55)	0.03 (0.01-0.04)	0.11 (0.05-0.17)	0.74 (0.68-0.81)
10-14 years	0.09 (0.05-0.14)	0.15 (0.10-0.20)	0.01 (0.00-0.02)	1.47 (1.34-1.60)	0.28 (0.21-0.36)	0.03 (0.01-0.04)	0.03 (0.004-0.05)	0.68 (0.58-0.79)
15-19 years	0.02 (0.005-0.04)	0.14 (0.10-0.18)	0	1.53 (1.34-1.71)	0.21 (0.13-0.29)	0.05 (0.03-0.07)	0.04 (0.004-0.08)	0.75 (0.67-0.82)
Female	0.34 (0.31-0.41)	0.30 (0.25-0.35)	0.04 (0.02-0.06)	NA	0.39 (0.30-0.48)	0.07 (0.05-0.08)	0.08 (0.05-0.11)	1.00 (0.86-1.06)
Male	0.37 (0.33-0.41)	0.29 (0.25-0.33)	0.06 (0.03-0.09)	NA	0.39 (0.31-0.47)	0.05 (0.04-0.07)	0.09 (0.05-0.13)	0.96 (0.88-1.05)
All	0.36 (0.31-0.41)	0.30 (0.26-0.33)	0.05 (0.03-0.07)	2.03 (1.89-2.17)	0.39 (0.32-0.47)	0.06 (0.05-0.07)	0.09 (0.07-0.11)	1.18 (1.06-1.30)

Key: HSV=Herpes Simplex Virus; EV=enterovirus. ADEM=acute disseminated encephalomyelitis. Data presented are per 100,000 population (95% confidence interval). NA=not available

[1] New data

Table 2.5 Aetiology of childhood encephalitis in England (1999-2011) [1]

Aetiology	Number of people admitted (1999-2011)	Male: female ratio	Percentage of total people admitted (n=6287)
Meningococcal encephalitis	0	N/A	0
Toxoplasma encephalitis	0	N/A	0
Encephalitis due to mosquito borne viruses	1	N/A	0.02
Tick borne virus encephalitis	4	1.0	0.06
Measles encephalitis	9	1.25	0.1
Adenovirus encephalitis	9	0.80	0.1
Mumps encephalitis	18	1.00	0.3
Listeria encephalitis	59	0.79	0.9
Enterovirus encephalitis	74	1.55	1.2
Herpes zoster encephalitis	91	0.86	1.4
Encephalitis due to other specified virus	137	1.11	2.2
Herpes simplex virus encephalitis	452	0.99	7.2
Varicella encephalitis	531	1.13	8.4
ADEM	613	1.04	9.8
Unspecified viral encephalitis[&]	1503	1.06	23.9
Encephalitis of unknown aetiology	3270	NA	52.0

Key: N/A=not applicable; NA= data not available. [&] subset of unknown aetiology

[1] New data

Enteroviral encephalitis

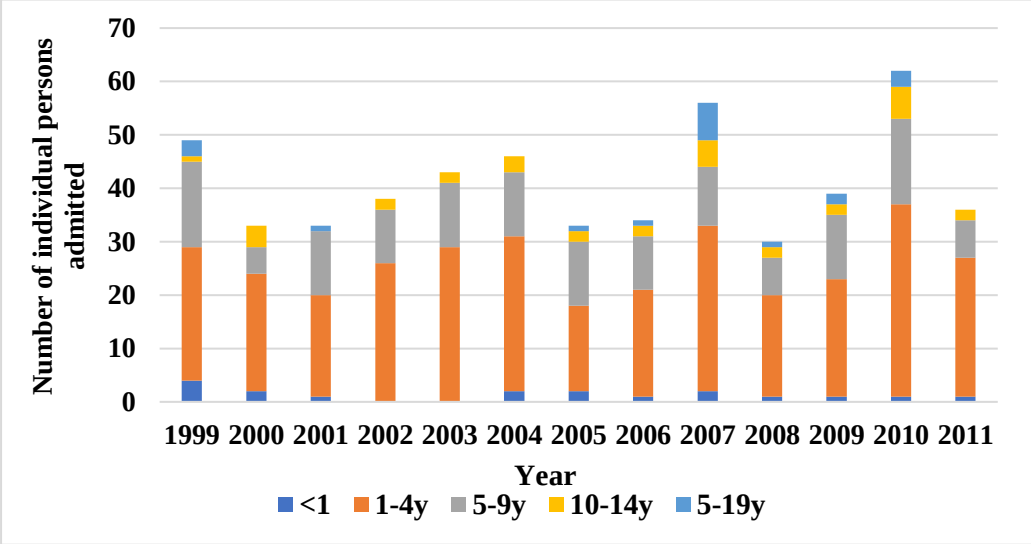
Hospital ARs for EV encephalitis (1995-2011) ranged from 0.01 to 0.06/100,000/year (average: 0.09/100,000/year; 95% CI 0.04-0.14) (Fig 2.19), peaking at 0.40/100,000/year in 2002, representing a 20-fold increase from 2001. This peak corresponded to an increase in admissions in the <1year age group. ARs (2003-2011) decreased but were higher in this period than between 1995 and 2001 (average hospital AR: 0.10/100,000/year (0.06-0.14) vs. 0.03/100,000/year (0.01-0.04) respectively; p=0.0002. Between 2002 and 2004, the mean hospital ARs for EV encephalitis were 0.03/100,000/year (95%CI 0.00-0.10) and 0.5/100,000/year (0.00-1.10) for males and females respectively (p=0.1000). Across the study period, the mean hospital AR for EV was highest in infants (<1 year age group) than for the other age groups and were 0.43/100,000/year (95%CI 0.005-0.85) for infants vs. 0.01 (0.02-0.18), 0.01 (0.003-0.021), 0.008 (0.00-0.02), and 0.001 (0.00-0.004) for the 1-4y, 5-9y, 10-14y and 15-19y age groups respectively; p=<0.0001). Between 1999 and 2011, there were 161 EV encephalitis admissions for 74 different people, giving an incidence rate of 0.05 (95%CI 0.03-0.07) in this period.

Varicella encephalitis

Hospital ARs for varicella encephalitis were lowest in 1995 (0.25/100,000/year, 95% CI 0.16 to 0.34) and highest in 2010 (0.61/100,000/year, 0.47-0.75). Between 1995 and 2011, admission rates for varicella encephalitis fluctuated with approximately 3 yearly peaks (Fig 2.19). Hospital ARs for males and females were closely aligned, however differences were observed for the different age groups and were highest in the 1-4y age group (1.18/100,000/year (95%CI 1.01-1.35) than for the <1y (0.36, 0.19-0.53), 5-9y (0.48/100,000/year, 0.39-0.57), 10-14y (0.08/100,000/year, 0.05-0.11) and 15-19y

(0.04/100,000/year, 0.01-0.07), p=<0.0001**.** Between 1999 and 2011, there were 838 varicella encephalitis admissions for 531 individual people, giving an overall incidence rate of 0.36/100,000/year (95%CI 0.31-0.41). Varicella encephalitis admissions predominantly occurred in people aged 1-4 years who accounted for 60.0% (320/531) (Figure 2.20).

Figure 2.20 Year on year admissions (0-19 years) for varicella encephalitis in England [1]



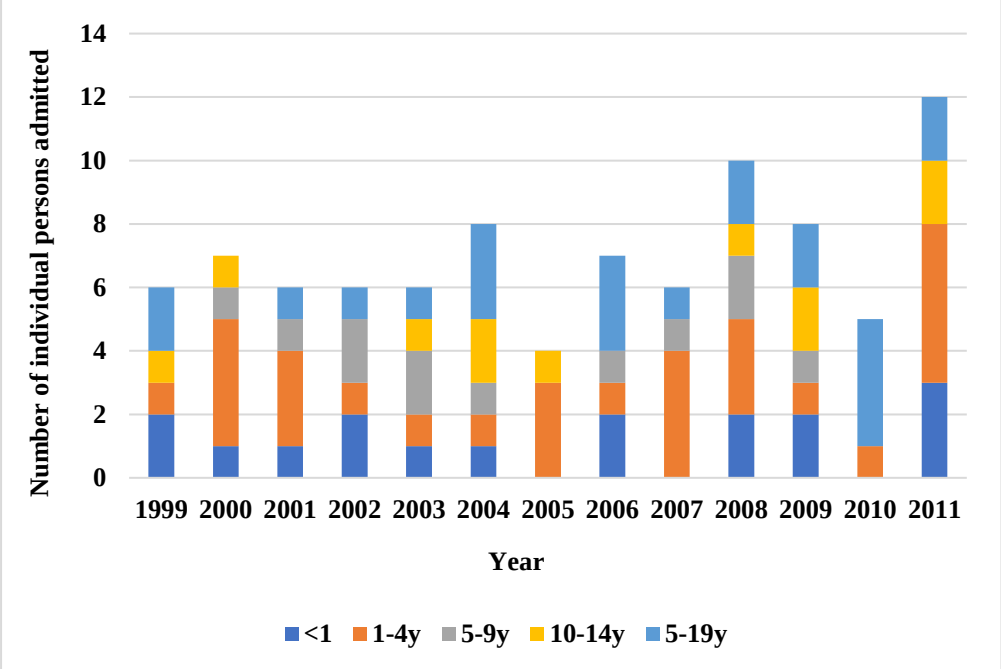
[1] New data

Herpes zoster encephalitis[1]

There were 120 herpes zoster encephalitis hospital episodes between 1995 and 2011 of which 69 (57.5%) were in females. This gives an overall hospital admission rate (1995-2011) of 0.06/100,000/year (95% CI 0.05-0.07). Hospital ARs for herpes zoster encephalitis fluctuated between 1995 and 2011 (Fig 2.19) with the highest number of admissions occurring in 2011 (n=14, 11.7%) (Fig 2.21).

Between 1999 and 2011, there were 108 admissions for 91 individual people with herpes zoster encephalitis, giving an incidence rate of 0.06/100,000/year (95%CI 0.05-0.07).

Figure 2.21 Year on year admissions (0-19 years) for herpes zoster encephalitis in England) [1]

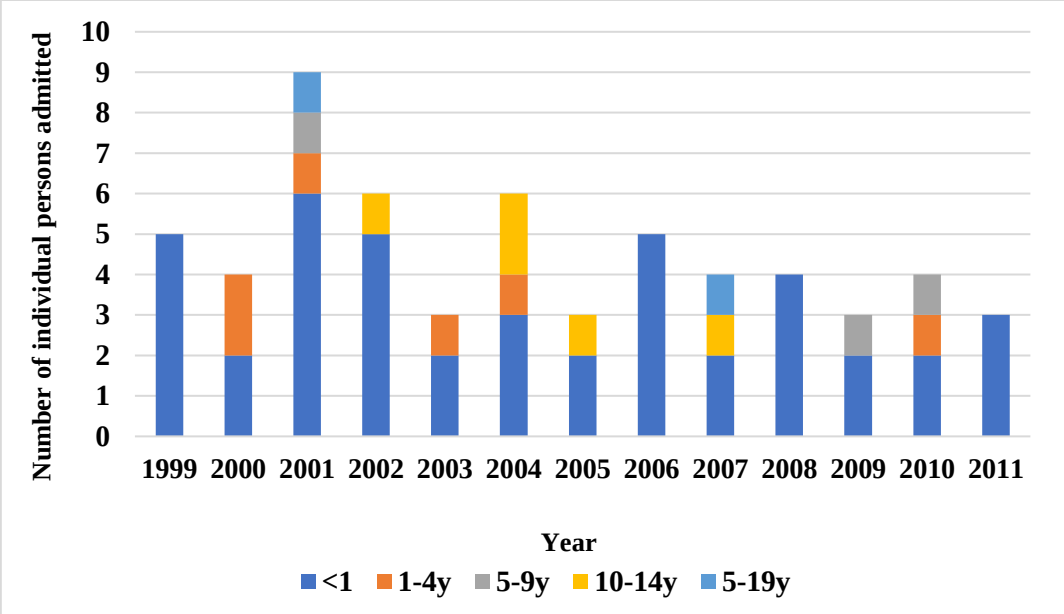


[1] New data

Listeria encephalitis [1]

There were 92 admissions coded as listeria encephalitis between 1995 and 2011 of which 46 (50%) were in males. The average hospital AR for listeria encephalitis between 1995 and 2011 was 0.05/100,000/year (95%CI 0.04-0.06) and most admissions occurred in 2001 (n=12, 13%). The number of people admitted from 2001 showed a downward trend (Fig 2.22). Year-on-year, admissions for listeria encephalitis occurred predominantly in infants who accounted for all episodes that occurred in 1999, 2006 and 2011 (Fig 2.22). Between 1999 and 2011, there were 71 admissions for 59 individual people, giving an incidence rate of 0.04/100,000/year (9%%CI 0.03-0.05) for listeria encephalitis.

Figure 2.22 Year on year admissions (0-19 years) for listeria encephalitis) in England [1]



[1] New data

Tick-borne encephalitis [1]

Between 1990 and 2011, there were a total of 11 tick borne encephalitis admissions (9 in females, 82%) resulting in an average hospital AR of 0.01/100,000/year (95% CI 0.002-0.008). Between 1999 and 2011, there were 5 admissions (3 in females) for 4 different people, giving an incidence rate of 0.003/100,000/year (95% CI 0.0002-0.005) during this period.

Meningococcal encephalitis [1]

ICD codes for meningococcal encephalitis were only available in the ICD 9 period. During this time, a total of 54 meningococcal encephalitis episodes occurred, with most cases (n=17, 31%) occurring in people aged 1-4 years. Most episodes occurred in 1990 (n=12), and 1993 and 1994 (n=11 for each year). Hospital ARs for meningococcal encephalitis (1983-1995) ranged (lowest to highest) between 0.02/100,000/year (95%CI 0.00-0.04) in 1995 and 0.1/100,000/year (95%CI 0.04-0.15) in 1990 (Figure 2.18), with an average hospital AR of for 0.03/100,000/year (95% CI 0.01-0.05).

Other specified viral encephalitis [1]

Between 1995 and 2011, hospital ARs for other specified viral encephalitis ranged from 0.05/100,000 (95%CI 0.01-0.10) to 1.19/100,000/year (0.11-0.27), average AR = 0.11/100,000/year; 95%CI 0.09-0.13. A rise in hospital ARs for 'other specified viral encephalitis' occurred between 2005 (0.07/100,000, 95%CI 0.02-0.12) and 2008 (0.19/100,000, 0.11-0.27), representing a 2.7 times increase during this period (Fig 2.19). From 1999 to 2011, there were 189 hospital episodes for 137 people (males=72,

[1] New data

53%), giving an incidence rate for ‘other specified viral encephalitis’ of 0.09/100,000/year (95% CI 0.06-0.12). No pattern in the age group distribution of admissions was observed.

2.4.6. Outcomes [1]

A summary of LOHS data is presented in Table 2.6. Of the 6287 persons admitted between 1999 and 2011, 224 in-hospital deaths (3.5%) were recorded. Including the 122 out of hospital encephalitis deaths, this gives a case fatality of 5.5%. The overall median cumulative LOHS (1999-2011) was 9 days (IQR 5-19), this was 7 days (3-25) for non-survivors and 9 days (5-18) for survivors. The proportion of persons admitted with a cumulative LOHS of ≥ 28 days was 1.7 times higher in those that died (24%) than those that survived (14%).

[1] New data

Table 2.6 Length of hospital stay for person based encephalitis admissions (1991-2011)

Cumulative LOHS (days)	All (frequency)	Deaths (frequency)	Survivors (frequency)
1	371	12	359
2	404	30	374
3	380	21	359
4	365	18	347
5	316	13	303
6	420	15	405
7	318	13	305
8	339	2	337
9	309	5	304
10	207	5	202
11	242	1	241
12	205	3	202
13	159	3	156
14	155	6	149
15	175	0	175
16	135	3	132
17	117	3	114
18	93	1	92
19	81	3	78
20	63	2	61
21	76	3	73
22	75	4	71
23	105	1	104
24	71	0	71
25	64	2	62
26	68	2	66
27	54	0	54
28+	920	53	867
Total	6287	224	6063

Key: LOHS= length of hospital stay

2.5. Discussion

This unique 33-year study is the first to report on long-term hospital admission trends for all-cause encephalitis in an exclusively paediatric population in England. It is also the first to quantify impact of the MMR vaccine on measles and mumps encephalitis admissions in England and to define the specific incidence of encephalitis due to the main infectious pathogens causing disease in England.

The data showed that, after a decline (1979-1994), admissions for all-cause encephalitis increased between 1995 and 2011. The age-related epidemiological profile of ADEM changed with more infants being admitted with the diagnosis from 1990 onwards. Measles and mumps encephalitis admissions reduced following introduction of the MMR vaccine. Admission rates for herpes viral encephalitis and encephalitis of unknown aetiology increased with the routine use of PCR testing.

While the exact reason for the initial decline (1979-1994) is unclear, it is likely that a major component is the reduction in measles and mumps encephalitis admissions with MMR vaccination (including a reduction in cases of unspecified aetiology that were in fact caused by mumps and measles). The reasons for the increase in all-cause encephalitis in the later years need consideration. Restriction of this finding to the ICD10 period makes it unlikely to be due to the transition in ICD codes and change in hospital coding practices. Another consideration is whether the increase could have been explained by diagnostic displacement with reclassification of cases of 'viral meningitis' as 'viral encephalitis'. This is unlikely since the increase in encephalitis admissions from 1995 is not mirrored by a decrease in viral meningitis cases[312] in the same time period. It is also unlikely that the observed increase in the later years simply represents an increase in multiple admissions per person with encephalitis. The increasing trend was similar using the person-based rates, i.e. only counting each person once, and after

adjusting for short hospital stays (which might not have been confirmed encephalitis), indicating a genuine increase in encephalitis incidence. Plausible reasons for this observation include increasing case ascertainment with the discovery of biomarkers such as anti-NMDAR antibodies and other biomarkers implicated in encephalitis,[196] recognition of clinico-radiological syndromes such as limbic encephalitis which would have historically been undiagnosed in some instances[313] and increased awareness and use of diagnostic tools such as EEG in children with unclear infections. There is also the possibility of emergence of new viruses implicated in encephalitis and changing neurovirulence such that pathogens that previously caused meningitis are now causing encephalitis.

Routine use of the MMR vaccine has resulted in reduction in disease burden from measles, mumps and rubella.[312,314] While its specific effect on the incidence of measles and mumps encephalitis has been demonstrated in some countries, [238,315] data for England are lacking. In this study, admissions for measles encephalitis were high prior to the mid 1980's, when the single measles vaccine was being used and when coverage was low (<60%) and insufficient to fully interrupt transmission.[316] Following introduction of the single-dose MMR vaccine in 1988 and an increase in vaccine coverage to > 90%,[316] possibly due to an increase in public awareness, a sustained decline was observed. The increase in measles encephalitis admissions observed in 1994 matches the increase in overall measles case notifications in England in the same year.[317,318] However, contrary to the notification data in which children over 10 years were most affected, admissions for encephalitis in this study were highest in the 1-4 year age group, possibly reflecting greater susceptibility of younger children to complications such as CNS manifestations.[319] Following the introduction of the two-

dose MMR schedule in 1996 with vaccine coverage sustained in excess of 80%,[316] admissions for measles encephalitis declined further and remained very low; this was similar for mumps encephalitis admissions.

Contrary to the measles data, the increase in mumps encephalitis admissions in 2005 mostly affected the 15-19 years age group, which was the predominantly affected age group in the 2005 outbreak in England and Wales.[314,320] Various reasons for the increased infection rate in the 15-19 years age group during the outbreak have been suggested. Most of the infection occurred in children who were not eligible for routine MMR vaccination post introduction in 1988. [320,321] In addition, herd immunity was possibly not sufficient to prevent transmission in this susceptible group.

The changing age-related epidemiological profile of ADEM is notable. ADEM admissions were high in the early 1980's, mostly affecting the 1-9 years age group, and were lower in the early 1990's, corresponding to a reduction in admission rates in the same age group. This finding mirrors the data for measles and mumps encephalitis admissions in the 1-9 year age group, suggesting that the ADEM rates in the 1980's might have been caused by measles and mumps. The lower ADEM rates in the early 1990s possibly reflect the impact of MMR vaccination in reducing measles and mumps infections, especially in the 1-9 year age group. The rising trend in ADEM admissions from the mid 1990's most probably indicates enhanced disease recognition with the widespread routine use of MRI scans[322] and increased case ascertainment following publication of a consensus definition guideline in 2007.[139] It may also be a true rise in ADEM cases due to the emergence of pathogens or environmental triggers that are yet to be identified. Interestingly, ADEM admissions were coded in infants from 1990 onwards having been virtually non-existent prior to this time, with admissions occurring

in three yearly peaks suggesting an unknown, possibly viral aetiological agent. There is a growing body of evidence showing an association between certain neuronal autoantibodies and acute demyelinating disease in children.[323] Thus the increasing ADEM trend may also reflect improved disease identification through awareness of this association with increasing testing for neuronal antibodies in children presenting with encephalopathy, where the cause is uncertain. Given the beneficial role of immunotherapy in the management of ADEM,[324] these findings are important since early consideration of this diagnosis, testing for neuronal antibodies and timely institution of immunotherapy could be worthwhile.

Post-PCR, herpes viral encephalitis admissions increased possibly reflecting enhanced aetiological diagnosis. Admissions for encephalitis without a specific diagnosis also increased. However, admissions for unspecified viral encephalitis remained unchanged. Uncertainty about aetiological diagnosis could lead to unnecessary delays in the institution of appropriate treatment, and result in poor outcomes.[242,325,326]Also, non-identification of a viral cause could lead to the institution of unnecessary treatment and limit appropriate public health prevention and control strategies. The high proportion of cases in this category could reflect the delays in obtaining laboratory results, affecting timely documentation in HES of an aetiological diagnosis. Additionally, variations in the breadth of PCR testing in different hospitals due to lack of a standard diagnostic algorithm for viral encephalitis in England, may lead to some aetiologies being missed. There may also be newer pathogens implicated in encephalitis[327] that are either not identified by currently available testing or do not have a specific ICD code.

Parechoviruses were only identified as a distinct genus from enteroviruses in 1999, and publication of the most recent ICD version in 1994 predates this. Therefore, no specific ICD code exists for this aetiology. In addition, specific testing for parechovirus only became available in 2006. It is therefore likely that encephalitis caused by parechovirus would have been coded as unknown aetiology. Similarly, there is increasing recognition of autoimmune encephalitis syndromes due to a range of antibodies to neuronal surface antigens.[14,20,328] These syndromes currently do not have a specific ICD code and may explain the high and increasing proportion of encephalitis admissions assigned to the unknown aetiology group, despite PCR testing. While available guidelines for the management of suspected viral encephalitis[213,329] may help standardise the laboratory diagnostic pathway, there remains an urgent need for research into improving aetiological diagnosis in encephalitis cases.

In the Western world, HSV encephalitis is thought to be the most common aetiological agent implicated in encephalitis, however there is some suggestion from published data that this may no longer be true. In the CEP, HSE was the third most common aetiological diagnosis after anti-NMDAR and VZV encephalitis [20] although this could reflect selection bias due to physicians referring only diagnostically challenging patients to the study. Similarly, in Canadian and Swedish studies of encephalitis in children,[4,9] HSV ranked second and seventh respectively as the cause of disease, with *Mycoplasma pneumoniae* being the most commonly identified pathogen in the Canadian study and tick-borne virus in the Swedish study. In a previous encephalitis study of adults and children in England, HSV was the most commonly identified viral pathogen in 19% of 203 individuals.[14] However, in this study, ADEM was the most common specific diagnosis (9.8%), followed by VZV (8.4%) and HSV (7.7%). This finding may reflect

better coding of ADEM and VZV encephalitis cases with improved diagnostics. In this study, listeria encephalitis predominated in infants with minimal contribution year-on-year from other age groups. A previous study of bacterial meningitis in England conducted between 2010 and 2011,[330] demonstrated an exclusive occurrence of listeria meningitis in neonates. A breakdown of age for those <1 year was not available in this study for a direct comparison, although it was observed that all listeria encephalitis admissions in 2011(one of the years covered in the bacterial meningitis study) were for infants. For the other year covered by the bacterial meningitis study (2010), there were two listeria encephalitis admissions in children >1 year. Listeria infection is common in the immunocompromised which could explain the admissions in children outside the neonatal period.

The case fatality in this study was 3.5% and increased to 5.5% with the inclusion of encephalitis out of hospital deaths. The case fatality of 3.5% is higher than was reported in paediatric studies conducted in Australia and Canada[4,16] where the reported case fatality was 2.6% and 2% respectively. The differences in case fatality could reflect methodological differences between this study and the two previous studies. In particular, in the Australian study, only principal diagnostic codes were considered for the study period when analysis of mortality was performed, whereas in this study, all diagnostic fields were considered. In addition, the Canadian study was conducted over one year only, which is not likely to be representative of overall mortality over a long period as has been considered in this study. The case fatality rate in this study is much lower than was reported in a paediatric study conducted in Pakistan,[22] possibly reflecting differences in the availability and accessibility of medical care. Higher case fatality rates have been reported in adult studies, [242,325,331] indicating better survival outcomes for children with encephalitis.

There are a few comparable paediatric studies. An Australian study in children ≤ 14 years (2000-2012)[16] reported an incidence estimate of 3.8-5.0/100,000 population. A 2-year surveillance conducted in Finland which included children aged 1 month to 15 years reported an incidence of 10.5/100,000 children.[8] A Japanese questionnaire based study (1984-1990) of encephalitis admissions for children ≤ 15 years reported an incidence rate of 3.3/100,000[5] while an earlier study of viral encephalitis in England reported an admission rate of 2.8/100,000 children.[30] Although the incidence rate for all encephalitis cases in this study, irrespective of LOHS (i.e. 4.02/100,000 population) is comparable to these studies, it is 2.6 fold lower than that reported in the Finnish study and 1.4 fold higher than was reported in the earlier study in England. The higher admission rates in the Finnish study may be due to the very short study period, which does not take into account year-on-year variation seen with encephalitis admissions. The earlier study in England[30] observed admission rates for viral encephalitis alone, which would explain the lower rate reported. A further study in England (2005-2009) of encephalitis admissions in children and adults[174] reported a best incidence estimate of 5.23/100,000 population after accounting for potential over diagnosis of HES only encephalitis cases. This is higher than the recalculated incidence rate in this study of 2.91/100,000 population and possibly reflects differences in the study populations and the methodology used in ascertaining incidence rates from 'true' encephalitis admissions. Of note, deaths from encephalitis without a corresponding HES record for it added only a small fraction to the total number of cases admitted between 1999 and 2011, providing some confidence in our estimation of disease incidence using HES data.

The main strength of this study is the longevity of the datasets, which are excellent resources for observing the impact of developments in the field of encephalitis on long-

term admission trends. The use of episode-based data reflects the impact of disease on the NHS, while the use of the linked data helps to reach a truer approximation of disease incidence. However, while the quality and consistency of HES data are generally well established, they are less so for encephalitis, possibly because precise laboratory diagnoses are sometimes not available at the time of discharge. HES data rely on accurate recording of clinical information in case records and correct coding which can be inconsistent, although there is some evidence that this is improving.[332]The difficulty with making a clinical diagnosis of encephalitis, and therefore subjectivity among clinicians, may have an impact on the validity of cases coded in HES. National data for England are lacking between 1986 and 1989; however, availability of ORLS data provides some insight to admission trends during this period. The use of different revisions of the ICD codes could impact on estimation of admissions and disease incidence for certain. An attempt to avoid this was made by calculating hospital ARs separately for the two ICD coding periods for aetiologies more likely to have been affected by this transition. Furthermore, the calculated IR is based on person-based data, which are only available in the ICD10 period. Not all ICD codes for specific encephalitis were included in this study, however the vast majority of those known to be prevalent in England were included. Ultimately, this study is the first of its kind, and provides useful data on long-term trends on encephalitis hospitalisations for the paediatric population in England and gives some insight to the disease burden on the healthcare system.

2.6. Conclusions

Childhood encephalitis admission rates in England declined between 1979 and 1994 but increased between 1995 and 2011. The impact of MMR vaccination may have

contributed to the initial decrease, but the exact reason for the later increase is unclear and warrants further investigation. The increasing availability and widespread use of MRI scans, increasing awareness and testing for neuronal autoantibodies and increased diagnostic certainty, have probably contributed to the rising trend in ADEM admissions. Strategies to enhance aetiological diagnosis in children with encephalitis are needed.

Hospital admissions for all-cause encephalitis in people less than 20 years old in England increased during the study period highlighting the need for close disease surveillance. Hospital admissions due to measles and mumps encephalitis declined substantially following the introduction of the 2-dose MMR vaccination schedule. The findings of this study demonstrate a change in the age-related epidemiological profile for ADEM, and an increase in ADEM admissions between 1995 and 2011, possibly reflecting enhanced disease identification with the use of MRI's. ARs for herpes viral encephalitis increased, reflecting improved ascertainment of aetiology particularly with the use of HSV PCR. However, ARs for encephalitis of unknown aetiology also increased, suggesting there is still room for improvement in aetiological diagnosis in children with encephalitis.

This study reports the most complete population based dataset in the UK of disease incidence for all-cause encephalitis in children in England between 1979 and 2011. It is also the first English study to demonstrate the impact of MMR vaccination on measles and mumps encephalitis admissions. Ongoing disease surveillance and identification of enhanced diagnostic techniques remain crucial.

3. A population based observational study of childhood encephalitis in children admitted to paediatric intensive care units in England and Wales

The clinical course of encephalitis can be variable and severe cases can occur, resulting in admission to the intensive care unit (ICU). Although there are studies describing the incidence of childhood encephalitis in England,[30,174,333] there are no studies that have reported data for children with severe encephalitis. Thus, the incidence of severe encephalitis in England is unknown. A previous study of encephalitis in England estimated the cost of encephalitis to the NHS to be £40 million per year.[174] This estimate was based on bed days of hospital occupancy and did not take into account other associated costs such as those incurred from ICU admission, expensive investigations and in-patient rehabilitation, and is therefore likely to be an underestimate. Data on the use of intensive care resources by children with encephalitis are necessary to complement pre-existing data and improve our understanding of the potential true cost burden of encephalitis.

The aim of this study was to describe a cohort of children with encephalitis admitted to paediatric intensive care units (PICU) in E&W over a 10-year period, and to define the PICU incidence and mortality rates. In addition, this study aimed to explore predictors of clinical outcomes in this cohort, specifically mortality and length of PICU stay.

3.1 Methods

3.2 Patient identification and datasets

Individuals aged 0 to 17 years with a primary or secondary diagnosis of encephalitis who were admitted to any of the 29 PICUs in E&W between January 1, 2003 and December 31, 2013 were included in this study. Patients were identified through a retrospective search of the Paediatric Intensive Care Audit national network (PICANet) database using Clinical Terms 3 (Read) codes, which were mapped to the WHO ICD-10 codes for encephalitis. PICANet includes demographic and clinical information on all children admitted to any PICUs in England and Wales. [334] The Clinical Terms 3 (Read) code is a five-character length code used to categorise each PICU admission.[335] Data available from PICANet on identified patients included in this study were age on admission, sex, the need for and duration of invasive ventilation (DOIV), renal support and vasoactive treatment, PICU length of hospital stay (LOHS), Paediatric Index of Mortality 2 (PIM2) scores on admission, and mortality. The PIM2 score (0-100%) is used to predict the risk of mortality for patients admitted to PICU. [336] Mortality was defined as any death that occurred while on PICU or within one calendar month following discharge from PICU.

3.3 Classification of cases and definitions

Cases were grouped into five age groups <1 year, 1-4 years, 5-9 years, 10-14 years and ≥15 years; and three main aetiological groups: 'infectious'(INF), if a causal pathogen featured in the diagnosis from PICANet, 'immune-mediated' (IMM), if a known causal autoantibody target (e.g. NMDAR) featured in the diagnosis or they had a diagnosis of a demyelinating form of encephalitis including ADEM and 'unspecified' (UNS), if they did not meet either of these criteria.

Patients in the INF encephalitis group were further categorised to two main subgroups: viral and bacterial encephalitis. The viral encephalitis subgroup comprised three subgroups: HSV, EV and VZV encephalitis. Patients were assigned to three PIM2 score groups: <10%, 10-20% and >20%.

3.4 Statistical analysis

The PICU encephalitis incidence and mortality rates were calculated using the mid-year (2008) population estimates for people in E&W aged 0-17years (11.8 million) obtained from the Office of National Statistics database.[337] Descriptive statistics were used to report demographic data and clinical characteristics. Skewed data were expressed as median and interquartile range (IQR). Chi-squared and Fisher's tests were used to compare categorical outcomes across the three main aetiological groups The Kruskall-Wallis test was used to compare continuous variables across the main aetiological groups. The Wilcoxon-Mann-Whitney test was used to compare continuous variables between the viral and bacterial subgroups and between survivors and non-survivors. The Wilcoxon-Mann-Whitney test was also used to compare average ADEM PICU incidence rates between 2003-2006 and 2008-2013. Length of PICU stay was log-transformed and ANOVA was applied to test its association with age, sex, PIM2 category, need for invasive ventilation and renal support, DOIV, duration of renal support and vasoactive treatment, and main aetiological group using linear regression analysis[1]. Results were then back-transformed to provide geometric mean estimates and their 95% CIs. Binary logistic regression was used to test the association between mortality and age, sex, PIM2 score, need for invasive ventilation and renal support, DOIV, duration of renal support and vasoactive treatment and main

[1] Linear regression analyses were performed by Alecia Nickless

aetiological group. A significance level of 0.05 was chosen for testing statistical hypotheses. Data were analysed using SPSS 22 and Graph pad prism 7.0.

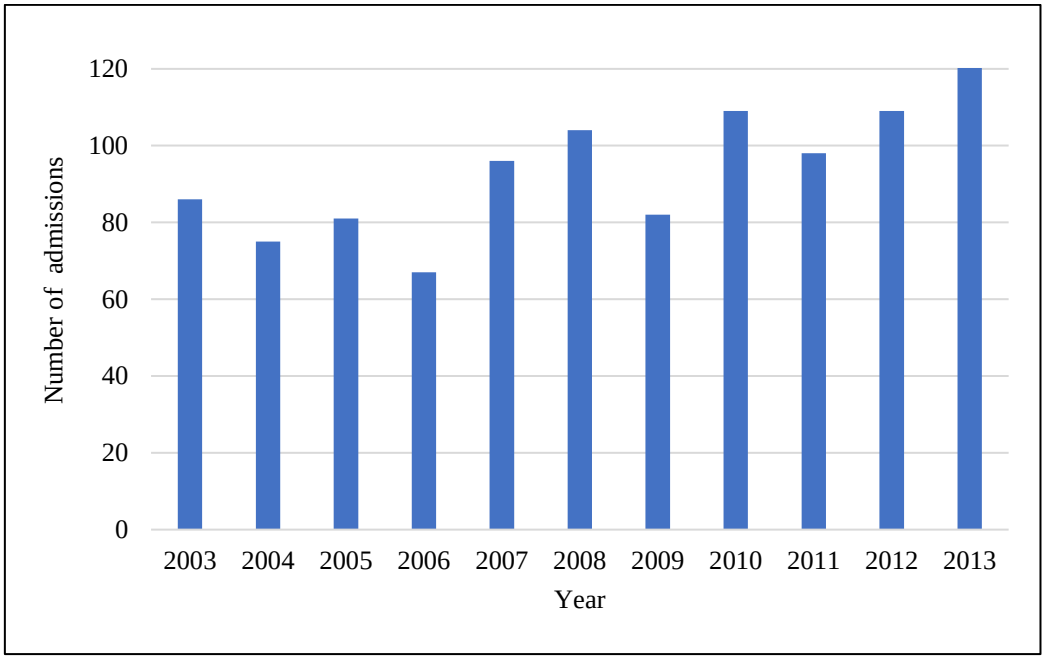
3.5 Ethical approval

Collection of personally identifiable data was approved by the Patient Information Advisory Group (now the NHS Health Research Authority Confidentiality Advisory Group).[338] Ethical approval was granted by the Trent Medical Research Ethics Committee, ref. 05/MRE04/17 +5.

3.6 Results

There were 1031 encephalitis cases admitted to PICU between 2003 and 2013, giving an average PICU incidence in E&W of 0.79/100,000 children (0-17years) per year (95%CI 0.74-0.84). Encephalitis accounted for 0.58% of all PICU admissions during the study period (total number of admissions=178686). Encephalitis PICU admissions varied by time period, with a gradual increase in the number of cases across the decade, and were highest in 2013 (n=124; 12.0%) and lowest in 2006 (n=67; 6.5%) (Figure 3.1).

Figure 3.1 Year on year encephalitis PICU admissions in England and Wales for people aged 0-17 years



All encephalitis

Baseline characteristics and outcomes for all admitted cases are shown in Table 3.1. Most cases (567/1031; 55.0%) were male. The median age at admission was 4.0 years (IQR1.0-10.0). The proportion of children in each age group category was highest in the 1-4 year group (n=330; 32%) and lowest in the ≥ 15years age group (n=61, 5.5%). Data on invasive ventilation were available for 1024 (99.3%) cases. In total, 808/1024 (78.9%) encephalitis cases received invasive ventilation compared with 11, 8492 of 17, 7654 (66.7%) non-encephalitis cases (unadjusted odds ratio (95%CI): 1.87(1.61-2.17); p= <0.0001). Data on DOIV was available for 806 cases. The median DOIV was 3.0 days (IQR 2.0-5.0). The median PIM2 score (%) on admission was 3.3 (IQR 1.9-4.8), most cases (n=699, 67.8%) had a PIM2 score of 1-5% on admission.

The median length of PICU stay (days) for all cases was 2.3 (IQR1.1-4.9). Overall, there were 87 deaths (8.4%) in patients with an encephalitis diagnosis compared with 7827

deaths (4.4%) in patients with a non-encephalitis diagnosis (unadjusted odds ratio, OR (95%CI): 1.99, 1.60-2.49), p=<0.0001. This puts the PICU encephalitis mortality rate in E&W (0-17 years) at 0.07 /100,000/year (95%CI 0.05to 0.08). Deaths in the encephalitis group accounted for 1.1% of all PICU deaths in the study period. The case fatality rates for the age group categories were 11.3% (<1y), 7.9% (1-4y), 9.6% (5-9y), 6.3% (10-14y) and 4.9% (15+y). A comparison of survivors versus non-survivors is shown in Table 3.2. The proportions of children who received invasive ventilation, renal support and vasoactive treatment were higher in the non-surviving group than in the surviving group (p=<0.0001). Those that died were ventilated for longer than those that survived (p=<0.0001). The PIM2 scores on admission were lower for those that survived than for those that died (p=<0.0001). There were no significant differences in age, sex distribution, main aetiological group split, and PICU length of stay between survivors and non-survivors.

Table 3.1 Baseline characteristics of children (0-17years) with encephalitis admitted to PICU in E&W

	All (N=1031)		INF (N=202, 19.6%)	IMM (N=83, 8.1%)	UNS (N=746, 72.4%)	P value[1]
Male	567 (55.0)		119 (58.9)	44 (53.0)	404 (54.2)	0.4503
Age (years)	4.0 (1.0-10.0)		2.0 (0.0-7.0)	5.0 (2.0-8.0)	5.0 (1.0-10.0)	<0.0001
Age category						
<1 y	195 (18.9)		68 (33.7)	3 (3.6)	124 (16.6)	<0.0001
1-4y	330 (32.0)		67 (33.2)	35 (42.2)	228 (30.6)	0.0917
5-9y	239 (23.2)		30 (14.9)	31 (37.4)	178 (23.9)	0.0002
10-14y	206 (20.0)		26 (12.9)	10 (12.1)	170 (22.8)	0.0013
≥ 15y	61 (5.9)		11 (5.4)	4 (4.8)	46 (6.2)	0.8422
Invasive ventilation	808/1024 (78.9)		155/202 (76.7)	62/83 (74.7)	591 /739 (80.0)	0.3751
DOIV	3.0 (2.0-5.0) (N=806)		3.0 (2.0-5.0)	4.5 (2.0 -10.0)	2.0 (2.0-5.0)	<0.0001
Vasoactive treatment (VT)	216/983 (22.0)		46 /200 (23.0)	14/79 (17.7)	156/704 (22.2)	0.6157
Duration of VT	3.0 (2.0-4.0) (N=100)		3.0 (2.0-4.0) (N=17)	2.0 (2.0-3.0) (N=7)	3.0 (1.0-4.5) (N=76)	0.3988
Renal support	50/890 (5.6)		5/194 (2.6)	12/76 (15.8)	33/620 (5.3)	0.0001
DORS	5.0 (3.0-8.0) (N=37)		12 (3.0 -15.0) (N=3)	5.5 (4.0-7.0) (N=10)	5.0 (1.0 -8.0) (N=24)	0.2571
PIM 2 score	3.3 (1.9-4.8)		3.4 (1.5 - 5.7)	2.9 (1.5-3.9)	3.4 (2.3-4.8)	0.0181
PIM2 score category						
<10%	961 (93.2)		180 (89.1)	81 (8.4)	700 (93.8)	0.0154
10-20%	26 (2.5)		8 (4.0)	1 (1.2)	17 (2.3)	0.2915
>20%	44 (4.3)		14 (6.9)	1 (1.2)	29 (4.9)	0.0585
Length of PICU stay (days)	2.3 (1.1-4.9)		2.5 (1.2-4.9)	3.5 (1.8-9.0)	2.1 (1.1-4.7)	0.0013
Long PICU stay	51 (4.9)		4 (2.0)	10 (12.0)	37 (5.0)	0.0018
Mortality	87 (8.4)		22 (10.9)	4(4.8)	61 (8.2)	0.2181

Key: Data shown are median (interquartile range, IQR) for continuous variables or number (%) for categorical variables. INF=infectious encephalitis, IMM=immune mediated encephalitis; UNS=encephalitis of unspecified cause; DOIV=duration of invasive ventilation; DORS=duration of renal support; PIM2= Paediatric Index of Mortality score, second version. N= number of children who received a particular treatment and had available data. Long PICU stay = any PICU length of stay > the 95th centile of the length of stay for all PICU encephalitis admissions of 16.7 days. E&W=England and Wales

[1] Kruskal Wallis Test / Chi Square Test

Table 3.2 Comparison between PICU encephalitis survivors and non-survivors in E&W

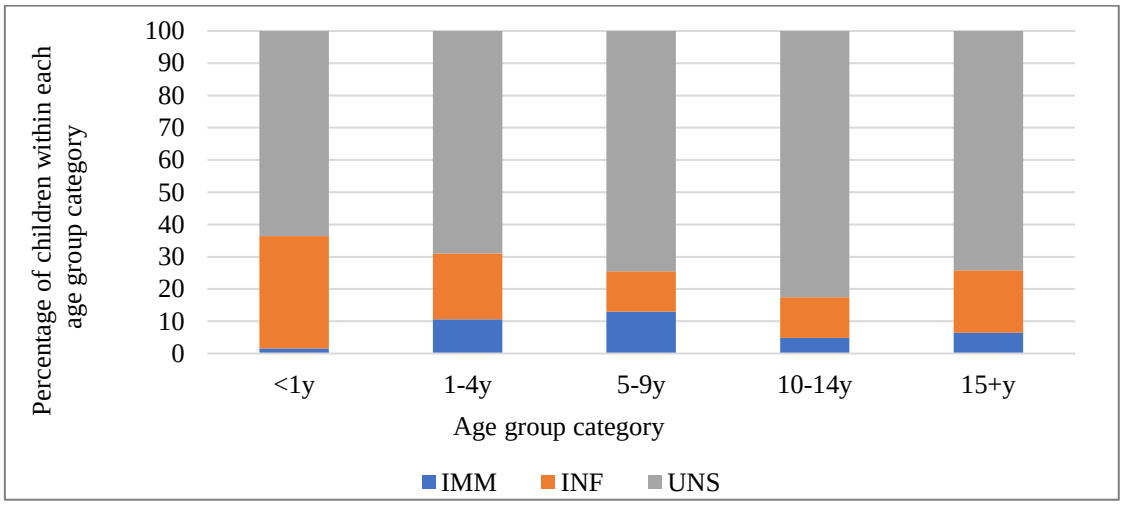
	Survived (N=944; 91.6%)	Died (N=87, 8.4%)	P value[1]
Sex (male)	523 (55.4)	44 (50.6)	0.4309
Age (years)	4.0 (1.0 -10.0)	4.0 (0.0-8.0)	0.0834
Age (weeks)	253 (77-538)	213 (46-253)	
Age category			0.3090
<1 y	173 (18.3)	22 (25.3)	
1-4 y	304 (32.2)	26 (29.9)	
5-9 y	216 (22.9)	23 (26.4)	
10-14y	193 (20.4)	13 (14.9)	
≥15y	58 (6.1)	3 (3.5)	
Aetiological group			0.2181
INF	180 (19.1)	22 (25.3)	
IMM	79 (8.4)	4 (4.6)	
UNS	685 (72.6)	61 (70.1)	
Invasive ventilation	726/939 (77.3)	82/85 (96.5)	<0.0001
DOIV	2.0 (2.0- 5.0) (N=724)	3.0 (2.0-5.3) (N=82)	<0.0001
Vasoactive treatment	161/901 (17.9)	55/82 (67.1)	<0.0001
Duration of vasoactive treatment	3.0 (1.8-4.0)	3.0 (1.8-5.0)	0.4624
Renal support	40/819 (4.9)	10/71 (14.1)	0.0042
Duration of renal support	5.0 (1.0-8.0) (N=31)	6.5 (4.5-12.8) (N=6)	0.1985
PIM2 score	3.3 (1.7-4.4)	8.2 (3.9-60.2)	<0.0001
PIM2 category			<0.0001
<10%	913 (96.7)	48 (55.2)	
10-20%	20 (2.1)	6 (6.9)	
>20%	11 (1.2)	33 (37.9)	
Length of PICU stay	2.2 (1.1-4.9)	2.5 (1.2 -5.5)	0.2855

Key: Data shown are median (interquartile range, IQR) for continuous variables or number (%) for categorical variables. INF=infectious encephalitis, IMM=immune mediated encephalitis; UNS=encephalitis of unspecified cause; DOIV=duration of invasive ventilation; DORS=duration of renal support; PIM2= Paediatric Index of Mortality score, second edition; Denominator=total number in the group or who received a particular treatment unless otherwise specified; N=number with available data. E&W=England and Wales

[1]Based on Kruskal Wallis Test / Chi Square Test

Cases were categorised into three main aetiological diagnosis groups: INF (n=202, 19.6%), IMM (n=83, 8.1%) and UNS (n=746, 72.4%). The proportion of patients assigned to each of the three main aetiological diagnosis group by age category is shown in Figure 3.2. The proportion of children with UNS encephalitis was highest across all age groups. The proportion of children in the INF group assigned to each age group category were 35% (<1yr), 20% (1-4y), 13% (5-9y and 10-14y), 19% (15+y). Summary statistics for each main aetiological diagnosis group are shown in Table 3.1. Children assigned to the INF group were younger (median, IQR: 2.0 years, 0.0-7.0) than those assigned to the IMM (5.0 years, 2.0-8.0) and UNS groups (5.0 years, 1.0-10.0); p=<0.0001. For those ventilated, the median DOIV was longer for the IMM than the UNS and INF groups (p=<0.0001). The proportion of children who received renal support was higher in the IMM than the UNS and INF groups (p=0.0001). Length of PICU stay was longer for children in the IMM than the INF and UNS groups (p=0.0013). PIM2 score on admission was lower for children in the IMM than the UNS and INF group (p=0.0181). There were no significant differences in the sex distribution, proportion of children that received invasive ventilation, and vasoactive treatment, duration of renal support and vasoactive treatment, and mortality across the main aetiological groups.

Figure 3.2 Main aetiological diagnosis group split by age group for children admitted to PICU in E&W with encephalitis



Key: IMM=immune mediated encephalitis, INF=infectious encephalitis, UNS=unspecified encephalitis, E&W=England and Wales

INF encephalitis

Cases in the INF group were further categorised into two main subgroups: viral (n=118/202; 58.4%) and bacterial (n=75/202; 37.1%). Nine (4.5%) further cases in the INF group had a diagnosis of malaria encephalitis. Summary characteristics for the viral and bacterial subgroups are shown in Table 3.3. Children assigned to the viral encephalitis subgroup were younger (median age (years), IQR: 1.0 year, IQR 0.0-6.0) than those assigned to the bacterial encephalitis subgroup (3.0 years, 1.0-9.0) (p=0.0039). There were no significant differences between children in the viral and bacterial encephalitis subgroups for the other outcomes that were assessed (Table 3.3).

The individual diagnoses for those assigned to the viral encephalitis group (n=118) were HSV (n=72, 61.0%), VZV (n=28, 23.7%), EV (n=10, 8.5%), influenza (n=3, 2.5%), mumps (n=2, 1.7%), post measles (n=1, 0.8%), and chronic echovirus encephalitis (n=2, 1.7%). The number of admissions year-on-year for the main viral aetiologies HSV, VZV

and EV are summarised in Figure 3.3. There were no significant differences in the baseline characteristics between the three main viral aetiologies.

Of those assigned to the bacterial encephalitis group (n=75), 65 (86.7%) had a diagnosis of meningococcal encephalitis, 6 (8.0%) had tuberculous (TB) encephalitis and 4 (5.3%) had encephalitis due to listeria infection.

The proportion of children for each infectious aetiology by age group is summarised in Figure 3.4. Most cases with a diagnosis of HSV or EV encephalitis were in the <1 year age group, whereas for malaria, VZV and TB encephalitis, most children were in the 1-4 years age group.

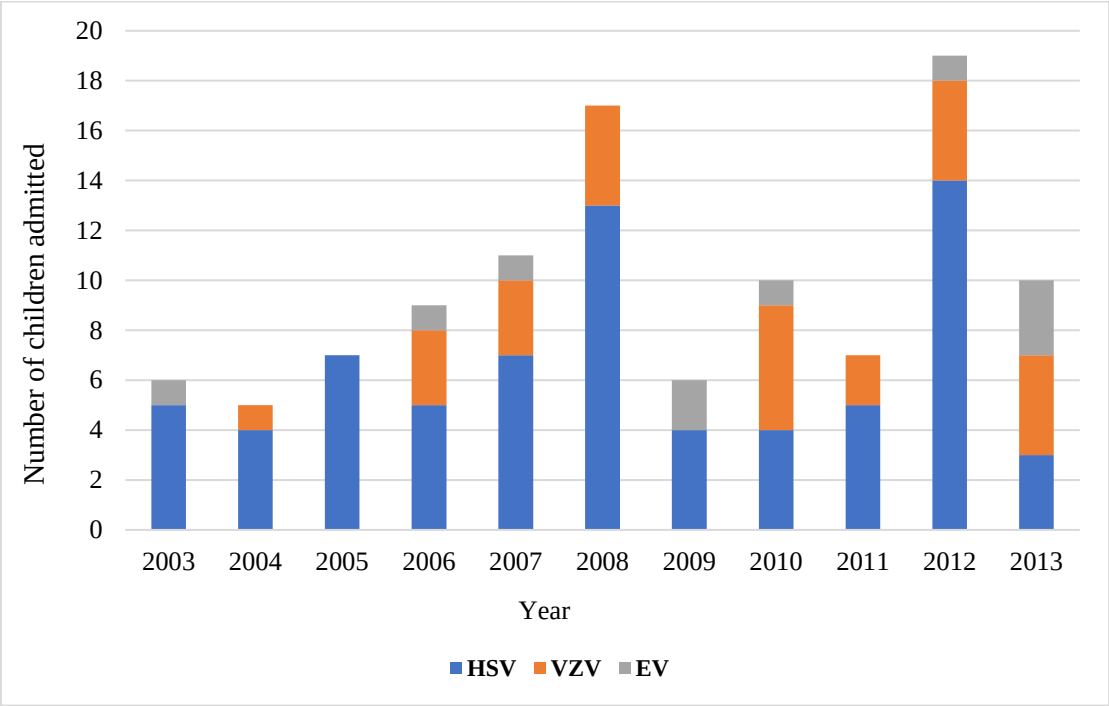
The calculated average PICU incidence and mortality rates for the main viral aetiologies are summarised in Table 3.4.

Table 3.3 Comparison of baseline characteristics between children with viral and bacterial encephalitis admitted to PICU in E&W

	Viral (N=118)	Bacterial (N=75)	P value
Sex (male)	68/118 (57.6)	46/75 (61.3)	0.6540
Age (years)	1.0 (0.0-6.0)	3.0 (1.0-9.0)	0.0039
Invasive ventilation	87/118 (73.7)	63/75 (84.0)	0.1115
DOIV	3.0 (2.0-5.0) (N=87)	3.0 (2.0 -5.0) (N=63)	0.7881
Vasoactive treatment	23/116 (19.8)	23/75 (30.7)	0.1183
Duration of vasoactive treatment	3.5 (2.0-5.0) (N=10)	3.0 (2.0-4.0) (N=7)	0.6121
Renal support	5/113 (4.4)	0/73 (0)	0.1584
Duration of renal support	12 (3-15) N=3	N/A	N/A
PIM2 score	3.3 (1.4-5.6)	3.9 (2.3-6.0)	0.3245
Length of PICU stay	2.5 (1.0 -4.9)	2.5 (1.6-5.0)	0.4565
Mortality	11/118 (9.3)	11/745 (14.7)	0.2573

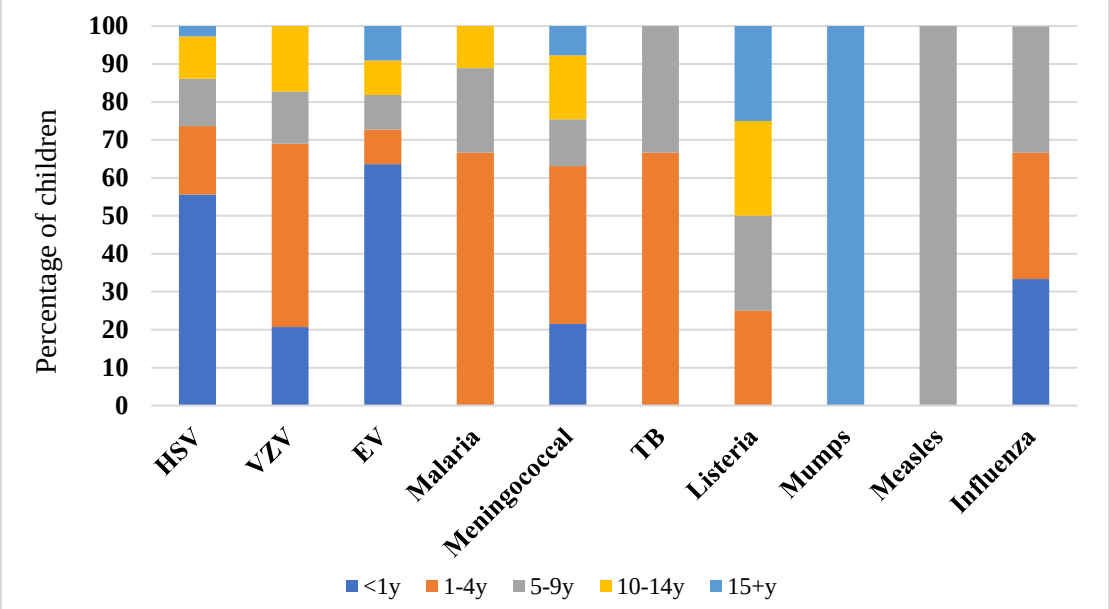
Data shown are median (interquartile range, IQR) for continuous variables or number (%) for categorical variables. INF=infectious encephalitis, IMM=immune mediated encephalitis; UNS=encephalitis of unspecified cause; DOIV=duration of invasive ventilation; DORS=duration of renal support; PIM2= Paediatric Index of Mortality score, second edition; Denominator is the total number in each group who received a particular treatment unless otherwise specified N=total number of children with available data, N/A=not applicable. E&W=England and Wales

Figure 3.3 Year on year PICU admissionsin E&W for the main viral encephalitis aetiologies



Key: HSV=herpes simplex virus, VZV=varicella zoster virus, EV=enterovirus. E&W=England and Wales

Figure 3.4 Proportion of children admitted to PICU in E&W with encephalitis by age group for each infectious aetiology



Key: HSV=herpes simplex virus, VZV=varicella zoster virus, EV=enterovirus, TB=tuberculous. E&W=England and Wales

Table 3.4 PICU incidence and mortality rates in E&W for the main viral aetiologies

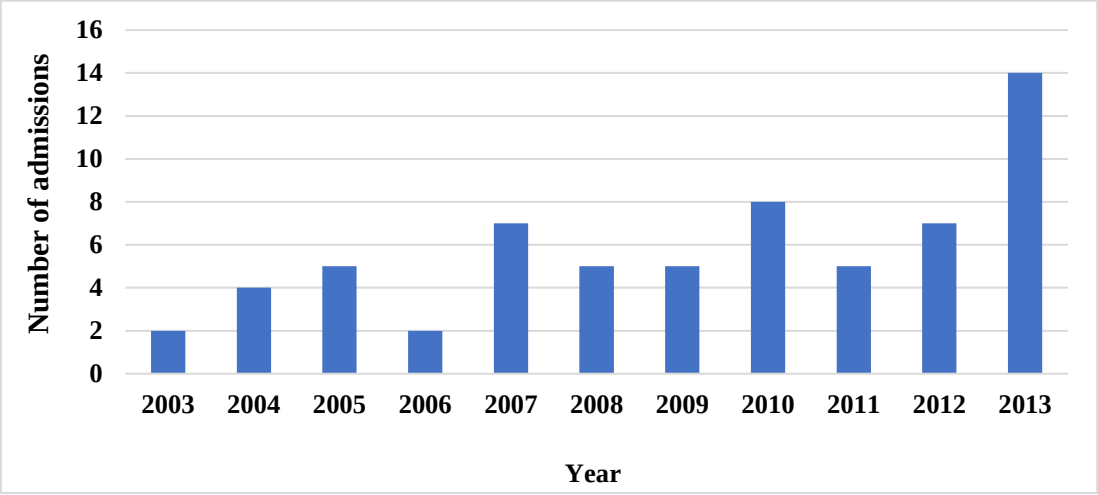
	HSV	VZV	EV
No. of admissions	72	28	10
PICU incidence rate	0.06/100,000/year (0.03-0.08)	0.02/100,000/year (0.01-0.03)	0.01/100,000/year (0.002-0.013)
No. of deaths	7	3	0
PICU mortality rate	0.005/100,000/year (0.003-0.008).	0.002/100,000/year (0.000-0.005)	0

Key: HSV=herpes simplex virus, VZV=varicella zoster virus, EV=enterovirus. E&W=England and Wales

IMM encephalitis

A total of 83 cases (8.1%) were assigned to the IMM group of which 74 (89.2%) had a diagnosis of ADEM, and most of these (n=40, 54.1%) were male. This gives an average PICU ADEM IR (0-17 years) in England and Wales at 0.06 per 100,000 per year (95%CI 0.03-0.08). An increasing trend in the number of ADEM admissions was seen over the study period (Figure 3.5)

Figure 3.5 Year on year ADEM PICU admissions in E&W for people aged 0-17 years



Key: E&W=England and Wales

Between 2003 and 2006, there were 13 ADEM admissions, giving an ADEM PICU incidence of 0.03/100,000/year (95%CI 0.01-0.05) in this period. Between 2008 and 2011, there were 56 ADEM admissions to PICU, giving an ADEM PICU incidence of 0.08/100,000/year (95%CI 0.04-0.12) during this period. The ADEM PICU incidence between 2008 and 2011 was significantly higher than between 2003 and 2006 (p=0.0190). There were 4 deaths (5.4%) in the ADEM group, giving an ADEM PICU mortality rate of 0.003/100,000/year (95%CI 0.000 – 0.008).

The median age in the ADEM group was 5.0 years (IQR 2.0-7.0). The numbers (%) of ADEM cases that received invasive ventilation, vasoactive treatment and renal support were 57 (77.0%), 13 (18.6%) and 9 (13.4%) respectively. The median duration in days (IQR) for each treatment was 5.0 (2.0-10.0) for invasive ventilation (n=57), 2.0 (2.0-3.0) for vasoactive treatment (n=7), and 5.0 (4.0-7.5) for renal support (n=8). The median PICU length of stay (days) and PIM2 score (%) on admission for the ADEM group were 3.5 (IQR 1.8-9.1) and 3.0 (1.7-3.9) respectively. The documented diagnoses for other cases in the IMM group included NMDAR encephalitis (n=4, 4.8%), Rasmussen's encephalitis (n=2, 2.4%), limbic encephalitis (n=1, 1.2%), Hashimoto encephalitis (n=1, 1.2%), and 'autoimmune encephalitis' (n=1, 1.2%).

Predictors of outcomes

In a binary logistic regression analysis with multiple covariates, risk factors associated with death were vasoactive treatment, DOIV, length of PICU stay and PIM2 score on admission (Table 3.5). The odds of dying for children who received vasoactive treatment would be expected to be 0.12 fold lower than the odds for those who did not (OR 0.122; 95%CI 0.045- 0.328, p=<0.001). For a ventilated child, a one day increase in the duration of ventilation (days) would be expected to increase the odds of the child dying by 1.9

times (OR 1.869; 95%CI 1.216-2.874, p=0.004). For each additional day spent on PICU, the odds of dying would be expected to decrease by 0.6 fold (OR=0.563; 95%CI 0.369-0.858, p=0.0076). For each one unit increase in the PIM2 score on admission, the odds of dying would be expected to increase by 1.1 times (OR 1.058; 95%CI 1.0333-1.0832, p=<0.0001).

Table 3.5 Logistic regression exploring factors associated with mortality in children (0-17 years) admitted to PICU with encephalitis in E&W

	Exp (B)	Lower CI	Upper CI	P value
Sex (male)	1.1884	0.5659	2.4956	0.6485
Age (years)	0.9667	0.8935	1.046	0.4003
Main aetiological category				0.8704
IMM	1.1676	0.471	2.8944	0.738
UNS	0.7448	0.154	3.6016	0.7141
Vasoactive treatment (yes)	0.1215	0.045	0.3277	<0.0001
Duration of vasoactive treatment	0.9134	0.7419	1.1245	0.3932
Renal support (yes)	0.8253	0.0947	7.1964	0.8621
Duration of renal support	1.0977	0.8606	1.4002	0.4527
Invasive ventilation (yes)	0.5774	0.0994	3.3552	0.5407
Duration of invasive ventilation	1.8694	1.2162	2.8736	0.0043
Length of PICU stay	0.5626	0.3688	0.8583	0.0076
PIM2 score on admission	1.058	1.0333	1.0832	<0.0001

Key: IMM=immune mediated encephalitis; UNS=unspecified encephalitis, PICU=paediatric intensive care unit; PIM2 score =Paediatric Index of Mortality score second edition; B=beta coefficient, Exp (B) =odds ratio, CI= confidence interval. E&W=England and Wales

In a linear regression analysis, mortality, renal support, vasoactive treatment, invasive ventilation, age group and main aetiological group predicted length of PICU stay (Table 3.6).

Table 3.6 Linear regression exploring factors associated with length of PICU stay in children aged (0-17 years) admitted to PICU with encephalitis in E&W

	OR	Lower CI	Upper CI	P value
Sex (male)	1.06	0.93	1.22	0.379
Invasive ventilation (yes)	2.43	2.05	2.87	<0.0001
Vasoactive treatment (yes)	1.84	1.54	2.21	<.0001
Renal support (yes)	2.28	1.68	3.08	<0.0001
Age (<1y)	1.21	1.02	1.45	0.0332
PIM2 category (<10)	1.19	0.80	1.77	0.3787
PIM2 category (≥10 &	1.44	0.81	2.54	0.2095
Aetiological category	1.59	1.24	2.04	0.0003
Aetiological category	1.01	0.85	1.19	0.9516
Died (yes)	0.71	0.53	0.96	0.0242

Key: Dependent variable: log length of PICU stay. Predictors in the Model: (Constant), died (yes), sex (male), age (<1y), invasive ventilation (yes), renal support (yes), aetiological category (IMM), aetiological category (UNS), vasoactive treatment (yes), PIM2 category. IMM=immune mediated encephalitis, UNS=unspecified encephalitis, CI=confidence interval, OR=odds ratio. E&W=England and Wales

The average PICU length of stay would be expected to be 0.71 times shorter in non-survivors compared with survivors (95%CI 0.527-0.956, p=0.0242). The PICU length of stay would be expected to be, on average, 2.3 times longer (95%CI 1.68-3.08, p=<0.0001) for children who receive renal support than those who do not (adjusting for sex, age group, mortality, need for invasive ventilation, vasoactive treatment, main aetiological group and PIM2 category). The PICU length of stay would be expected to be on average1.8 times longer for children who receive vasoactive treatment (95%CI 1.54-2.21, p=<0.0001) compared with those who do not (adjusting for sex, age group, mortality, need for invasive ventilation, renal support, main aetiological group and PIM2 category). The PICU length of stay would be expected to be on average 2.4 times longer (95%CI 2.05-2.87, p=<0.0001) for those who receive invasive ventilation compared with those

who do not (adjusting for sex, age group, mortality, need for vasoactive treatment, renal support, main aetiological group and PIM2 category). The length of PICU stay for children aged less than 1 year would be expected to be on average 1.2 times longer (95%CI 1.02 to 1.45, p=0.0332) than that for children older than 1 year (adjusting for sex, mortality, need for invasive ventilation, renal support and vasoactive treatment, main aetiological group and PIM2 category). The length of PICU stay for children < 1 year would be expected to be on average 1.6 times longer (95%CI 1.24-2.04, p=0.0003) for children with IMM encephalitis compared with those with INF encephalitis (adjusting for sex, age group, mortality, need for invasive ventilation, renal support and vasoactive treatment, and PIM2 category).

3.7 Discussion

This is the first study to describe all-cause encephalitis admissions to PICU and to define the PICU encephalitis incidence and mortality rates in E&W. It is also the first study to show usage of intensive care resources by children with encephalitis in E&W. From these data, the PICU incidence and mortality rates for all-cause encephalitis in children aged 0 to 17 years in E&W were estimated to be 0.79/100,000children/year (95%CI 0.74-0.84) and 0.07 /100,000children/year (95%CI 0.05-0.08) respectively. The data suggest a high use of intensive care resources by children with severe encephalitis and gives an insight into the burden of severe encephalitis. Particularly, mortality and requirement for invasive ventilation were higher in children admitted to PICU who had a diagnosis of encephalitis compared with those who had a non-encephalitis diagnosis. Additionally, factors associated with mortality were identified, notably vasoactive treatment, PIM2 score on admission, duration of invasive ventilation, and PICU length of stay.

In this study, an encephalitis diagnosis was made in 0.58% of all PICU admissions and

mortality in children with a diagnosis of encephalitis accounted for 1.1% of all PICU deaths in the study period. The proportion of deaths in this study was 8.4%. These figures are relatively lower than was reported in a previous study of PICU encephalitis admissions in Hong Kong in which encephalitis admissions accounted for 2.7% and 11.8% of all PICU admissions and deaths respectively,[339] and the proportion of deaths was 28%. The Hong Kong study was a single centre study of children less than 12 years with a diagnosis of infectious encephalitis. Furthermore, the catchment population in that study was 1.1 million, of which approximately 25% were children less than 12 years. Thus, differences in methodology, and the size and structure of the sampling population may account for the relative differences in the proportion of deaths reported in the previous study and this study. The case fatality rate in this study was 2 times lower than reported in an adult French ICU study,[340] and 3 times lower than was observed in a North Indian study of ICU admissions of all ages in which only 18% of cohort were <18 years,[256] indicating worse outcomes in adults.

The findings of this study reflect the data presented in Chapter 2 of this thesis, with an observed year on year increase in the number of ADEM admissions. Particularly, the PICU ADEM incidence for the period after publication of the consensus definition for ADEM (2008-2013) was significantly higher than for the period preceding this. While this observation is likely to reflect an increase in case recognition, the possibility that this increase was due to a true rise in the number of cases cannot be ruled out. Furthermore, the observations in the ADEM cohort in this study are similar to previously reported data on ADEM PICU admissions in E&W[341] in terms of PICU ADEM incidence rate, age distribution, proportion of cases requiring invasive ventilation (including the duration of ventilation) and vasoactive treatment. The proportion of ADEM cases that were

ventilated in this cohort is similar to the findings in an earlier adult ADEM PICU study in France. [342] The PICU ADEM mortality rate and proportion of deaths in this study were two times lower than previously reported in E&W.[341] Additionally, the proportion of deaths in the ADEM group in this cohort was 4.5 times lower than was reported in the adult French cohort.[342] The lower proportion of deaths in this study could reflect an improvement in outcomes for patients with ADEM due to improved case ascertainment with the more widespread use of MRI, and therefore, timelier institution of immunomodulatory treatments. Also, the French ADEM cohort [342] were adults and the higher mortality rate could reflect the more severe disease course and poorer outcomes in adults with ADEM compared to children.[343]

This study gives useful insight into the burden of severe encephalitis and provides valuable information on the potential additional cost burden from severe encephalitis. Eighty percent of patients with a diagnosis of encephalitis admitted to PICU received invasive ventilation while 20% and 6% respectively received vasoactive treatment and renal support during the PICU admission. In particular, the proportion of cases who required invasive ventilation was higher in those with an encephalitis diagnosis than among those who had a non-encephalitis diagnosis. Additionally, the odds of dying for those with an encephalitis diagnosis was twice that for those with a non-encephalitis diagnosis. In a previous encephalitis study in England,[174] the cost to the NHS was estimated at £40 million per year. However, this estimate did not take into account additional cost implications from admission to intensive care. With a median PICU encephalitis length of stay of 2.3 days (IQR 1.1-4.9), and a population size of 11.8 million, an incidence rate of 0.79/100,000/year equates to 214 bed days of intensive care occupancy per year. Based on a PICU bed-day cost of £1932,[344] the PICU encephalitis

bed cost is estimated at approximately £414,230 (IQR 198,111-882,495) per year. This does not take into account treatment, staff and other variable costs such as costs for procedures and treatment costs. In a study of PICU admissions in Spain,[345] the estimated mean fixed cost of an admission was $608 per patient per day with a mean variable cost of approximately $218 per patient per day. In another study of PICU admissions,[346] the mean incremental cost of mechanical ventilation was estimated at $1,522 per day and in this study. Based on a median length of PICU stay of 2.3 days, and a median duration of invasive ventilation of 3 days, these cost estimates suggest an additional cost burden of childhood encephalitis from PICU admission of approximately £565, 000 per year.

Factors associated with mortality and PICU length of stay were evaluated in this study. Knowledge of outcome predictors is important in the management of children with encephalitis and considering these outcomes could lead to better outcomes. In this study, for children with severe encephalitis, mortality was predicted by vasoactive treatment requirement, DOIV, length of PICU stay, and PIM2 score on admission. The association between PIM2 score and mortality further validates the usefulness of this tool as a predictor of mortality. Requirement for invasive ventilation, renal support and vasoactive treatment was higher in non-survivors than survivors, in keeping with the findings of the Hong Kong study,[339] possibly reflecting a more severe clinical course in non-survivors compared with survivors. However contrary to previous data from the Hong Kong study in which 89% of the non-survivors were male,[339] a difference in the sex distribution of survivors and non-survivors was not observed in this study. Notably, PICU length of stay would be expected to be longer for patients who required invasive ventilation, vasoactive treatment and renal support compared with those patients who did not require these treatments, again indicating a more severe illness and thus slower recovery in those

receiving these interventions. Other clinical predictors of outcomes in encephalitis have been described in several studies,[261,340,342,347,348] and factors including a Glasgow Coma Score of <8, thrombocytopaenia, cerebral oedema and a longer interval between hospitalisation and ICU admission have been associated with poor outcomes. Data were not available to investigate these previously reported clinical predictor factors in this study. Similar to previous reports, [113,261] there was no association between sex and mortality in this study, although this has been reported in other studies.[22,178] In a previous study in Finland, [113] children with HSE had a 12-fold greater risk of death than those with encephalitis due to other viral aetiologies. In this study, no difference was observed in the proportion of deaths across the main viral aetiology groups. Nonetheless, validation of these findings using prospectively collected data is warranted. Data from an ongoing study of encephalitis ICU admissions across Europe[349] might be useful in validating these findings.

The main strength of this paper is that it provides across-the-board data on encephalitis admissions to all PICUs in E&W during the study period. However, there are some limitations. PICANet data rely on accurate reporting of clinical information and recording onto the database and data were missing for some of the outcomes of interest. Furthermore, mapping of Read codes to WHO ICD 10 codes is not entirely straightforward and some encephalitis cases may have been missed. We did not have access to microbiological and radiological data so there is the possibility of incorrect assignation of cases particularly to the UNS encephalitis group. Since readmission to PICU is possible, the use of admission rates as a proxy for incidence rate could overestimate the true incidence. Linkage of the PICU data to national data contained in HES would have provided a richer dataset to provide an accurate account an estimation

of the true incidence of severe encephalitis in E&W. Nevertheless, reported readmission rates to PICU are low, between 2 and 4%,[350,351] therefore a significant overestimation of the PICU encephalitis incidence rate is less likely.

3.8 Conclusion

This study provides useful information to complement existing data sources to inform on the burden of encephalitis. Despite being rare, encephalitis potentially constitutes a significant cost for the NHS. Future studies are warranted to accurately quantify the cost of encephalitis and to enhance appropriate diagnosis and treatment. Knowledge of outcome predictors will aid better management of children with encephalitis and might help to improve outcomes.

4 Intravenous immunoglobulin for the treatment of encephalitis in children: a systematic review and meta-analysis

4.1 Introduction and background

The review of the literature in Chapter 1 as well as data presented in Chapter 2 of this thesis highlight the potential health, economic and social impact of encephalitis. The extent of brain inflammation in encephalitis is suggested to be a key determinant of outcomes.[352] Attenuation of such inflammation either as a direct effect or through modulation of the immune response may therefore improve clinical outcomes. Consequently, biologic agents like IVIG that possess anti-inflammatory and/or immunomodulatory properties might have a role in the treatment of encephalitis.[353]

Data from studies presented in Chapter 1 (section 1.10) of this thesis suggest a potential beneficial effect from IVIG in encephalitis. However, for the information from these reports to be useful, evaluation of the quality of the evidence is needed to make a conclusive statement on the role of IVIG in encephalitis.

Systematic reviews and meta-analyses provide a basis to inform health care decisions. Of particular importance are systematic reviews of randomised controlled trials (RCTs), which offer the highest level of evidence from which robust inferences e.g. regarding treatment effect could be made. To date, two systematic reviews of IVIG use in encephalitis have been published. One was a systematic review of immune therapy in AIE,[354] and the other was a systematic review of IVIG in neurological disorders.[355] None of these reviews included RCTs, studies of children with infectious encephalitis, nor a meta-analysis of findings.

The objective of the work described in this chapter was to systematically review evidence from RCTs on the role of IVIG in the treatment of childhood encephalitis.

4.2 Declaration

The methods and results of this systematic review presented here have been published in the Cochrane Systematic review database.[356] This systematic review was registered with the Cochrane Multiple Sclerosis and Rare Diseases of the CNS Group and a protocol was published prior to undertaking this review.

4.3 Methods

4.3.1 Review Objective

The main objective of this review was to assess the efficacy and safety of IVIG as add-on treatment for children with encephalitis.

4.3.2 Study selection and rationale

Only RCTs were included in this review.

4.3.3 Study population

This review included only trials conducted in children (6 weeks to 16 years) with acute (symptoms present within 24 hours prior to hospitalisation) or subacute (symptoms present between 24 hours and four weeks prior to hospitalisation) encephalitis. Trials including children with chronic encephalitis e.g. Rasmussen's encephalitis were excluded.

4.3.4 Intervention that were assessed in this review

The intervention assessed in this review was IVIG (irrespective of dose, time of commencement, and duration of treatment), used either alone or in combination with standard care, as defined in eligible trials.

4.3.5 Outcomes assessed in this review

There are no internationally agreed outcomes for encephalitis. Outcomes for this review were chosen following consultation with two paediatric neurologists based in a tertiary centre in London, UK.

Primary Outcome

- *Proportion of participants with a poor outcome (i.e. significant disability) at 6 months after treatment, as a binary outcome.* Any validated disability assessment scale was acceptable, including any of the following:
  - (i) Glasgow Outcome Scale Extended, GOSE[357]
  - (ii) Liverpool Outcome Score, LOS[358]
  - (iii) modified Rankin Scale, mRS[359]

Significant disability was defined as a score of > 2 on the GOSE and mRS and > 4 on the LOS.

- *Proportion of participants with at least one serious adverse event at 6 months after treatment*

Any definition or classification of serious adverse event used in a trial was acceptable. Where the method of classifying an adverse event was not reported, the decision was

made a priori to use the International Conference on Harmonisation (ICH) Guideline.[360] This guideline defines a serious adverse event as any adverse event that results in any of the following outcomes: is life-threatening, results in death, requires prolongation of hospital stay, results in persistent or significant disability/incapacity, congenital abnormality.

Secondary outcomes

Short-term outcomes (i.e. during the hospitalisation period)

- Length of hospital stay (either as continuous or categorised data, as reported in the trial)
- Proportion of participants requiring invasive ventilation and duration (either as continuous or categorised data, as reported in the trial).
- Time to resolution of fever (either as continuous or categorised data, as reported in the trial)
- Time to stop spasms (either as continuous or categorised data, as reported in the trial).
- Time to regain consciousness (either as continuous or categorised data, as reported in the trial).
- Time to resolution of neuropathic symptoms (either as continuous or categorised data, as reported in the trial).

Long term outcomes (up to 6 months after the hospitalisation period)

- Cognitive impairment as a binary outcome (yes/no), assessed using any validated age-appropriate psychometric instrument including but not limited to the following: (i) Bayley Scales of Infant Development,[361] (ii) Wechsler Preschool

and Primary Scale of Intelligence,[362] (iii) Wechsler Intelligence Scale for Children.[363]

- Poor adaptive functioning as a binary outcome (yes/no) using any validated age-appropriate scale including (i) Vineland Adaptive Behaviour Scales,[364,365] (ii) Adaptive Behaviour Assessment System,[366] (iii) Behavior Assessment System for Children Parent Rating Scales.[367] Where adaptive scores alone were reported, scores less than two standard deviations from the mean was to be used as an indicator of poor adaptive functioning.
- Quality of life assessment scores assessed using any validated age-appropriate tool including but not limited to the following: (i) Pediatric Quality of Life Inventory (PedsQL),[368] (ii) Health-related quality of life.[369]
- Diagnosis of epilepsy or ongoing seizures

4.3.6 Literature search [1]

Using an optimally sensitive strategy developed for the identification of RCTs,[370] a systematic search without any language or date restrictions, and based on a combination of free-text and Medical Subject Headings (MeSH) terms as illustrated in the Appendix of this thesis was conducted[2]. The following databases were searched:

- Cochrane Central Register of Controlled Trials (CENTRAL 2016, Issue 9)
- MEDLINE (PubMed) (1966 to 30 September 2016)
- EMBASE (Embase.com) (1974 to 30 September 2016)
- Cumulative Index to Nursing and Allied Health Literature (CINAHL) (EBSCO host) (1981 to 30 September 2016)

[1] Search of the main databases was conducted by the Trial Search Coordinator of the Cochrane Multiple Sclerosis and Rare Diseases of the Central Nervous System

- ClinicalTrials.gov (clinicaltrials.gov); World Health Organization (WHO) International Clinical Trials Registry Portal (ICTRP) search portal (apps.who.int/trialsearch/) (30 September 2016)
- Global Health Library (Virtual Health Library (www.globalhealthlibrary.net/php/index.php) (30 September 2016)[2]
- Science Citation Index Expanded (SCI-EXPANDED) & Conference Proceedings Citation Index-Science (CPCI-S) (Web of Science) (30 September 2016)[2]

In addition, reference lists of included studies and related reviews were screened for potentially eligible trials. Experts working in the field of encephalitis were also contacted to enquire about any ongoing trials or unpublished data.

4.3.7 Screening of articles

The studies identified by the literature search were imported into a systematic review software, DistillerSR,[371] for screening. Publications were linked and true duplicates were excluded. Multiple reports of the same study were compared and the most comprehensive report was used. Articles were screened at two levels by two independent reviewers, using a review specific eligibility form. Level one screening involved a review of title or abstracts (or both) of all studies retrieved from the search. The full text of reports of trials reporting on the use of IVIG for children with encephalitis identified at Level 1 screening were retrieved. Level 2 screening involved a comprehensive assessment of the trials reported in the retrieved full text articles to assess their eligibility for inclusion in the review. Reasons for non-inclusion of an identified study were documented. Where there

[2] A search of these databases was conducted by MI and NGM

were discrepancies in selected articles for inclusion, these were discussed and a decision about inclusion was reached by consensus.

4.3.8 Data extraction and management

A review specific data extraction sheet was developed to collect information relevant to the objectives of this review as well as data on study and participant characteristics, interventions used in each study, and participant follow-up. Additional information regarding potential sources of bias including conflicts of interest for the trial investigators, and source of funding for each trial were obtained. Where required data were unclear or missing, authors were contacted for clarification or to provide the missing data. Extracted data were manually entered, managed and analysed in the Review Manager 5 software (RevMan 2014).

4.3.9 Assessment of risk of bias in included studies

Two independent reviewers assessed the risk of bias in included studies. The Cochrane Collaboration's methodology[372] was used. This describes a number of domains to be judged to determine the adequacy of a trial, including (i) selection bias, relating to the process of sequence generation and allocation concealment, (ii) performance bias which relates to blinding of trial participants and personnel, (iii) detection bias which relates to blinding of trial outcome assessors, (iv) attrition bias, considering completeness of outcome data provided, and (v) reporting bias, relating to the selective reporting of study outcomes. To assess the risk of reporting bias, reported outcomes in a trial were compared with those pre-specified in the trial protocol, where this was accessible in the public domain.

4.3.10 Classification of studies

To determine the methodological quality of included studies, the risk of selection, detection, and attrition bias were considered. Based on published guidance,[372] three risk of bias categories were assigned: (i) low risk of bias where bias was plausible but unlikely to have seriously altered the trial results; (ii) high risk of bias in situations where an identified bias was considered would seriously weaken confidence in the trial results, and (iii) unclear risk of bias where a plausible bias raised some doubt about the study findings but there was insufficient information to permit judgement of low or high risk.

A trial was rated as having an overall low risk of bias if none of the three domains was considered to have a high risk of bias, (ii) high risk of bias when at least one of the three domains were judged to have a high risk of bias, and (iii) unclear risk of bias in all other cases.

The risk of bias assessment of each included study was taken into consideration in the interpretation of the results of this review.

4.3.11 Criteria for assessing quality of safety data for all studies

The risk of bias for serious adverse events was assessed by considering specific factors such as the methods used to identify and evaluate adverse events occurring in a trial, which might significantly affect adverse event reporting. This was done by asking two main questions:

- Did the researchers actively monitor for serious adverse events (low risk of bias), or did they simply provide spontaneous reporting of serious adverse events that arose (high risk of bias)?

- Did the authors define serious adverse events according to an accepted international classification and report the number of serious adverse events?

4.3.12 Measures of treatment effect and unit of analysis

Statistical analysis of extracted data was carried out using RevMan 2014. In all studies, the unit of analysis was the individual patient. To determine the size of a treatment effect, risk ratios (RR) and mean differences (MD), along with their 95% confidence intervals (CIs) were calculated for dichotomous and continuous data respectively, and a p value of <0.05 was considered statistically significant.

4.3.13 Assessment of heterogeneity in included studies

Statistical heterogeneity in trial results was assessed using the Chi-square (Chi^2) test.[373] Based on four predefined Chi^2 statistic thresholds, heterogeneity was classified as either (i) low (Chi^2 of 0% to 40%), (ii) moderate (Chi^2 of 30% to 60%), (iii) substantial (Chi^2 of 50% to 90%, or (iv) considerable (Chi^2 of 75% to 100%). A p value of < 0.10 for the Chi^2 statistic was considered to be significant.

4.3.14 Data synthesis

Meta-analysis

A meta-analysis of study results was conducted where more than one study reported on an outcome and the data that were reported in a similar way (e.g. using same units). Qualitative description of study results was done for outcomes that were not homogenously reported.

For the pooled analysis, a random-effects model was used where significant statistical heterogeneity was found and a fixed-effect model where study results were significantly homogenous.

For studies involving multiple intervention groups, a separate meta-analysis synthesising results of each of the different comparisons of interventions for each of the different outcomes was performed.

Subgroup analysis

Since clinical outcomes for encephalitis may vary depending on factors such as aetiology, baseline immune status, dose, and timing of IVIG administration as well as timing of standard therapy given, subgroup analyses taking into consideration these factors were planned.

Sensitivity analysis

A sensitivity analysis was planned to assess whether treatment effect varied with the exclusion of studies with high or unclear risk of bias.

4.3.15 Data presentation

Using the GRADEpro software[374] and based on Cochrane methodology and recommendations,[372] the quality of evidence for each review outcome was graded.

4.4 Results

The process of study identification and selection is shown in Figure 4.1. The search strategy retrieved 822 titles (MEDLINE (160), Embase (433), the Cochrane Library (11), CINAHL (57) Clinicaltrials.gov (1), Web of Science (23), Global Health Library (135), and DARE (2). One additional record was retrieved through Google search. Of the 681 articles remaining after removal of duplicates, 668 were excluded following Level 1 screening because the titles/abstracts of the articles did not suggest that the report related to a trial reporting on the use of IVIG in the treatment of encephalitis in a group of children. Therefore, 13 articles of 13 different studies had the potential to be included in this review. Following review of the full article for all 13 papers three were considered eligible for inclusion in this review.

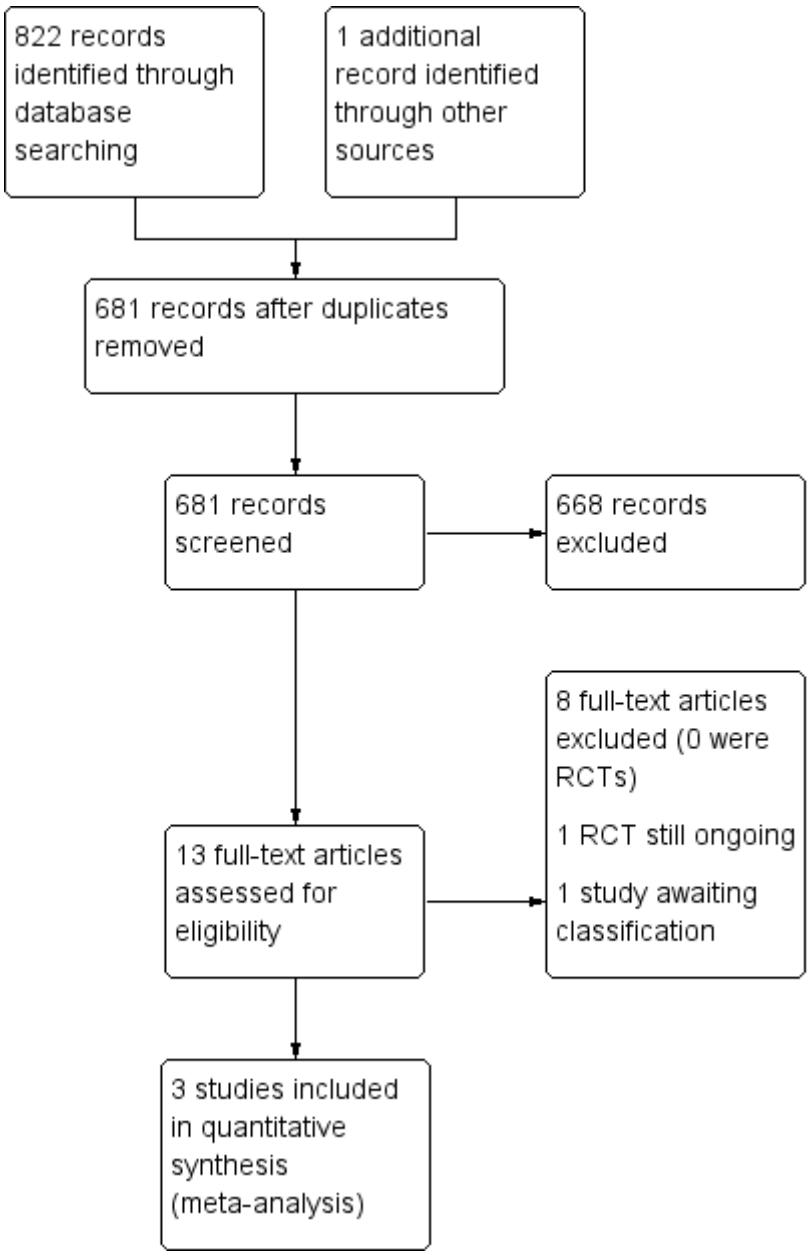


Figure 4.1 Flow diagram showing process of selection of studies

One ongoing trial in the UK of IVIG in childhood encephalitis[375] was identified but was not included since results of the study were not available at the time of the review.

One trial was awaiting classification at the time of conducting this review.[376] This was a Chinese study of high-dose immunoglobulin in 72 adults and children (aged 14-72 years) with severe viral encephalitis in which participants were randomly allocated to one of two groups - treatment group (n=35) and control group (n=37). Those in the treatment group

received IVIG at a dose of 400mg/kg for 5 consecutive days while those in the control group received standard care only. The findings of this study were presented as aggregate data and the results for children were not presented separately. An attempt was made to contact the authors but no contact details were found at the time of the analysis of this review. Thus, it was not possible to include data from this study in the review.

4.4.1 Summary of included studies

Up to 30 September, three studies RCTs of IVIG in children with encephalitis met the criteria to be included in this review. Two of the three studies[377,378] were published in Chinese and were translated to English[1].

Chen *et al*[378] described the findings of an RCT of IVIG in addition to standard treatment in children with viral encephalitis. Between February 2000 and October 2004, 62 children aged 7 months to 12 years with a diagnosis of viral encephalitis defined were enrolled. The authors reported that the definition of encephalitis for entry to the study was based on the guidelines and symptoms described in "Applied Pediatrics", sixth edition. Children enrolled in the trial were and randomly split into two groups – an observation (treatment) group of 32 children, and a control group of 30 children. The proportion of boys enrolled to the study was 58.1%. The mean (± standard deviation (SD) ages for children in the observation and control groups were 6.16 ± 2.38 years and 5.88 ± 2.46 years respectively. Baseline characteristics i.e. gender, age, clinical symptoms and signs, severity of disease, and duration of hospital stay did not differ significantly between the study groups. Children in both groups received conventional treatment which included treatment for fever and spasms, for reduction of intracranial pressure, maintenance of

[1] Translation was done by Kathy Xu of the Bodleian Libraries, University of Oxford.

hydration, electrolyte, and acid-base balance, application of hormones and antivirals, prevention and treatment of infections, and 'upkeep of morale'. In addition, the observation group received IVIG at a dose of 400 mg/kg/day for three to five days.

Outcome measures included (i) time to resolution of fever (ii) time to seizure control, (iii) time taken to regain consciousness, and (iv) duration of hospital stay. The authors concluded that children who received IVIG treatment had significantly quicker recovery time in terms of days to reduction of fever, regaining of consciousness level, and resolution of spasms. Additionally, the authors reported a significantly shorter duration of hospital stay in the observation compared with the control group.

Rayamajhi *et al* [277] reported results from a pilot feasibility RCT conducted in two centres in Nepal from May to June 2009. Participants were aged 1 to 14 years who were suspected to have Japanese encephalitis and met the following diagnostic criteria were enrolled: history of fever that lasted less than 14 days, altered consciousness defined as Glasgow Coma Score less than 15 with or without a history of seizures, and consistent CSF findings, i.e. white cell count less than 1000 cells/mm3 with no organisms on Gram stain and CSF: plasma glucose ratio greater than 40%. Exclusion criteria the occurrence of a single seizure lasting less than 15 minutes with full recovery of consciousness within 60 minutes which was assumed to indicate a diagnosis of febrile convulsion; positive blood slide or rapid antigen test for Plasmodium falciparum parasites, clinical or laboratory (or both) features suggestive of bacterial meningitis; GCS < 3, artificial ventilation, and absence of signs of spontaneous respiration and oculocephalic reflex. Ethical approval and informed consent were obtained prior to study commencement. Enrolled children were randomly allocated to receive either IVIG 400 mg/kg/day or an equivalent volume of 0.9% saline (placebo) for five days. Randomisation was stratified by centre using a

variable block size of four, six, or eight, selected on a random basis. Participants were assessed at discharge and after three to six months.

The reported primary objective was to assess the feasibility of a multicentre placebo-controlled RCT of IVIG in a resource-poor setting. Reported secondary outcomes were death or neurological sequelae at discharge or follow-up, and adverse events. Additional outcomes were comparison of anti-Japanese encephalitis virus (anti-JEV) antibody titres between children treated and not treated with IVIG.

A total of 23 children were screened of which 22 met the eligibility criteria and were enrolled. One child was not enrolled due to meeting an exclusion criterion. The proportion of enrolled boys was 54.5%. Baseline characteristics of children in the study groups were comparable. The authors concluded that a trial of IVIG in Japanese encephalitis in Nepal is feasible and that IVIG may augment the development of neutralising antibodies in JEV-positive people. Further information on missing data required for this review including method of randomisation, allocation concealment, blinding of outcome assessors) was sought from the main author of one of the included studies[379] without success.

The third included study was a single centre RCT of 80 children (49 males, 31 females) between 1 and 6 years with a diagnosis of hand, foot and mouth disease (HFMD) complicated by viral encephalitis.[377] The study was conducted in a general hospital in China between January 2009 and January 2013. The aim of the study was to compare the clinical efficacy of adjunctive treatment with gammaglobulin and interferon in HFMD complicated by viral encephalitis. The diagnosis of HFMD was based on published definition guidelines by the Chinese Department of Ministry of Health. Diagnostic criteria for encephalitis were not specified in the article. Exclusion criteria included

immune suppression and reduced consciousness. Ethical approval and consent were obtained.

Enrolled children were randomly assigned to three groups: experimental group one (n= 27), experimental group two (n=26), and a control group (n=27); according to a random-sampling table. There was no statistically significant difference between the groups in age, gender, and duration of illness.

Participants in the control group was given 'conventional treatment' which included rehydration, reduction of intracranial pressure, nutritional support, and antimicrobial treatment. Participants in experimental groups 1 and 2 received intravenous gamma globulin (400 mg/kg/day for three consecutive days) and interferon alfa-2h (one or two sprays per time, three times per day on three consecutive days) respectively, in addition to the conventional treatment. The outcomes measured were time for fever to subside, convulsions to be under control, rashes to subside, neuropathic symptoms to subside, and mean stay in hospital. Adverse events occurring in children in the two experimental groups were compared. Based on their findings, the authors concluded that the treatment of children with HFMD complicated by encephalitis with gamma globulin and human interferon can relieve, and reduce sequelae.

4.4.2 Excluded studies

Eight studies were excluded at level two screening due to their study design (Table 4.1), three of these were single case reports and four were case series and one was a non-randomised controlled trial.

Table 4.1 Characteristics of excluded studies

First author, publication year	Reason for exclusion
Gelli 1974 [380]	Single case report describing use of human anti-measles immunoglobulin in measles encephalitis
Unay 2004 [381]	Single case report describing use of IVIG in acute disseminated encephalomyelitis (ADEM) associated with hepatitis A infection
Samileh 2007 [382]	Case series of children with ADEM, not randomised, no control group
Girija 2010[378]	Case series of children with ADEM, no control group
Bhatt 2012 [280]	Quasi-controlled trial of IVIG compared with standard therapy in children with acute encephalitis syndrome complicated by myocarditis. Not randomised study
Erol 2013[136]	Case series of children with ADEM, not randomised, no control group
Incecick 2013[292]	Case series of children with ADEM, not randomised, no control group
Růžek 2013 [383]	Single case report of IVIG use in tick borne encephalitis

4.4.3 Risk of bias in included studies

A summary of the risk of bias assessment for the individual studies included in this review are detailed Table 4.2 and an overall summary is shown in and Figure 4.2.

Allocation (selection bias)

Random sequence generation

All three included studies[277,377,378] were described as RCTs. Complete information on the method of randomisation was described in only two of the studies[277,377] and were judged appropriate, therefore a low risk of selection bias was assigned to both studies. The exact method of randomisation was not reported for one study,[378] so an unclear risk of bias was assigned.

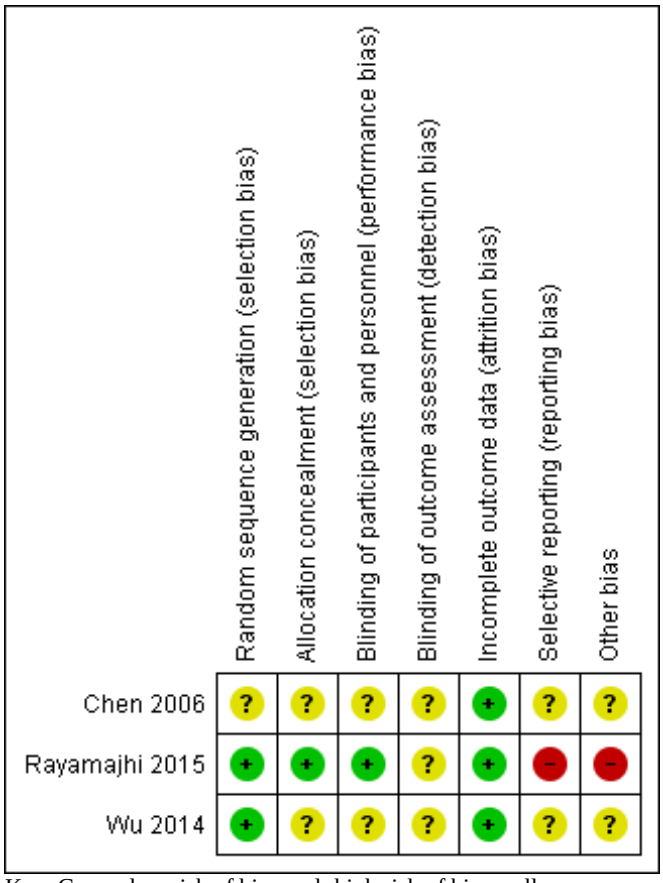
Allocation concealment

The method of allocation concealment was described for one study.[277] The authors reported that the randomisation code for each participant was kept in sealed envelopes. To maintain blinding, due to the subtle visual differences between IVIG and 0.9% saline, masking was applied to the study drug before administration by independent investigators. Furthermore, empty vials of the study drug were concealed in paper bags prior to discard. This was judged to be adequate and a low risk of bias for allocation concealment was assigned. For the other two studies,[377] [378] the method of allocation concealment was not provided so the risk of bias for allocation concealment was judged to be unclear.

Table 4.2 Risk of bias table for the included studies

Chen (2006)[378]		
Bias	Author's judgement	Support for judgement
Random sequence generation (selection bias)	Unclear	Random allocation but method of randomisation not described.
Allocation concealment (selection bias)	Unclear	No information was provided
Blinding of participants (performance bias)	Unclear	No information was provided
Blinding of outcome assessment (detection bias)	Unclear	No information was provided
Incomplete outcome (attrition bias	Low	No dropouts and all participants were included in outcome assessment
Selective reporting (reporting bias)	Unclear	Study protocol was not available in the public domain
Other bias	Unclear	• Small sample size and no justification for sample size provided so unclear if large enough to detect significant differences in the outcomes between the study groups. • No information on funding provided.
Rayamajhi (2015)[277]		
Random sequence generation (selection bias)	Low	Random allocation, stratified by centre.
Allocation concealment (selection bias)	Low	Randomisation code concealed and opened by independent investigators.
Blinding of participants (performance bias)	Low	All investigators and care providers blind to study drug.
Blinding of outcome assessment (detection bias)	Unclear	No information provided.
Incomplete outcome (attrition bias	Low	Numbers and reasons for withdrawals provided, numbers lost to follow-up similar in each group.
Selective reporting (reporting bias)	High	• The primary outcome reported in the published article differs from primary outcome specified in the published protocol in ClinicalTrials.gov. • Some adverse event outcomes pre-specified in the study protocol such as infusion site reaction, diarrhoea, rise in blood pressure, and change in urinary output were not reported in the article. • Details of participant death provided for treatment group but not placebo group.
Other bias	High	• Sample size calculation was not based on the pre-specified primary stated in the study protocol. • Small sample size • Authors' group affiliated to funding body. • Imbalance in number of participants who received corticosteroid therapy. • Recruitment period unclear, authors reported 2 different recruitment periods: May-June 2009 and May-September 2009.
Wu (2014)[377]		
Random sequence generation (selection bias)	Low	Random allocation using random sampling table
Allocation concealment (selection bias)	Unclear	No information provided
Blinding of participants (performance bias)	Unclear	No information provided
Blinding of outcome assessment (detection bias)	Unclear	No information provided
Incomplete outcome (attrition bias	Low	No dropouts and all participants included in outcome assessment
Selective reporting (reporting bias)	Unclear	Study protocol was not available in the public domain
Other bias	Unclear	• Small sample size and no sample size calculation so unclear if sample size large enough to detect significant differences between study groups. • No information on funding provided. • Diagnostic criteria used for encephalitis not specified in paper

Figure 4.2 Risk of bias summary for the included studies



Key: Green=low risk of bias, red=high risk of bias, yellow =unclear risk of bias

Blinding (performance bias and detection bias)

Rayamajhi *et al* [277] reported that all investigators, care providers, and participants were blind to the treatment allocation. However, it is unclear whether blinding extended to the outcome assessors. Therefore, the study was rated as having a low risk for performance bias but an unclear risk for detection bias. No information on blinding was provided for the other two studies[377,378] and were assigned an unclear risk for performance and detection bias.

Incomplete outcome data (attrition bias)

A flow chart detailing participant progress through the study and numbers lost to follow up was provided by Rayamajhi *et al* [277] and loss to follow up was balanced between both treatment groups. The reasons for non-inclusion of participants numbers in some laboratory outcome assessments were provided. Therefore, the study by Rayamajhi *et al* [277] was judged to have a low risk for attrition bias. All participants in the other two studies were assessed for the study outcomes at discharge, with no loss to follow up reported so both studies[377,378] were judged to have a low risk for attrition bias.

Selective reporting (reporting bias)

Discrepancies were observed in the outcomes specified by Rayamajhi *et al* [277] when compared with the pre-specified outcomes in the study protocol for one study. Therefore, the study by Rayamajhi *et al* [277] was assessed to have a high risk of reporting bias as high. The protocols for the other included studies[377,378] were not publicly available so it was not possible to determine the risk of reporting bias, therefore an unclear risk was assigned to both studies.

Other potential sources of bias

The sample sizes for all three studies were small. Justification of the choice of sample size was not provided for two studies[377,378] whereas for one study,[277]the s ample size calculation was based on a tertiary outcome. Therefore, it was not possible to determine adequacy of the sample sizes in assessing the primary outcomes for all three studies. In the study reported by Rayamajhi *et al*,[277] there was an imbalance in the number of participants in both study groups who received corticosteroid therapy, a potential confounder of a treatment effect, and this was not considered in the analyses.

Furthermore, the main authors' group provided funding for the study hence conflict of interests could not be excluded.

Thus, the study by Rayamajhi *et al* was rated as having a high risk for other bias whereas the studies by Chen and Wu *et al* [377,378] were considered to have an unclear risk for other bias. Due to the limited number of included studies in this review (fewer than 10), investigation of the potential for publication bias could not be assessed using a funnel plot.

Risk of bias for adverse events

A summary of the risk of bias assessment for adverse events is shown in Table 4.3. The definition criteria and method of assessment of adverse events was reported by Rayamajhi *et al* [277] and was assigned a low risk of bias for adverse events. Similar information was not provided for the other two studies, [377,378] thus the risk of bias for adverse events was judged as unclear for both studies.

Table 4.3 Risk of bias summary for adverse events

Study (First author, year)		
	Did the researchers actively monitor for AEs or did they simply provide spontaneous reporting of AEs that arose?	Risk of bias
Chen, 2006[378]	Information not provided	Unclear
Rayamajhi, 2015[277]	The authors reported that participants were monitored for AEs from first day of commencing treatment until death or discharge	Low
Wu, 2014 [377]	Information not provided	Unclear
Study (First author, year)		
	Did the authors define serious adverse events (SAEs) according to an international classification and report the number of SAEs	Risk of bias
Chen, 2006[378]	Information not provided	Unclear
Rayamajhi, 2015[277]	Adverse events were graded using WHO recommendations and reported to an independent data safety committee.	Low
Wu, 2014[377]	Information not provided	Unclear

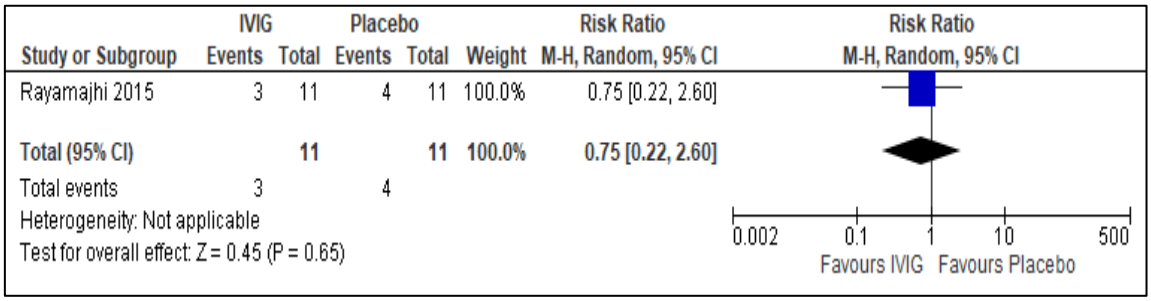
4.4.4 Effects of interventions

4.4.4.1 Primary outcome

Significant disability

Proportion of participants with significant disability (= 'poor outcome') assessed at six months after treatment. Only one study[277] reported on the proportion of participants with significant disability. Disability was assessed using a validated assessment tool (Liverpool Outcome Score, LOS) at three to six months after discharge from hospital. Based on the predefined criteria for significant disability in this review i.e. Liverpool Outcome Score of 2 or less, 3/11 (27.3%) participants in the IVIG group versus 4/11 (36.4%) in the placebo group had significant disability at three to six months (RR 0.75, 95% CI 0.22 to 2.60; P = 0.65) (Figure 4.3).

Figure 4.3 Forest plot for significant disability at 6 months (IVIG versus placebo)



Key: IVIG=intravenous immunoglobulin, CI=confidence interval, M-H=Mantel-Haenszel method.

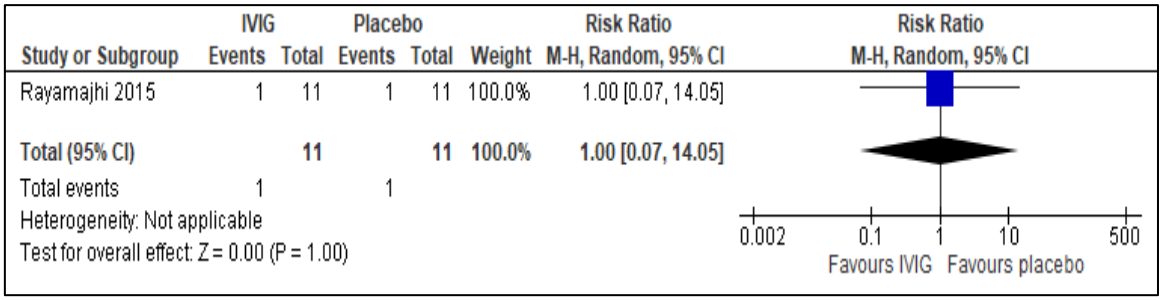
Proportion of participants with at least one serious adverse event

All three studies reported on adverse events. Rayamajhi *et al* [277] applied the WHO recommendations for grading adverse events and based on this, reported on SAEs including death, melaena, and hypotension.

For the other included studies for which a clear definition of serious adverse event was not provided,[377,378] the ICH guideline was used to categorise each reported adverse event and based on the criteria, none of the adverse events reported in these two studies met the

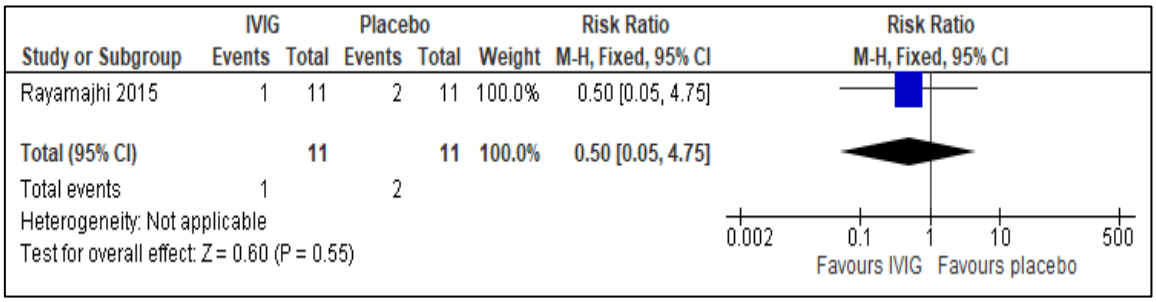
SAE definition. Therefore, only the study by Rayamajhi *et al* [277] contributed to this analysis. Rayamajhi *et al*[277] reported occurrence of as least one SAE in 1/11 participants in both the IVIG, and placebo group (RR 1.00, 95% CI 0.07 to 14.05; P = 1.00) (Figure 4.4). The proportion of deaths in the same study was 1/11 versus 2/11 in the IVIG and placebo groups respectively (RR 0.50, 95% CI 0.05 to 4.75; P = 0.55) (Figure 4.5).

Figure 4.4 Forest plot for serious adverse event (IVIG versus placebo)



Key: IVIG=intravenous immunoglobulin, CI=confidence interval, M-H=Mantel-Haenszel method.

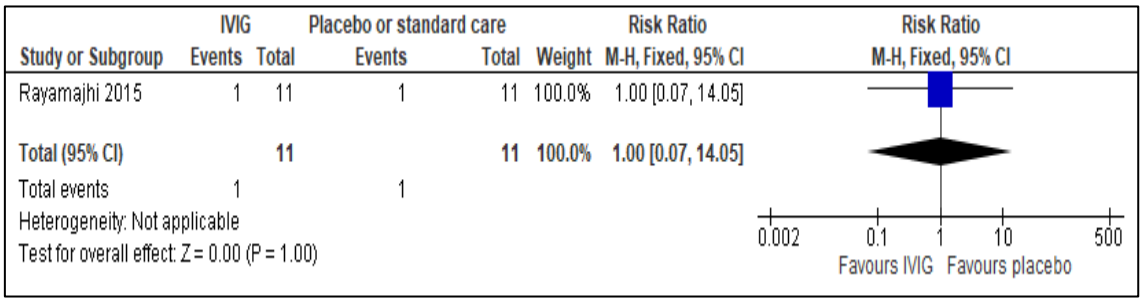
Figure 4.5 Forest plot for mortality (IVIG versus placebo)



Key: IVIG=intravenous immunoglobulin, CI=confidence interval, M-H=Mantel-Haenszel method.

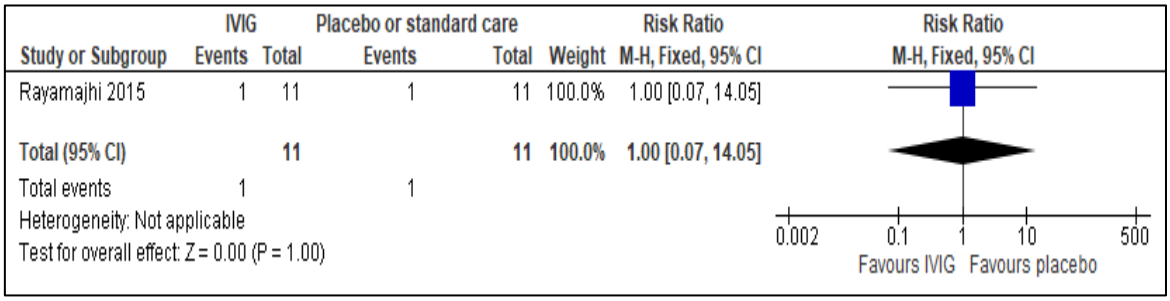
The authors also reported a non-significant difference in the number of participants in each study group that experienced either hypotension or melaena (RR 1.00, 95% CI 0.07 to 14.05; P = 1.00) (Figures 4.6 and 4.7).

Figure 4.6 Forest plot for hypotension (IVIG versus placebo)



Key: IVIG=intravenous immunoglobulin, CI=confidence interval, M-H=Mantel-Haenszel method.

Figure 4.7 Forest plot for melaena (IVIG versus placebo)



Key: IVIG=intravenous immunoglobulin, CI=confidence interval, M-H=Mantel-Haenszel method.

Summary of main findings

A Summary of the main outcomes of this review is presented in Table 4.4.

Table 4.4 **Summary of findings for the main outcomes**

Intravenous immunoglobulin compared with standard care or placebo for childhood encephalitis

Outcomes	**Illustrated comparative risks** (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Assumed risk (control) **Standard care or placebo**	**Corresponding risk (intervention)** **IVIG ± standard care**				
Significant disability at 3-6 months assessed with: Liverpool Outcome Score RR (M-H random effects analysis, 95%CI)	364 per 1,000	**273 per 1,000** (80 to 945)	**RR 0.75** (0.22 to 2.60)	22 (1 RCT)	⊕◯◯◯ VERY LOW [a,b,c,d,e]	Unclear risk of detection bias and high risk of publication bias. Small sample size. Study not powered on primary outcome
At least one serious adverse event RR (M-H random effects analysis, 95%CI)	91 per 1,000	**91 per 1,000** (6 to 1,000)	**RR 1.00** (0.07 to 14.05)	22 (1 RCT)	⊕◯◯◯ VERY LOW [a,b,c,d,e]	Unclear risk of detection bias and high risk of publication bias. Small sample size. Study not powered on primary outcome
Length of hospital stay MD, random effects analysis (95%CI)	The mean length of hospital stay was 13.26	The mean length of hospital stay in the intervention group was 4.54 lower (7.47 lower to 1.61 lower)	-	116 (2 RCTs)	⊕◯◯◯ VERY LOW [d,f,g,h,i]	Unclear risk of selection bias in one study. Unclear risk of allocation concealment, performance and detection bias in both studies.

*__The basis for the assumed risk (e.g. the median control group risk across studies) is provided in footnotes. The corresponding risk__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI). **Key: CI:** Confidence interval; **RR:** Risk ratio; **MD:** Mean difference ; M-H: Mantel Haenszel method

GRADE Working Group grades of evidence
High certainty: Further research is very unlikely to change our confidence in the estimate of the effect
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate
Low quality: Further research is very likely to have an important impact on our confidence in the effect estimate and is likely to change the estimate
Very low quality: We are very uncertain about the estimate

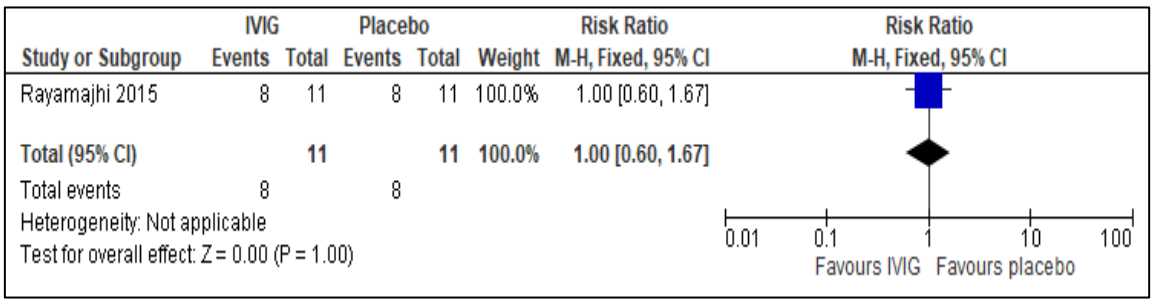
Key: a. Unclear risk of detection bias, b. Conflicts of interest could not be excluded, c. Study of Japanese encephalitis in Nepal therefore, results not generalisable to other forms of encephalitis in other settings, d. Small sample size in studies contributing to this outcome, e. Some predefined outcomes were not reported, f. Unclear risk of performance and detection bias in both studies contributing to this outcome, g. Unclear risk of selection bias in one study contributing to this outcome assessment, h. High proportion of variation in point estimates, i. Both studies involved only children with viral encephalitis. RR=risk ratio, RCT=randomised controlled trial, CI=confidence interval.

4.4.4.2 Secondary outcomes

Significant disability at the time of hospital discharge

Only Rayamajhi *et al*[277] assessed disability at discharge and reported significant disability (based on the criteria defined above) in 8/11 participants in each treatment group (RR 1.00, 95% CI 0.60 to 1.67; P = 1.00) (Figure 4.8)

Figure 4.8 Forest plot for significant disability at discharge (IVIG versus placebo)



Key: IVIG=intravenous immunoglobulin, CI=confidence interval, M-H=Mantel-Haenszel fixed effect method.

Glasgow Coma Score at discharge

Only Rayamajhi *et al*[277] assessed Glasgow Coma Score at discharge and reported a no significant difference between the treatment groups (IVIG: median score =14, range 3 - 15; placebo: median score =14, range 7-15; p = 0.53)

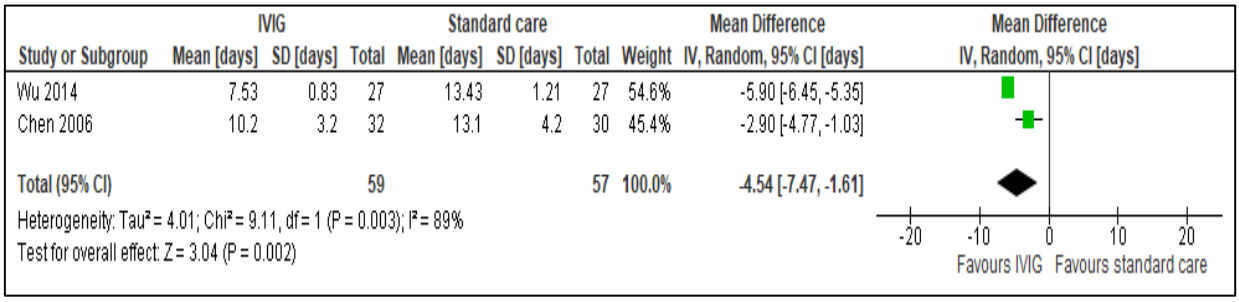
Length of hospital stay (LOHS)

LOHS comparison: IVIG vs. standard care

All three studies reported on LOHS. This was reported as mean (standard deviation, SD) in two studies,[377,378] and as median (range). [277] Therefore, only two studies[377,378] contributed to the pooled analysis for this outcome which showed significant difference in favour of IVIG (MD -4.54 days, 95% CI -7.47 to -1.61; P = 0.002) (Figure 4.9). When the median LOHS was assessed, Rayamajhi *et al* [277] found no significant difference

between the treatment groups (IVIG: median 13 days, range 9-21; placebo: median 12 days, range 6 to 18; p value 0.59).

Figure 4.9 Forest plot for length of hospital stay (IVIG versus standard care)

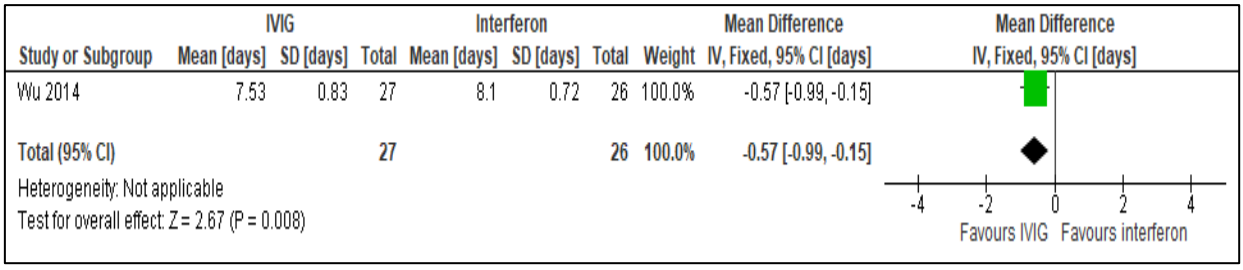


Key: SD=standard deviation, CI=confidence interval, IVIG=intravenous immunoglobulin.

LOHS comparison: IVIG vs. interferon

Wu 2014 also compared mean length of stay between participants receiving IVIG or interferon in addition to standard care and reported a significant difference in favour of IVIG (MD -0.57, 95% CI -0.99 to -0.15; P = 0.008) (Figure 4.10).

Figure 4.10 Forest plot for length of hospital stay (IVIG versus interferon)



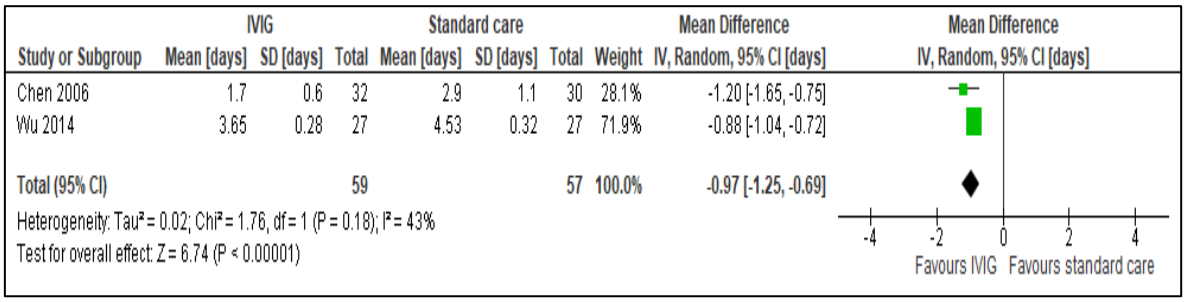
Key: SD=standard deviation, CI=confidence interval, IVIG=intravenous immunoglobulin.

Time to fever resolution

Time to resolution of fever: IVIG versus standard treatment

Two of the included studies[377,378] reported on days to fever resolution as means (SDs). Pooled analysis showed a significant difference in favour of IVIG (MD -0.97 days, 95% CI -1.25 to -0.69; P < 0.00001) (Fig 4.11).

Figure 4.11 Forest plot for time to fever resolution (IVIG versus standard care)

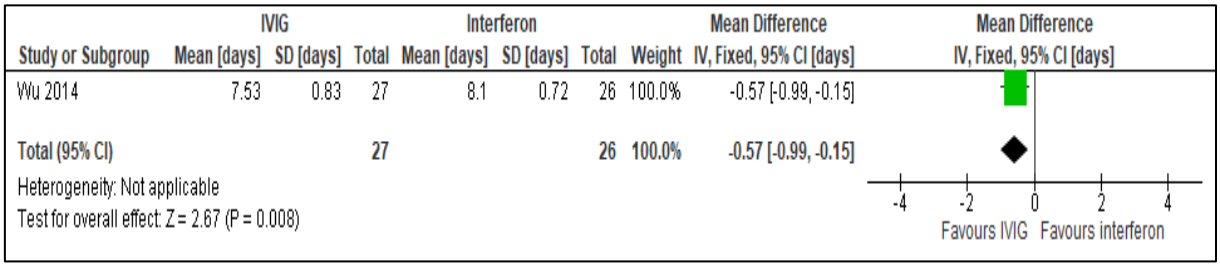


Key: SD=standard deviation, CI=confidence interval, IVIG=intravenous immunoglobulin.

Time to resolution of fever: IVIG vs interferon

Wu *et al*[377] compared days to fever resolution between participants receiving IVIG or interferon in addition to standard care and reported a statistically significant difference in favour of IVIG (MD -0.27 days, 95% CI -0.42 to -0.12; P = 0.0006) (Fig 4.12).

Figure 4.12 Forest plot for time to fever resolution (IVIG versus interferon)



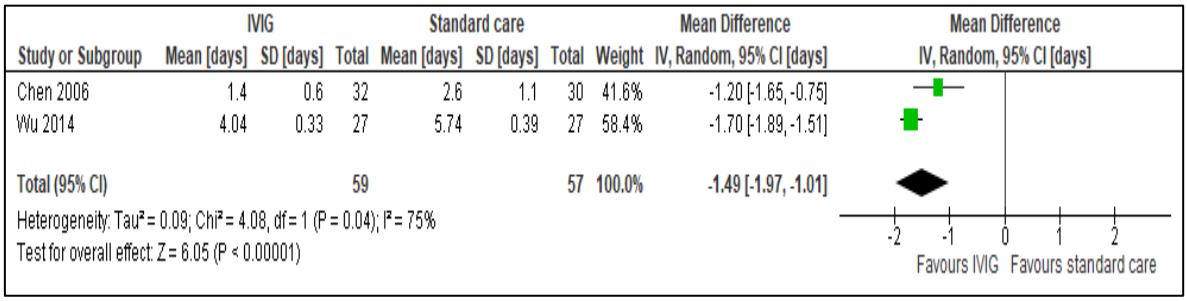
Key: SD=standard deviation, CI=confidence interval, IVIG=intravenous immunoglobulin.

Time to stop spasms

Time to stop spasms: IVIG versus standard care

Two of the included studies[377,378] reported on days to stop spasms as means (SDs). Pooled analysis of the results from both studies demonstrated a significant difference in favour of IVIG when compared with standard care (MD -1.49 days, 95% CI -1.97 to -1.01; P < 0.00001) (Fig 4.13)

Figure 4.13 Forest plot for time to stop spasms (IVIG versus standard care)

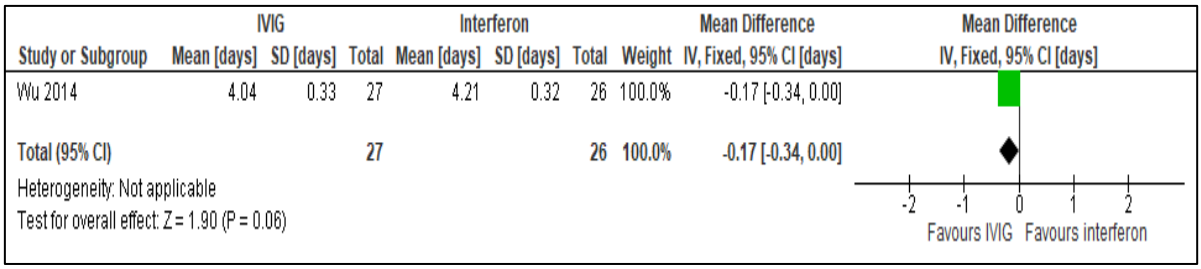


Key: SD=standard deviation, CI=confidence interval, IVIG=intravenous immunoglobulin.

Time to stop spasms: IVIG versus interferon

Wu *et al*[377] also compared time to stop spasms between the IVIG and interferon group and found a non-statistically significant difference between groups (MD -0.17, 95% CI -0.34 to 0.00; P = 0.06) (Figure 4.14).

Figure 4.14 Forest plot for time to stop spams (IVIG versus interferon)

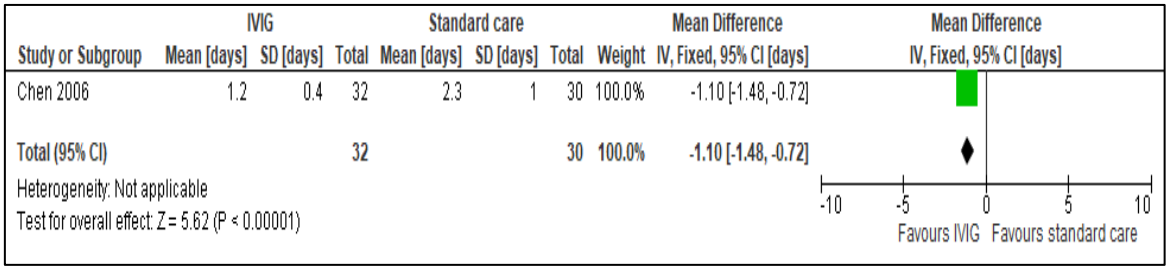


Key: SD=standard deviation, CI=confidence interval, IVIG=intravenous immunoglobulin.

Time to regain consciousness

Only one study[378] reported on time to regain consciousness as mean (SD) and found a statistically significant difference in favour of IVIG when compared with standard care (MD -1.10, 95% CI -1.48 to -0.72; P < 0.00001) (Fig 4.15)

Figure 4.15 Forest plot for time to regain consciousness (IVIG versus standard care)



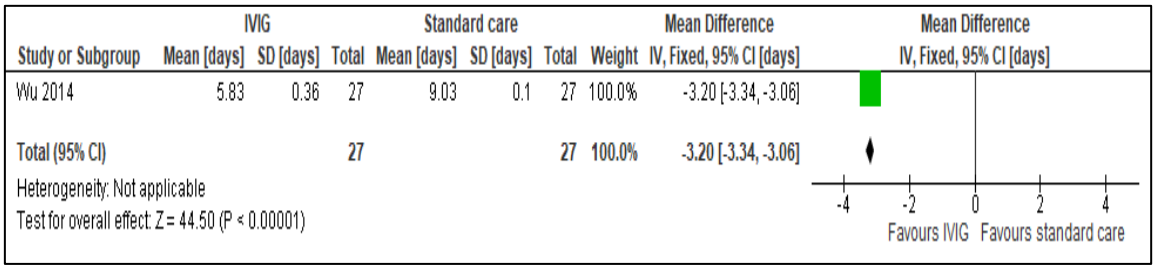
Key: SD=standard deviation, CI=confidence interval, IVIG=intravenous immunoglobulin.

Time to resolution of neuropathic symptoms

Time to resolution of neuropathic symptoms: IVIG vs. standard care

Wu *et al*[377] reported time to resolution of neuropathic symptoms as mean (SD) and found a statistically significant difference in favour of IVIG when compared with standard care (MD -3.20 days, 95% CI -3.34 to -3.06; P < 0.00001) (Fig 4.16).

Figure 4.16 Forest plot for time to resolution of neuropathic symptoms (IVIG versus standard care)

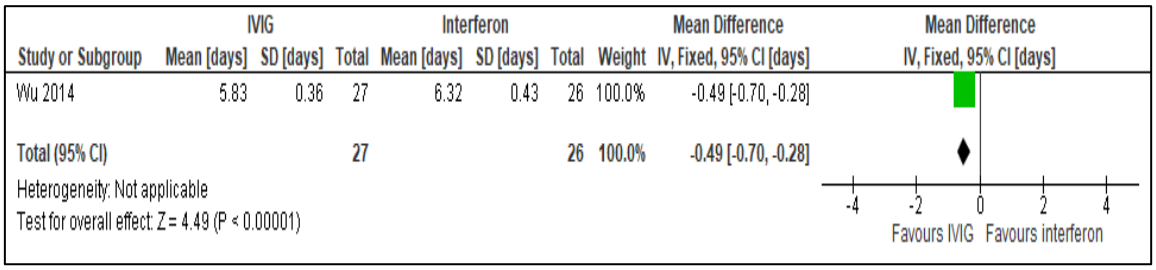


Key: SD=standard deviation, CI=confidence interval, IVIG=intravenous immunoglobulin.

Time to resolution of neuropathic symptoms: IVIG vs. interferon

Wu *et al*[377] reported a statistically significant difference in favour of the IVIG when compared with interferon (MD -0.49 days, 95% CI -0.70 to -0.28; P < 0.00001) (Fig 4.17).

Figure 4.17 Forest plot for time to resolution of neuropathic symptoms (IVIG versus interferon)



Key: SD=standard deviation, CI=confidence interval, IVIG=intravenous immunoglobulin.

Other review outcomes

None of the included studies reported on other outcomes of interest in this review including proportion of participants requiring invasive ventilation and duration of ventilation, cognitive impairment, adaptive functioning, quality of life assessment, seizure frequency and new diagnosis of epilepsy.

4.5 Discussion

This is the first systematic review and meta-analysis of RCTs of IVIG in childhood encephalitis. Three RCTs that involved 138 participants: 70 participants treated with IVIG either alone or in addition to standard care and 68 participants treated with either standard care or placebo contributed to this review data. Only one study of Japanese encephalitis of 22 children (11 children in each treatment group) [277]contributed to the primary outcomes of this review. The main conclusion based on the findings of that study was that IVIG treatment did not offer additional benefit over placebo when specific outcomes (proportion of participants with significant disability and with at least one serious adverse event) were considered. Furthermore, in the same study[277] the median length of hospital stay and Glasgow Coma Score at the time of hospital discharge were

similar for children with Japanese encephalitis who received IVIG treatment and those who received placebo.

Based on the findings of two other studies of viral encephalitis in children, [377,378]evidence of very low quality suggested a beneficial effect from IVIG in reducing the mean length of hospital stay, time to resolution of spasms, neuropathic symptom, time to regain consciousness, and time to resolution of fever. However, the inherent limitations in the included studies make it impossible to draw definite conclusions on the efficacy of IVIG in children with encephalitis.

Two studies are worth discussing. Bhatt and colleagues[280] reported a quasi-control trial of IVIG versus standard therapy in children with acute encephalitis syndrome complicated by myocarditis. A total of 83 children (2 months to 12 years) were enrolled at a tertiary hospital in India and were assigned to receive either IVIG or standard care. Due to the availability of IVIG on set days only, patients who presented on Monday and Friday were allocated to the IVIG group and patients who presented on other days were allocated to the standard therapy group. The primary outcomes were mortality and left ventricular function. The authors reported a borderline significant difference in mortality between both study groups. There was 1/26 (3.8%) vs. 13/57 (22.8%) deaths in the IVIG vs. standard care group respectively, with a reported risk difference of 0.17 (95%CI=0.02-1.22), p=0.005. A significant difference was also observed in the mean ejection fraction between both study groups in favour of IVIG. The authors reported a mean ejection fraction (standard deviation) of 49.5 (9.04) vs. 35.9 (19.6) in the IVIG vs. standard care group respectively and a risk difference of 13.6 (5.13-22.06), p=0.001. No significant difference was found when the secondary outcomes were considered, which included duration of hospital stay, proportion with neurological sequelae at discharge, duration of invasive ventilation and mean inotrope score.

The second study by Fan *et al* [376] enrolled 72 participants with severe viral encephalitis who were randomised to receive IVIG (n=35) in addition to standard care or standard care alone. The authors reported a significant difference in favour of IVIG for all outcomes that were considered which included time to resolution of fever, to stop spasms, to regain consciousness, to resolution of paralysis, to return of mental state, and mean duration of hospital stay. This study included patients between 14 and 72 years. The results of the study were presented as an aggregate, data for children were not presented separately.

The clinical relevance of the findings of this review is worth considering. For two of the included studies, the outcome measures were not clearly defined. Both of these studies reported on outcomes including time to resolution of fever, to stop spasms and resolution of neuropathic symptoms. It was unclear from both studies what specific definitions were used for these outcome measures (e.g. fever). In addition, the effect of confounders such as concomitant use of antipyretics, and anticonvulsants were not accounted for in the analysis of a treatment effect. Furthermore, for this review, outcome measures that were deemed important for the assessment of children with encephalitis were selected however several of these outcomes such as cognitive functioning, adaptive functioning, and quality of life were not investigated in the included studies. Additionally, follow up of participants beyond the hospitalisation period occurred in only one of the included studies[277] and was only for 3 to 6 months. Therefore, the impact of IVIG on longer term (beyond 6 months) outcomes cannot be determined from these studies. Assessment of long-term outcomes is extremely important in the management of people with encephalitis since up to 60% of affected people can experience long-term morbidity including those patients that appear to have made full recovery at discharge.

How generalisable are the findings of this review?

There were differences in the eligibility criteria used in the included studies. Furthermore, all three studies were conducted in lower middle-income countries: Nepal[277] and China.[377,378] Also, all three studies were conducted in children with viral encephalitis. These factors limit generalisability of the review findings to higher income countries and to other forms of encephalitis.

Limitations of this review

The main limitations of the findings of this review are the low number of studies contributing to the meta-analysis, the small sample size in each of the included studies, the short follow-up period, and the inherent methodological limitations in each of the included studies, which might have a role in the final assessment of efficacy and safety of IVIG treatment.

Implications for future research

Well-designed randomised controlled trials are needed to investigate the role of IVIG in all forms of encephalitis and to provide evidence to inform clinical practice on the management of children with encephalitis. Future trials should include a clear and objective definition for encephalitis, which is aligned with the published consensus definition for encephalitis,[194] to ensure a homogenous population. There is the need for internationally agreed core outcome measures for clinical trials in childhood encephalitis. For future trials, consideration should be given to clinically relevant outcomes including disability, cognitive functioning, and adaptive skills and, where possible, these should be assessed using validated tools. Outcome measures should be clearly defined and where

there are confounders of a treatment effect, these must be considered in the analysis. Long-term follow-up (beyond the hospitalisation period) needs to be assessed.

4.6 Conclusion

This is first systematic review of RCTs of IVIG in encephalitis that incorporates a meta-analysis. It is also the first systematic review of IVIG use in viral encephalitis. The findings highlight the need for well-designed RCTs to reach a definitive conclusion on the role of IVIG in childhood encephalitis.

5 Immunoglobulin in the Treatment of Encephalitis (The IgNiTE study)

5.1 Background and rationale for the design of the study

As indicated in Chapter 1 (section 1.10), studies of IVIG in encephalitis suggest a possible benefit in improving outcomes. However, there are inherent limitations in those studies which make it impossible to reach a definite conclusion on the role of IVIG in encephalitis. The largest study to date reporting a benefit from IVIG is a multi-institutional cohort study of 577 patients (211 children, 37%) with anti-NMDAR encephalitis.[216] Although in that study, participants received IVIG in combination with other immunomodulatory treatments and without a subgroup analysis, it is not possible to assign the treatment effect observed to IVIG alone. A Cochrane systematic review of IVIG in encephalitis presented in Chapter 4 highlights the paucity of well-designed RCTs of IVIG in encephalitis. Although the findings of the Cochrane review suggest some benefit from IVIG in the treatment of children with viral encephalitis, the quality of the evidence was judged to be very low. Studies have suggested that the extent of brain inflammation that occurs in encephalitis is fundamental to determining outcomes, [352] indicating that the goal of treatment should be early intervention aimed to attenuate and limit extensive brain inflammation.

The IgNiTE trial described in this Chapter is the first ever RCT assessing the role of early intervention with IVIG treatment in children with all-cause encephalitis. Due to the slow recruitment, enrollment to the IgNiTE trial was paused in September 2017, with the potential to resume this in March 2018, pending confirmation of further funding provision This Chapter describes the IgNiTE study and details present aggregate results from an interim analysis of data from the currently enrolled participants. Some of the problems encountered with setting up of the study, and recruitment of participants are discussed.

5.2 Methods

5.2.1 Study description

The IgNiTE trial is a multi-centre randomised, placebo controlled, double blind trial aimed at evaluating the early use of IVIG treatment in children with all-cause encephalitis. A total of 308 children were planned to be recruited over a period of 42 months, from 30 NHS hospitals in the UK. The planned study duration was 60 months, which included 12 months follow up of participants.

5.2.2 Participants selection and recruitment

Inclusion criteria

The inclusion criteria were adapted from the Consensus Statement of the International Encephalitis Consortium case definition:[194]

Major criteria[1]

1) Age: 6 weeks to 16 years

2) Acute (within 24 hours) or sub-acute (between 24 hours and 4 weeks) onset of altered mental state (reduced or altered conscious level, and/or irritability, and/or altered personality or behavior, and/or lethargy) not attributable to a metabolic cause

3) Parent/guardian/legal representative able to give informed consent

Minor criteria

At least two of:

(a) fever $\geq 38^{o}C$ within 72 hours before or after presentation to hospital

[1] All 3 of the major criteria were required

(b) brain imaging evidence consistent with encephalitis or immune-mediated encephalopathy that is either new from prior studies or appears acute in onset

(c) CSF pleocytosis >4 white cells per microlitre

(d) generalised or partial seizures not fully attributable to a pre-existing seizure disorder

(e) new onset focal neurological signs (including movement disorders) for >6 hours

(f) abnormality on EEG that is consistent with encephalitis and not clearly attributable to another cause.

Exclusion criteria

The exclusion criteria used in the IgNiTE trial are summarised in Table 5.1

Table 5.1 Exclusion criteria for the IgNiTE trial

1) High clinical suspicion of bacterial meningitis or TB meningitis (for example: presence of frankly purulent CSF; CSF white cell count (WCC) >1000/microlitre; bacteria on Gram stain and/or culture)
2) Receipt of any IVIG product during the index admission where this was administered prior to obtaining written informed consent for the IgNiTE study
3) Traumatic brain injury
4) Known metabolic encephalopathy
5) Toxic encephalopathy (i.e. encephalopathy secondary to exposure to intoxicants, including alcohol, prescription or recreational drugs)
6) Hypertensive encephalopathy/posterior reversible encephalopathy syndrome
7) Pre-existing demyelinating disorder; pre-existing antibody mediated CNS disorder; pre-existing CSF diversion
8) Ischaemic or haemorrhagic stroke
9) Children with a contra-indication to IVIG or albumin (i.e. history of anaphylactic reaction to IVIG or albumin, known total IgA deficiency and history of hypersensitisation)
10) Known hypercoagulable state
11) Significant renal impairment defined as GFR of 29mls/min/1.73m2 and below (Chronic Kidney Disease Stage 4)
12) Known hyperprolinaemia
13) Known to be pregnant
14) Any other significant disease or disorder which, in the opinion of the Investigator, may either put the participants at risk because of participation in the trial, or may influence the result of the trial, or the participant's ability to participate in the trial
15) Participants who were being actively followed up in another research trial involving an investigational medicinal product (IMP) where the IMP was thought to potentially have an immunomodulatory or neuroprotective effect
16) Any other condition which, in the opinion of the investigator, may have interfered with the ability to fulfil study requirements, especially relating to the primary objective of the study (this included plans to be outside the UK for more than 12 months after enrolment)

Key: TB meningitis=tuberculous meningitis, CSF=cerebrospinal fluid, CNS=central Nervous System, GFR = Glomerular filtration rate, IgA=immunoglobulin A, UK=United Kingdom.

5.3 Study objectives

Primary Objective

The primary objective of the IgNiTE study was to compare neurological outcomes between participants treated with IVIG or placebo, in addition to standard care. This was assessed at 12 months after randomisation, using the paediatric version of the Glasgow Outcome Score Extended (GOSE-Peds).

Secondary objectives

- to compare (1) clinical, (2) further neurological, and (3) radiological outcomes between participants treated with IVIG or placebo, in addition to standard therapy
- to confirm the safety of IVIG treatment for children with encephalitis
- to identify the proportion of children with immune-mediated encephalitis
- to determine the effect of IVIG treatment on neuronal autoantibody levels in children with immune-mediated encephalitis

Further neurological outcomes were assessed using various age specific questionnaires and outcome scores including the following:

a. Strength and Difficulties Questionnaire
b. Adaptive Behaviours Assessment System-Second Edition
c. Pediatric Quality of Life scoring algorithm
d. Liverpool Outcome Score
e. Gross Motor Function Classification System

A blinded neuropsychology assessment was conducted at 12 months after randomisation. In addition, clinical information on the use of anti-epileptic medication and new diagnosis of epilepsy were obtained throughout the study.

An optional, research specific brain MRI scan was performed around 6 months after randomisation, where consent for this was obtained.

Tertiary objectives

- to explore clinically relevant neuroimaging predictors of childhood encephalitis
- to explore predictors of neurological outcomes in children with encephalitis
- to explore radiological patterns associated with different types of encephalitis
- to understand the host inflammatory pathways in encephalitis, the relationship with clinical parameters, and the effect of IVIG treatment on these pathways

Participants in the IgNiTE study were followed up for a total of 12 months after randomisation, with assessments at 4-8 weeks after discharge, and at 6 and 12 months after randomisation.

Specific objectives for this thesis

Given the planned duration of the IgNiTE study, and the timeframe for this thesis, all the above objectives are not covered in this thesis. IgNiTE was a double blinded study and blinding was still in place at the time of writing, therefore assessment of efficacy of IVIG treatment in encephalitis is beyond the scope of this Thesis.

The objective of this Chapter was to describe a cohort of participants enrolled to the IgNiTE study from December 2015 to January 2018 by reporting on the following:

- Demographics

- Clinical presentation
- Results of routine clinical laboratory results
- Radiological investigation results
- Electroencephalogram (EEG) results
- Adverse events
- Clinical and neurological outcomes

5.4 Description of neurological outcome measures reported in this thesis

5.4.1 GOSE-Peds

The GOSE-Peds is a modified version of the GOSE, a gold standard for measuring outcomes in adults with traumatic brain injury. The GOSE-Peds provides a developmentally appropriate structured interview necessary to evaluate children across different age groups. Its use has been validated and found to be sensitive to both severity of injury and to recovery over time, at least 6 months after brain injury and has been suggested as useful in guiding treatment in the early phases of recovery from brain injury.[357] Based on the response to each of the questions, a score was assigned, which put the participant into one of 8 categories: 1-Upper Good Recovery, 2-Lower Good Recovery, 3-Upper Moderate Disability, 4-Lower Moderate Disability, 5-Upper Severe Disability, 6-Lower Severe Disability, 7-Vegetative State, and 8-Death. 'Good recovery' was defined as a GOS-E - Peds score of 2 or lower and a score of >2 indicated 'poor recovery'. The GOSE-Peds was administered by a research nurse and the study neuropsychologist (who were blinded to treatment allocation) at 6 and 12 months after randomisation, respectively.

5.4.2 Liverpool Outcome Score (LOS)

The LOS is a validated tool for assessing the level of disability after encephalitis in infants and children. Originally designed to assess disease burden following Japanese Encephalitis,[358] its use has been extended to other forms of encephalitis. The LOS has been adopted by the WHO and is being used in many resource poor settings, but is equally applicable to patients in the UK.

In the IgNiTE trial, the LOS was administered at 4-8 weeks after discharge and at 12 months after randomisation and was completed by the participant and/or parent. From this, a total score (sum of scores for all questions) was calculated. In addition, an outcome score, the lowest score for any single question asked were documented. Based on the outcome score only, participants were assigned to one of 5 outcome categories: 5-Full recovery, 4-Minor sequelae, 3-Moderate sequelae, 2-Severe sequelae, and 1-Death. 'Good recovery' was defined as a LOS of 5 and a score of ≤ 4 indicated 'poor recovery'.

5.4.3 Gross Motor Function and Manual Ability Classification Systems

The Gross Motor Function Classification System (GMFCS) is a five-level classification system originally designed for children with cerebral palsy and assesses gross motor function, based on self-initiated abilities.[384] The GMFCS assesses motor function in three areas - walking, sitting and standing. The Manual Ability Classification System (MACS) is a corresponding classification of manual ability and was applied from 4 years of age.[385] For both classification systems, Level 1 indicated no limitations, while Levels 2-5

indicated varying degrees of limitation in performing an assigned task. The GMFCS and MACS were completed by the participant and/or parent at 4-8 weeks after discharge and 12 months following randomisation.

5.5 Participant identification and eligibility assessment

Potential participants were identified through various routes which included review of medical handover notes, scouting the wards for new admissions with a suspected diagnosis of encephalitis, and review of routine clinical investigation results. Following identification of a potentially eligible patient, their clinical presentation was matched against the study inclusion criteria. Parents or guardians of the patients meeting the study criteria were approached by a member of the clinical team to seek their interest in the study. If interest was indicated, a member of the research team approached the family to confirm eligibility and to seek consent. Assent from the patient was also required if they were ≥6 years of age and judged to have sufficient mental capacity to provide this.

5.6 Randomisation, blinding, and study treatment

Enrolled patients were randomised in a 1:1 ratio to receive 2 doses (1g/kg/dose) of either IVIG or matching placebo, in addition to standard care. Randomisation was performed soon as possible after consent was obtained, and within the time window for administration of the first dose of study drug. This was done using an online randomisation system (Sortition). Stratification factors accounted for at randomisation were age (< 1 year, 1-4 years, 5-9 years, 10-14 years and ≥15 years) and steroid treatment at the time of randomisation. The trial was a double blinded trial in which neither participants, their parents/guardians, not the investigators were aware of the treatment

allocation. However, for practical reasons around the administration of the study treatment, the clinical trials pharmacist at each site had to be unblinded.

The IVIG used in the study was Privigen (100mg/ml solution)[2], with a shelf life of 3 years. The placebo was a mixture of 0.9% saline +0.1 human albumin solution[3], which had a shelf life of 6 months. The addition of albumin to saline was necessary to make the placebo visually identical to IVIG. Also, similar packaging and labelling were applied to both study treatments. A tear off label distinguishing both treatments was taken off at the time of dispensing by the unblinded pharmacist at each recruiting site.

5.7 Timeline for administering study treatment

The first dose of study treatment was to be given within 120 hours from presentation to hospital for the illness. Inter-hospital transfers are not unusual for patients with encephalitis and this could happen a few days into an admission such that if the patient was eligible for the IgNiTE study, there would have been little time left within the 120 hour window following transfer to approach the family and obtain consent. This would have led to non-inclusion of a proportion of this cohort of patients. Since the most common reason for transfer is the need for intensive care support, transfer patients represent the sicker of the cohort. Thus, to allow the incorporation of transfer patients as far as was possible, such that the IgNiTE cohort was truly representative of children with encephalitis in the UK, a modified time window was applied for transfer patients only. This was 72 hours from admission to the transfer hospital, only if this provided more time than if 120 hours from the initial presentation at the referring hospital was considered. In

[2] Provided by CSL Behring

[3] Manufactured at the Aseptic Manufacturing Pharmacy Unit of the Royal Liverpool and Broadgreen University Hospital NHS Trust, UK, under current Good Manufacturing Practice conditions, under its Manufacturer/Importers license

all cases, the second dose of study treatment was to be given 24-36 hours from the first dose.

5.8 Stratification factors

Several reports have suggested an association between age and outcomes, thus age could be a confounding variable. Evidence from the most recently published epidemiologic data[14] and an unpublished observation,[386] the study design suggested that 10-30% of children with encephalitis would have an autoimmune form of encephalitis and could have received corticosteroid therapy prior to enrolment to the study, which is an additional confounder. Therefore, age and steroid use at randomisation were considered as stratification factors to ensure balance between the treatment groups. The effect of the steroid use after randomisation was to be studied in an exploratory analysis.

5.9 Assessment of safety

Participants were monitored during, and 20 minutes after administration of the study treatment for adverse events. Adverse events of special interest were reported in the first five days following each dose of study treatment, serious adverse events (SAEs) were reported up to 6 months after randomisation, and serious adverse reactions were reported up to 12 months after randomisation. The IgNiTE trial had both Data Safety and Trial Steering Committee oversight.

5.10 Sample size calculation

A sample size of 308 (154 per treatment group which includes 10% attrition rate) provided 90% power and 5% level of significance (2-sided) to detect at least 20%

clinically significant treatment difference from 43% in the "good recovery" rate (i.e. GOS-E-Peds score ≤2) by 12 months after randomisation. The baseline recovery rate of 43% was based on the finding of a Public Health England Study, [14] while a 20% effect size was based on the finding of an observational study of IVIG in encephalitis.[216]

5.11 Data management

Trial data were managed through a password protected central web based database (OpenClinica™). Data were anonymised and participants were identified using a unique trial specific number.

5.12 Ethics and funding

Ethical approval was obtained from the Oxfordshire Research Ethics Committee A (14/SC/1416, EudraCT No 2014-002997-35) and registered on Clinicatrials.gov (identifier NCT02308982). Funding was provided by the National Institute for Health Research, Efficacy and Mechanism Evaluation Theme. IVIG and funding for the placebo were provided by CSL Behring.

5.13 Trial coordination and conduct

The trial was coordinated through the Oxford Vaccine Group, a UK Clinical Research Collaboration registered clinical trials unit working in collaboration with the Primary Care trials unit at the University of Oxford (registration number: 52), and was sponsored by the University of Oxford. The trial was fully compliant with International Conference on Harmonisation Good Clinical Practice. [387]

5.14 Data analysis

Presented in this Chapter are the results from an interim analysis of available data from enrolled participants as at 15th January 2018. Due to the slow recruitment, only a small number of participants had been enrolled to IgNiTE and are included in these analyses. Descriptive statistics were used to report on available data. Continuous variables are presented as median (IQR). Categorical variables are presented as total count (n) and percentage (%). Spearman's correlation analysis was used to compare LOS and GOSE-Peds scores.

5.15 Trial progress

Recruitment commenced in December 2015 and at the time of suspension, a site initiation visit (SIV) had been conducted in 29 sites, three of these were done within 3 prior to recruitment suspension (Table 5.2). Twenty-one (72%) sites had opened, for three of these, site opening occurred within 4 months prior to recruitment suspension. For an additional 5 sites that were not open, an SIV had been conducted at least over 1 year prior to suspension of recruitment (Table 5.2). The median time from SIV to site opening was 4 months (range 2.2-9.2).

Table 5.2 IgNiTE recruiting sites showing the sequence of activity at each site

No.	Site	SIV date	Date opened	Date of 1st recruit
01	Oxford University Hospitals	26/08/2015	23/12/2015	24/02/2016
02	Guy's and St Thomas's - Evelina	06/10/2015	19/05/2016	30/11/2016
03	St George's Hospital	12/10/2015	22/05/2016	27/10/2016
04	Alder Hey Children's Hospital	16/11/2015	30/03/2016	19/05/2017
05	University Hospitals Bristol	24/11/2015	25/08/2016	N/A
06	North Manchester Children's Hospital	30/11/2015	30/06/2016	19/05/2017
07	University Hospital Southampton	10/12/2015	N/A	N/A
08	Great Ormond Street Hospital	14/12/2015	01/03/2016	21/09/2016
09	Barts and the London (Royal London)	15/01/2016	04/05/2016	N/A
10	Sheffield Children's Hospital	14/01/2016	26/05/2016	25/05/2017
11	NHS Grampian	24/02/2016	07/06/2016	24/11/2016
12	Ninewells (Tayside Health Board)	25/02/2016	17/06/2016	N/A
13	Heart of England	02/03/2016	01/06/2016	15/02/2017
14	South Tees - James Cook Hospital	07/03/2016	N/A	N/A
15	Birmingham Children's Hospital	21/03/2016	N/A	N/A
16	Royal Hospital for Sick Children, Edinburgh	21/04/2016	22/07/2016	N/A
17	Imperial – St Mary's Hospital	20/06/2016	11/10/2016	13/02/2017
18	Cambridge - Addenbrooke's	09/08/2016	N/A	N/A
19	Nottingham University Hospitals	01/09/2016	07/11/2016	22/11/2016
20	University Hospitals of North Midland	19/09/2016	09/03/2017	N/A
21	Hull Royal Infirmary	27/10/2016	09/02/2017	27/02/2017
22	Leeds Teaching Hospital	24/10/2016	16/03/2017	N/A
23	York Teaching Hospital	31/10/2016	11/05/2017	N/A
24	Central Manchester University Hospital	29/11/2016	N/A	N/A
25	Royal Cornwall Hospitals	15/12/2016	18/07/2017	N/A
26	Royal Preston Hospital	26/01/2017	13/04/2017	N/A
27	Leicester Royal Infirmary	22/08/2017	N/A	N/A
28	Royal Berkshire Hospital	09/06/2017	N/A	N/A
29	Royal Exeter and Devon	30/06/2017	N/A	N/A

Key: Sites highlighted in grey had problems with set up and had not opened at the time of recruitment halt despite having had a site initiation visit. N/A=not applicable.

5.16 Results

Recruitment

The process of participant selection is shown in Fig 5.1. Of 884 screened participants, 672 (76%) were excluded. For 111 of those excluded (63%), the reason for exclusion was not sufficiently detailed. These patients were screened using an old log which was subsequently modified to capture more detailed information on the reasons for exclusion.

For at least 561 of excluded patients (63%), the reason for exclusion was due to not meeting the inclusion criteria. Of 212/884 (24%) who met the inclusion criteria 52 (25%) were approached and 18 enrolled, from 12 sites. This gives an overall consent rate of 35%. The median time from site opening to a first recruit was 6.1 months (range 0.5-13.8). A total of 77 patients who met the inclusion criteria (36%) were not approached. For 10 of these (13%) who were screened using the old log, the reason for non-approach was not documented. Of the remaining 67 participants, 65 (97%) were excluded because they met at least one exclusion criterion, 15 (23% of those excluded, 7% of those who met the inclusion criteria) of these were out of the study timeline for the first dose, and 20 (31% of those excluded, 9% of those who met the inclusion criteria) were due to prior or planned IVIG treatment as part of routine clinical care. Overall, participant recruitment at each site lagged significantly behind estimated recruitment (Table 5.3)

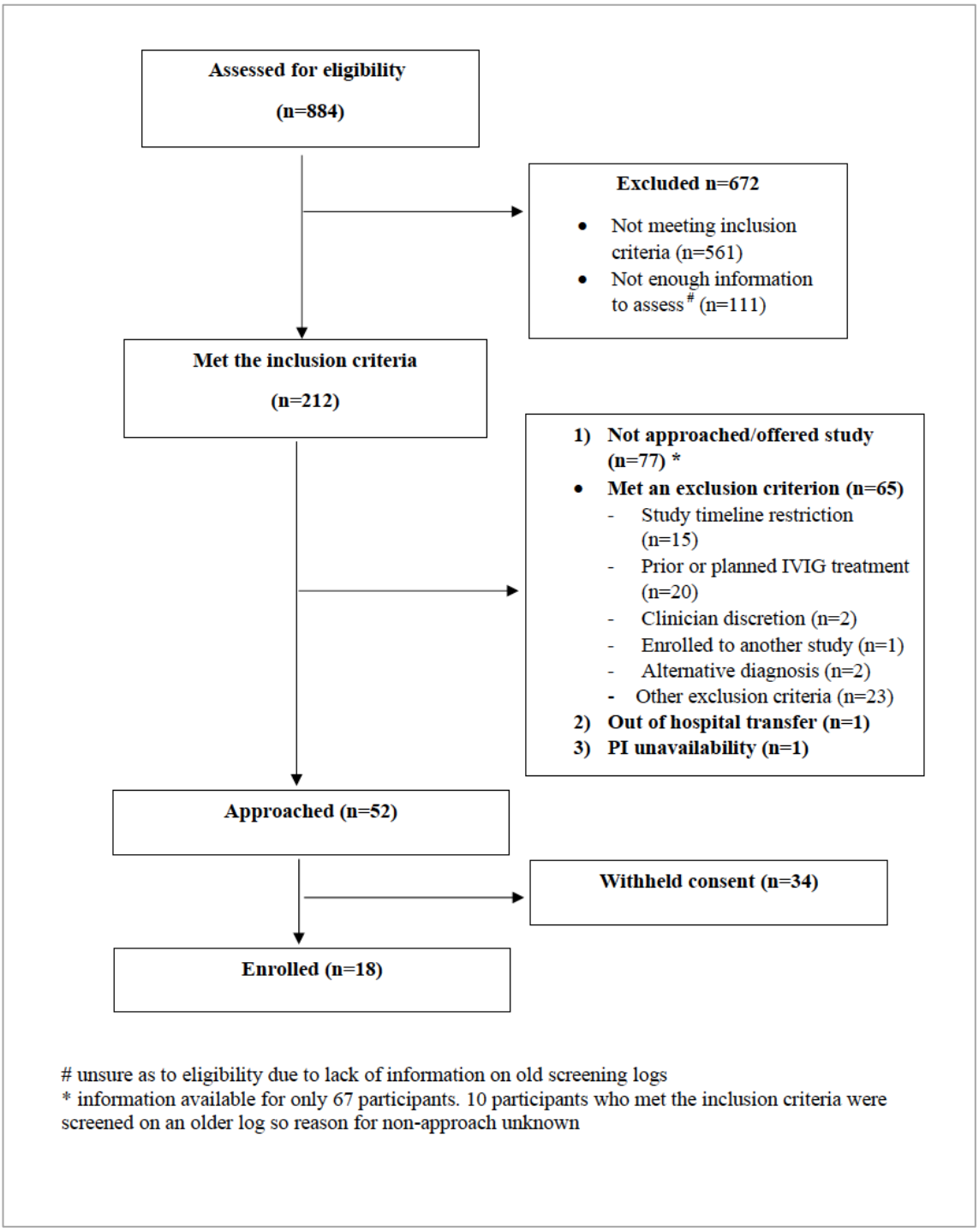


***Figure 5.1** Consort diagram showing the process of participant selection*

Table 5.3 Number of participants recruited against estimated recruitment for each open site[1]

Site	Number of whole months since opening	Estimated recruitment for number of months open	Actual recruitment
Oxford University Hospitals	21	7-8	3
Great Ormond Street Hospital	19	2-3	1
Alder Hey Children's Hospital	18	11-12	3
St George's Hospital	18	4-5	1
Barts and the London (Royal London)	17	2-3	0
Guy's and St Thomas's - Evelina	16	10-11	2
Sheffield Children's Hospital	16	1-2	1
NHS Grampian	16	4-5	2
Heart of England	16	3-4	1
Pennine - North Manchester Children's Hospital	15	4-5	1
Ninewells (Tayside Health Board)	15	0-1	0
Royal Hospital for Sick Children, Edinburgh	14	2-3	0
University Hospitals Bristol	13	3-4	0
Imperial – St Mary's Hospital	11	5-6	1
Nottingham University Hospitals	11	1-2	1
Hull Royal Infirmary	7	1-2	1
University Hospitals of North Midland	6	1-2	0
Leeds Teaching Hospital	6	2-3	0
Royal Preston Hospital	5	0-1	0
York Teaching Hospital	4	0-1	0
Royal Cornwall Hospitals	2	1-2	0

Key: NHS=National Health Service

Progress of the enrolled 18 participants through the study is summarised in Figure 5.2. In total, three participants were withdrawn from the study. Two of these were prior to receipt of the study treatment, one was due to concerns regarding staffing capacity at the recruiting hospital and the other was due to planned out of hospital transfer. The latter was re-enrolled at the transfer hospital. Parental consent was withdrawn for one participant before the 6-month time point.

[1] Data as at September 2017

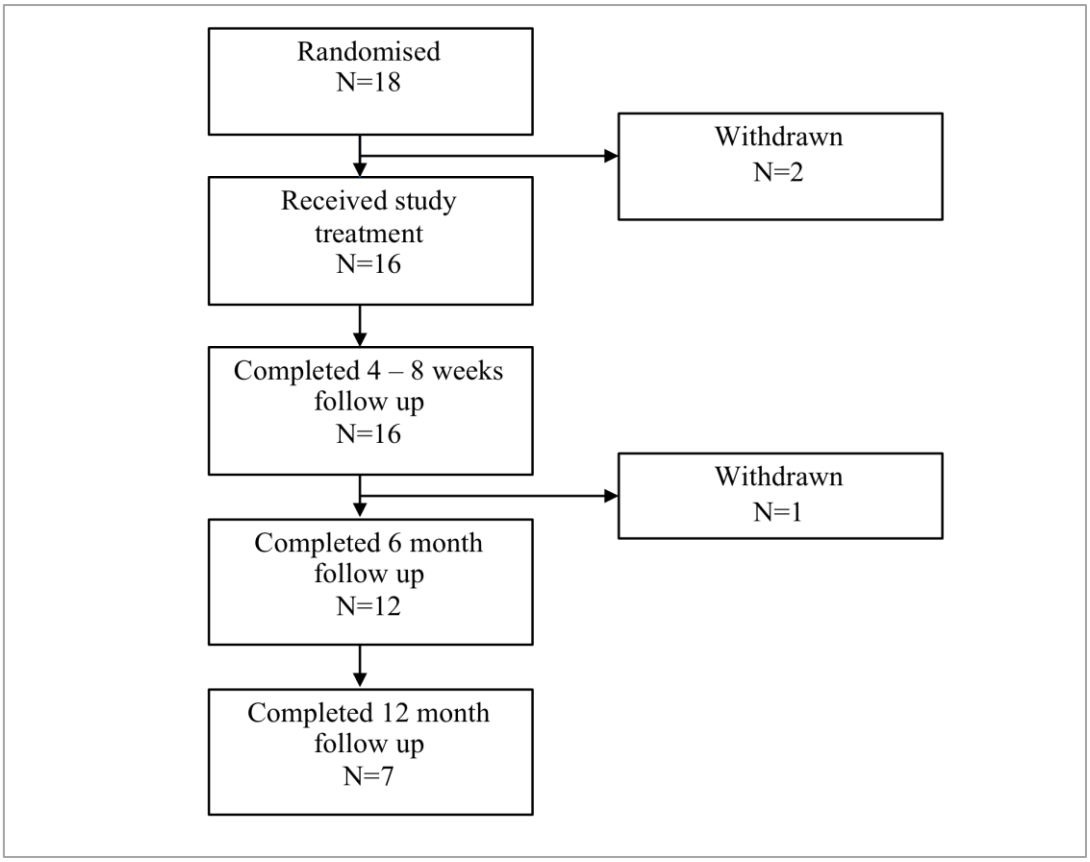


***Figure 5.2** Number of participants in the study and follow up time points completed. Not all data from all time points were available at the time of writing this thesis*

Demographic data

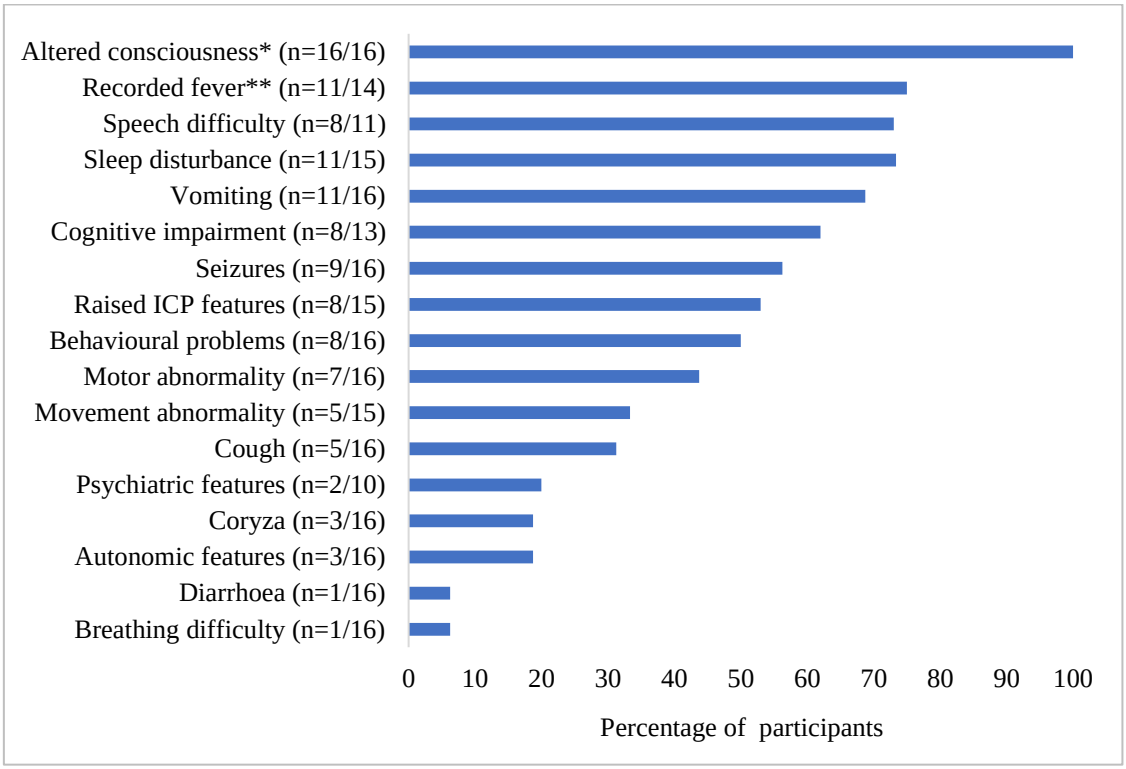
Baseline demographic and medical history data were available for 17 participants, 9 (53%) were male and 16 (94%) were White. Thirteen children were previously healthy, while 3/17 (18%) were reported to have existing problems affecting speech and language at the time of enrolment. Of 15 participants with available data, 13 (87%) were transferred from another site to the recruiting hospital. The reason for transfer was available for 9 participants, 6 (67%) needed intensive care support and 3 (33%) required specialist neurology input. The median weight on admission (n=15) was 20 kg (IQR 12-48). The median time to first presentation from illness onset (n=16) was 2 days (IQR 0.3-5.0). At presentation, a history of fever was obtained for 12/16 (75%) participants, for one participant, this information was missing. The date of fever onset was not known for one participant. For the remaining 11 participants with reported fever, 9 (82%) cases had

a fever on the same day as illness onset. The median highest recorded home temperature in 10/12 (83%) participants reporting a fever was 38.2^{o}C (IQR 38.0-39.0). The recorded highest temperature within 24 hours of admission was available for 14 participants and for 11 (79%) of these, the temperature was ≥ 38^{o}C. At presentation, the median highest recorded temperature within 24 hours of admission was 38.4^{o}C (IQR 37.9-38.6).

Clinical features

Figure 5.3 gives a summary of clinical features at presentation. Seizure history was missing for one participant. A history of seizures was reported for 9/16 (56%) participants, 5 males (56%), of which 5/9 (56%) were generalised, 1/9 (11%) focal and 2/9 (22%) complex. Seizure type was not documented for one participant. At presentation, 3/9 (33%) participants had experienced one seizure, 2/9 (22%) had experienced 2 seizures, and 4/9 (44%) had three or more seizures. Glasgow coma score (GCS) was available for 14/15 (93%) participants, 3/13 (23%) had a score of 7 or less, 6/13 (46%) had a score between 8 and 14, while 4/13 (31%) had a score of 15. The GCS assessment was incomplete for one participant. The median total score of the first GCS assessment done following presentation (n=13) was 13 (IQR 8-15).

Figure 5.3 Presenting clinical features for the IgNiTE cohort



*Key: Denominators indicate total number of children with available results or assessed for each symptom. *Major inclusion criterion ** Defined as a temperature reading of ≥ 38ºC and based on maximum temperature within 24 hours of first presentation. ICP=intracranial pressure.*

Laboratory investigation results

Blood investigations

A summary of routine clinical blood investigation results is presented in Table 5.4. Admission bloods were missing for 2/17(12%) participants. The median times to the first (n=15) and second set (n=13) of blood results from first presentation and illness onset were 2.0 days (IQR 0.3-4.5) and 3.0 days (1.0-4.5) respectively. The C-reactive protein (CRP) was raised (>5mg/dL) in 7/13 (54%) on the first set of bloods and in 6/10 (60%) of participants on the second set. The median CRP[1] level was 17.2 mg/dL (IQR 9-151) and 14.5mg/dL (9.3-184.3) on the first and second blood test results respectively.

[1] For participants with a CRP level > 5mg/dL

Table 5.4 Results from the first two sets of blood tests done following hospital presentation

Blood test (unit)	1st set of bloods of blood investigations	2nd set of blood investigations
Hb (g/dL)	12.7 (11.4-13.6) N=15	11.6 (10.8-13.7) N=10
WBC $x10^9$/L	11.6 (6.4-14.6) N=15	9.7 (5.9-15.1) N=10
Neutrophil count $x10^9$/L	7.7 (4.2-10.7) N=14	5.9 (3.9-11.0) N=10
Lymphocyte count $x10^9$/L	1.9 (1.5-3.7) N=13	1.5 (1.3-1.7) N=10
Platelet count $x10^9$/L	305 (251-382) N=15	254 (184-301) N=10
Urea (mmol/L)	4.4 (3.5-5.0) N=13	3.5 (2.5-4.0) N=12
Sodium (mmol/L)	138 (136-141) N=13	140.0 (137-142) N=12
Creatinine (umol/L)	41 (26-56) N=13	43 (26-54) N=11
ALT (U/L)	18.0 (10-26) N=11	13.0 (10-19) N=9
Albumin (g/L)	41.5 (34.5-46.3) N=14	35.5 (31-38.5) N=10
Bilirubin (umol/L)	5.5 (5.0-9.8) N=12	6.0 (4.5-9.0) N=9
Serum glucose (mmol/L)	7.6 (6.3-10.4) N=5	6.8 (6.0-7.6) N=3
CRP (mg/dL)[1]	17.2 (9-151) N=7	14.5 (9.3-184.3) N=6
Ammonia (umol/L)	28 (20.8-32)	N/A

Data are presented as median (interquartile range). Key: Hb=haemoglobin, WBC=white blood cell count, ALT=alanine transferase, CRP= C- reactive protein, N/A=not applicable. N=number of participants with available data.

Lumbar puncture and CSF analysis

Information on whether an LP was performed at first presentation was available for 15/17 (88%) participants and an LP was performed in 10 (67%). Of the 5 participants who did not have an LP, 1 was due to thrombocytopaenia, 1 was due to planned transfer to another hospital and a reason was not documented for the remaining three (60%). The median time to first LP from presentation was 1.0 day (IQR 1.0-3.0). The cerebrospinal fluid (CSF) was clear on visual inspection for all 10 participants who had an LP. The CSF white cell count (WCC) was not documented for 1 participant. CSF pleocytosis (> 4 white cells/ mm^3) was documented in 5/9 (56%) participants. The CSF of two of the nine participants (22%) with available CSF results had a CSF WCC of <1/mm^3, 7/9 (78%) participants had a CSF WCC of ≥1/mm^3. The median CSF WCC for those with a count of ≥1/mm3 (n=7) was 15 white cells/mm3 (4-86). The median CSF WCC for those with pleocytosis (n=5) was 64 white cells/mm3 (14-94). Three of 10 (30%) participants with available results had a raised CSF protein level (>0.4g/l). The median CSF protein count (n=10) was 0.35 g/l (IQR 0.26-0.94). For one participant, a CSF glucose level was not documented the median CSF glucose level in the remaining nine participants was 3.3mmol/L (2.9-4.1). The CSF culture and PCR results were known for 9/10 (90%) participants. The CSF culture was negative in all 9 (100%), and the CSF PCR was negative in 8/9 (89%) participants. For one participant (11%) the CSF PCR test was positive for VZV.

Other microbiology

Information on whether a viral or bacterial throat swab was performed was not available for 3 participants. Of the remaining 14, seven (41%) each either did not have a bacterial

swab done or had a negative result. Of 14 participants with available data on viral throat swab, 9 (64%) did not have a viral throat swab, 2 (14%) had a negative result, while 3 (21%) had a positive result. Of the 3 positive viral swab PCR results, 2 (67%) were positive for Rhinovirus and 1(33%) for EV. Information on whether a nasopharyngeal aspirate (NPA) PCR test was done was available for 15 participants and this test was not done in 10/15 (67%) participants. Of those that had an NPA PCR test (n=5), 3 (60%) were positive, 1 for adenovirus, 1 for rhinovirus and 1 for both *Streptococcus pneumoniae* and *Haemophilus influenza*. Of 15 participants with data on stool PCR, this test was not done in 13 participants. Of the two participants who had a stool PCR, one tested positive for adenovirus and the other was negative. One participant had endotracheal secretions taken for PCR testing and this was positive for human coronavirus.

Autoantibody testing

Data on autoantibody testing were available for 15/17 participants, and autoantibody testing was performed in 8/15 (53%) participants.

Of the 8 participants who had autoantibody testing, testing for NMDAR antibodies was not done in 1 (13%) case. Of the remaining 7 cases, the result (both CSF and serum) was unknown in 1 (14%) case, and in another, the serum but not CSF result was known. Of 2 participants with available paired[1] anti-NMDAR antibody test results, the result was negative in both samples for both participants. Of the 8 participants who had an autoantibody test, anti-NMDAR antibodies were tested for in the serum but not CSF in 3 (38%) cases.

Of the 8 participants who had autoantibody testing, testing for VGKC autoantibodies was not done in 2 (25%) cases. Of the remaining 6 participants, the results (in both CSF and

[1] In both CSF and serum

serum) were unknown in 1 (25%) case and in another, the serum but not CSF result was known. Of 2 participants with paired VGKC results available, the result was negative in both serum and CSF for both participants. Of the 8 participants who had an autoantibody test, VGKC antibodies were tested for in the serum but not CSF in 2 (25%) cases (25%). Anti-GQ1B antibodies were detected in the serum of one participant who had negative serum and CSF NMDAR and VGKC antibody results. Data on oligoclonal band (OCB) testing were not available for 5/17 (29%) participants. Of the 12 (71%) with available data, OCB testing was not done in 9 (75%) cases and the result was negative in all 3 participants that were tested.

Neuroimaging and EEG

Data on brain MRI scan performed during the admission were available for 15/17 (88%) participants. Fourteen (93%) participants had a brain MRI scan while 1(7%) did not. The median time to first brain MRI from illness onset and first presentation (n=14) was 5 days (IQR 2.0-9.3), and 2.0 days (1.0-4.3) days respectively. The first brain MRI scan performed following presentation to any hospital was abnormal for 9/14 (64%) participants. Summary characteristics of participants by first brain MRI scan finding are shown in Table 5.5. Six of nine (67%) male participants had an abnormal first brain MRI, while for 2/5 (40%) males had a normal first brain MRI scan. Four of nine (44%) participants with an abnormal first brain MRI scan presented with seizures and focal neurology while 5/9 (56%) had a normal scan. One of six participants (17%) with abnormal first brain CT scan also had an abnormal first brain MRI scan but this was normal in 5/6 (83%) participants. Three quarters of participants with a normal first brain MRI scan had an abnormal first EEG while a quarter had a normal first EEG. Data on the area of the brain involvement were not available for 1 participant. Of the remaining 8 participants with an abnormal first brain MRI, all (100%) had white matter involvement,

2 (25%) had grey matter involvement and 2 (25%) had brainstem involvement. Four of the 8 participants (50%) with an abnormal first brain MRI scan had multiple areas of involvement. Specific regions of involvement are shown in Table 5.6. Five participants had a repeat MRI scan (Table 5.6). Three of these had an abnormal first scan which normalised by the repeat scan for 2 (67%) participants but remained abnormal for 1 participant (33%).

Table 5.5 Characteristics of participants based on the first brain MRI scan finding

	Abnormal brain MRI (n=9)	Normal brain MRI (n=5)
Sex, male	6/9 (67)	2/5 (40)
Seizures, yes	4/9 (44)	3/5 (60)
Focal neurology, yes	4/9 (44)	3/5 (60)
Abnormal first CT scan	1/6 (17)	0/4 (0)
Abnormal EEG*	5/7 (71)	3/4 (75)
Median age (IQR)	4.5 (2.6-13.9)	2.7 (0.6-7.9)
Median time (days) to scan from first presentation (IQR)	2.0 (1.5-4.5)	1.0 (0.5-4.5)
Median time (days) from illness onset (IQR)	3.5 (3.5-7.5)	2.0 (1.5-19)

Key: EEG report not available for 3 participants (abnormal MRI scan group=2, normal MRI scan group=1), MRI= magnetic resonance imaging, EEG =electroencephalogram, IQR=interquartile range. Data are counts (%) unless otherwise specified.

Table 5.6 Regions of brain involvement as documented on brain MRI scan reports

No.	1st MRI scan	Area of involvement/classification	2nd MRI scan	Area of involvement
1	Abnormal	Frontal, parietal, temporal, occipital, insular (W, G)	Abnormal	Basal ganglia Hippocampus (G)
2	ND	N/A	Normal	N/A
3	Abnormal	White matter* (W)	Abnormal	Parietal (W)
4	Abnormal	Frontal, insular, temporal and parietal (W, G)	Normal	N/A
5	Normal	N/A	ND	N/A
6	Abnormal	White matter * (W)	ND	N/A
7	Abnormal	UNK	ND	N/A
8	Data not available	N/A	Data not available	N/A
9	Normal	N/A	ND	N/A
10	Data not available	N/A	Data not available	N/A
11	Abnormal	Centrum ovale, pons (B, W)	ND	N/A
12	Normal	N/A	Normal	N/A
13	Abnormal	Pons, central cerebellum (B, W)	ND	N/A
14	Abnormal	Corpus callosum (W)	ND	N/A
15	Normal	N/A	ND	N/A
16	Normal	N/A	Normal	N/A
17	Abnormal	Subcortical white matter, frontal (W)	ND	N/A

Key: *Physical copy of scan report specifying specific area of involvement was not available, N/A=not applicable, ND=not done, W=White matter, G=Grey matter, B=brainstem

Data on the first CT scan following admission were available for 14/17 (82%) participants of these, 11 (79%) had a brain CT scan on admission. The median time to the first CT scan from illness onset (n=11) was 3.0 days (IQR 0.0-3.0). Seven CT scans (64%) were done on the same day of first presentation, 3 (27%) were performed a day later and 1(9%) was performed 2 days after first presentation. The first CT scan following presentation to hospital was normal in 10/11 (91%) of participants.

An EEG was performed on admission in 12/15 (80%) participants. The median time to the first EEG from illness onset was 5.0 days (3.0-8.3) and 3.0 days (1.3-4.0) from first presentation. EEG reports were available for 11 participants, 2 of these were normal and 9 (75%) were abnormal. All the abnormal scans were reported to show slow activity and the findings were generalised in 5/9 (56%) and focal in 4/9 (44%) participants with an abnormal EEG. Summary characteristics of participants by first EEG report are shown in Table 5.7. Six of nine (67%) participants with an abnormal first EEG presented with seizures while 3/9 (33%) did not. Three of nine (33%) participants who had an abnormal EEG presented with focal neurology and 6/9 (67%) did not. One in seven (14%) participants who had an abnormal first EEG also had an abnormal first CT scan, whereas the first CT scan was normal in 6/7 (86%) participants with an abnormal first EEG. Five of eight (63%) participants with an abnormal first EEG also had an abnormal first MRI scan while 3/8 (38%) did not. Two thirds of participants with a normal first EEG presented with focal neurology.

Table 5.7 Participant characteristics based on the result of the first EEG following admission

	Abnormal EEG	Normal EEG
Sex, male	5/9 (56)	1/3 (33)
Seizures, yes	6/9 (67)	0/3 (0)
Focal neurology, yes	3/9 (33)	2/3 (67)
Abnormal first MRI	5/8* (63)	2/3 (67)
Abnormal first CT scan[&]	1/7 (14)	0/2 (0)
Median age (IQR)	7.7 (2.2-13.3)	8.2 (1.1-15.8)
Median time (days) to scan	2.0 (1.0-4.5)	4.0 (4.0-4.0)
Median time (days) from illness	4.0 (3.0-10.5)	5.0 (5.0-9.0)

Key: *MRI report not available for 1 participant, [&]CT scan report not available for 3 participants (2 in the abnormal EEG group, and 1 in the normal EEG group), MRI= magnetic resonance imaging, EEG =electroencephalogram, IQR=interquartile range. Data are counts (%) unless otherwise specified.

Diagnoses

An aetiological diagnosis was suspected or confirmed for 7 participants - Acute necrotising encephalitis (n=1), HSV encephalitis (n=1), VZV encephalitis (n=1), brainstem encephalitis suspected Bickerstaff (n=1), and Epstein Barr Virus infection (n=1). Data entry was incomplete for the participant with a diagnosis of HSV encephalitis and the PCR result had not been entered onto the database at the time of data download. One participant had *PCDH19* mutation and a diagnosis of seizure disorder was made while another participant was subsequently diagnosed with Citrullinemia. One participant was found to have 16p13.11 duplication but it was not clear from data entry whether this was thought to have caused the illness presentation.

Outcomes

Clinical

Data on admission to PICU during the admission were missing for two participants. Of the remaining 15 participants, 9 (60%) were admitted to PICU during the admission of which 8 (89%) required invasive ventilation, and 2 (22%) required vasoactive treatment. None of the participants admitted to PICU required renal support. The median duration of ventilation (n=8) was 2.5 days (IQR 2.0-4.5). The median duration of PICU stay (n=8) was 5.5 days (IQR 3.0-9.3). One participant was readmitted to PICU for mechanical ventilation. The overall median length of hospital stay (n=14) was 11 days (IQR 7-20). All participants were alive at discharge. Data on outcomes at discharge were not available for two participants. Of the remaining 15 participants, 12 (80%) had persisting symptoms, while 3 (20%) did not have persisting symptoms at discharge. Persisting symptoms at discharge included motor difficulty (n=9, 75%), movement difficulty (n=5, 42%), problems with cognition (6/9, 67%), sleep difficulty (7/11, 64%). Four of 12 children

(33%) had at least one of the behavioral problems assessed. At the time of discharge, the behaviors of 8/10 (80%) children were reported to be abnormal by a parent. A breakdown of the individual symptoms within these broad categories is presented in Table 5.8. Some participants who had persisting symptoms were judged too young to be assessed for some of symptoms (e.g. headache) and in some cases, there were missing data for some symptoms. Therefore, the denominators in Table 5.8 represent the total number of participants who had persisting symptoms at discharge, were assessed for a particular symptom, and had available data.

Table 5.8 Persisting symptoms at discharge

Symptom assessed	Number reporting symptom (%)*	Symptom progression		
		Some improvement (n, %)	Worsened n (%)	New onset n (%)
Headache	3/8 (38)	3 (100)	0	0
Sleep problems	7/11 (64)	3 (43)	1 (14)	3 (43)
Cognitive				
Concentration/attention	3/6 (50)	2 (67)	1 (33)	0
Memory difficulty	2/4 (50)	2 (100)	0	0
Difficulty finding words	4/8 (50)	3 (75)	1(25)	0
Problem with speech	5/9 (56)	4 (80)	1 (20)	0
Problems with vision	1/9 (11)	1 (100)	0	0
Other cognitive	2/6 (33)	2 (100)	0	0
Seizures	3/12 (25)	2 (67)	1 (33)	0
Motor				
Weakness	6/12 (50)	4 (67)	2 (33)	0
Reduced mobility	7/12 (58)	4 (57)	3 (43)	0
Movement				
Abnormal	3/12 (25)	2 (67)	1 (33)	0
Ataxia	2/8 (25)	1 (50)	1 (50)	0
Behavioral				
Hyperactivity	1/8 (13)	1 (100)	0	0
Agitation	4/11 (36)	2 (50)	2 (50)	0
Aggression	0/9 (0)	N/A	N/A	N/A
Obsessive compulsion	0/5 (0)	N/A	N/A	N/A
Anxiety	0/7 (0)	N/A	N/A	N/A
Depression	0/5 (0)	N/A	N/A	N/A
Psychiatric problems	0/4 (0)	N/A	N/A	N/A

Key: *Denominator represents number of children assessed for each outcome and with available data.
N/A= not applicable

Neurological

GMFCS at discharge

At discharge, the GMFCS was completed for 11 (walk domain); 13 (sit domain) and 12 (stand domain) participants. Table 5.9 shows the individual scores for the different domains, and for each participant. 5/11 (45%) participants could walk without limitations, while 6/11 (55%) could, but with limitations. One participant was wheelchair dependent. Ten of 13 children (77%) were able to sit independently, one child (8%) was able to sit with support, while 2/13 children (15%) were unable to sit, even with support. Nine of 12 participants were able to stand independently (75%) whereas 3/12 (25%) were able to stand only with support.

Table 5.9 GMFCS scores at the time of discharge

No	Participant age (years)	Walking	Sitting	Standing
1	1	Not assessed	4	Not assessed
2	1.1	Not assessed	1	1
3	1.8	2	2	3
4	2.7	1	1	1
5	3.7	5	4	3
6	4.5	1	1	1
7	7.7	2	1	1
8	7.8	2	1	3
9	8.2	2	1	1
10	13	1	1	1
11	13.5	1	1	1
12	14.9	2	1	1
13	15.8	1	1	1

Key: GMFCS=Gross Motor Function Classification System, Walking: 1= walks without limitations, 2= walks with limitations, 5=transported in a wheel chair. Sitting: 1 = independently able to sit, 2=able to sit with support, 4= unable to sit; Standing: 1=independently able, 3=able with support

MACS at discharge

Five of 15 children were below the age of 4 years and did not complete a MACS assessment. Of 10 participants >4 years, 7 (70%) completed the assessment. Five of the 7 children (71%) were able to handle objects easily and successfully (MACS level 1), 1

child was able to do this but with somewhat reduced quality and/or speed (MACS level 2), while 1 child could not handle objects and had severely limited ability to perform even simple actions (MACS level 5).

LOS at 4-8 weeks

Liverpool outcome score data at 4-8 weeks were available for 12 participants and 8 of these (67%) had a poor outcome (i.e. LOS ≤4) (Table 5.10). Participant characteristics for the LOS groups are shown in Table 5.11. Five of the eight who made a poor recovery were male (63%), as were 1/4 (25%) with a good recovery. The median age (years) for those with poor recovery was 3.2 (IQR 1.2-7.8) and 10.6 (5.4-13.4) for those with good recovery. The median length of stay (days) for those with poor recovery was 11.0 (IQR 7.3-16.3) and 7.5 (6.3-11.0) for those with good recovery. Two of four participants (50%) who made good recovery and 5/8 (63%) with poor recovery had seizures on admission while 2/4 (50%) and 3/8 (38%) in the good and poor recovery groups respectively had focal neurology on admission. One of four children (25%) with good recovery and 1/8 (13%) with poor recovery were on steroid treatment at the time of randomisation. The first EEG was abnormal in 5/7 (71%) and 1/3 (33%) participants in the poor and good recovery groups respectively. All participants with a poor recovery (8/8, 100%) had persisting symptoms at discharge as did 2/4 (50%) in the good recovery group.

Table 5.10 LOS and GOSE Peds scores for participants enrolled to IgNiTE

Age	LOS at 4-8 weeks	LOS at 12 months	GOSE-Peds score at 6 months	GOSE-Peds at 12 months
1 year	3-Moderate Sequelae	N/A	3-Upper Moderate Disability	1-Upper Good Recovery
13 months	4-Minor Sequelae	N/A	1-Upper Good Recovery	N/A
1.8 years	2-Severe Sequelae	N/A	1-Upper Good Recovery	1-Upper Good Recovery
2.7 years	3-Moderate Sequelae	N/A	6-Lower Severe Disability	6-Lower Severe Disability
3.7 years	2-Severe Sequelae	2-Severe Sequelae	6-Lower Severe Disability	6-Lower Severe Disability
4.5 years	5-Full Recovery	N/A	1-Upper Good Recovery	N/A
7.7 years	3-Moderate Sequelae	N/A	3-Upper Moderate Disability	N/A
7.8 years	3-Moderate Sequelae	N/A	5-Upper Severe Disability	N/A
8.2 years	5-Full Recovery	N/A	1-Upper Good Recovery	N/A
13years	5-Full Recovery	N/A	1-Upper Good Recovery	1-Upper Good Recovery
13.5 years	5-Full Recovery	5-Full Recovery	1-Upper Good Recovery	1-Upper Good Recovery
15.8 years	3-Moderate Sequelae	5-Full Recovery	1-Upper Good Recovery	1-Upper Good Recovery

Key: GOSE-Peds = Glasgow Outcome Score Extended – Pediatric version, LOS=Liverpool Outcome Score
N/A = data not available either because the participant has not reached the time point or because the data had not been entered onto the database.

Table 5.11 Characteristics of participants based on LOS categorisation at 4-8 weeks following discharge from acute care

	Good recovery (i.e. LOS =5) (N=4, 36%)	Poor recovery (i.e. LOS = ≤4) (N=8, 67%)
Median age (years), IQR	10.6, 5.4-13.4	3.2 (1.2-7.8)
Sex (male)	1/4 (25)	5/8 (63)
Steroid use (yes)	1/4 (25)	1/8 (13)
Seizures on admission	2/4 (50)	5/8 (63)
Focal neurology on admission	2 /4 (50)	3/8 (38)
MRI abnormality	2/3 (67)	5/8 (63)
EEG abnormality	1/3 (33)	5/7 (71)
CSF pleocytosis	1/1 (100)	3/4 (75)
ICU admission	2/4 (50)	4/8 (50)
Length of hospital stay	7.5 (6.3-11.0)	11 (7.3-16.3)
Persisting symptom present at discharge	2 /4 (50)	8 (100)

Key: Data presented are counts (%) or median, interquartile range (IQR). Denominators represent the number of participants assessed for each outcome and with available data. MRI=magnetic resonance imaging, LOS = Liverpool Outcome Score, EEG=electroencephalogram, CSF=cerebrospinal fluid, PICU = paediatric intensive care unit.

LOS at 12 months

LOS data at 12 months post randomisation were available for 3 participants (Table 5.10). Two participants (67%) made good recovery (score of 5) while one participant (33%) made poor recovery (score of 2). The latter was a 3-year-old male who presented with generalised seizures, a history of fever, and focal neurology and had ongoing speech and language difficulty prior to presentation. His initial investigations at presentation showed CSF pleocytosis (CSF WCC =12/mm^3) and an abnormal brain MRI, CT scan and EEG. This participant was not on steroid treatment at the time of randomisation. He was admitted to PICU for 16 days and received invasive ventilation for 8 days during this time. The participant was admitted in hospital for a total duration of 156 days and was discharged to a neurorehabilitation centre. At the time of discharge from hospital, the participant had persisting symptoms, including cortical blindness. The participant's LOS at 12 months was unchanged from the score at 4-8 weeks. This participant was diagnosed with acute necrotising encephalitis.

The second participant with available LOS at 12 months was a 13-year-old female who presented with complex seizures, but not focal neurology. She was not on steroid therapy at the time of randomisation. Her initial investigations showed a normal EEG. An LP and brain MRI scan were not done on admission but her initial CT scan was normal. She was admitted to PICU for 3 days, received invasive ventilation for 2 days, and was discharged home after 7 days. She had persisting symptoms at the time of discharge. Her LOS at 12 months was 5 (full recovery) and was unchanged from the score at 4-8 weeks.

The third participant with had a LOS of 5 (full recovery) at 12 months was a 15-year-old male who presented with fever and focal neurology but no seizures. He was not on steroid treatment at the time of randomisation. His initial CT scan done on day 1 following

admission was normal but a brain MRI scan done on day 4 of the admission showed white matter changes. An LP was not done on admission but an EEG done on the same day of admission was normal. The participant was not admitted to PICU, was admitted for 11 days and had persisting symptoms at discharge.

GOSE-Peds at 6 months

GOSE-Peds scores at 6 months were available for 12 participants (Table 5.10). Seven (58%) had made good recovery (score of ≤ 2) while 5/12 (42%) made poor recovery. Summary characteristics for participants in both GOSE-Peds outcome groups are shown in Table 5.12. The median age (years) for those with poor recovery was 3.7 (IQR 1.8-7.7) and 8.2 (1.8-13.5) for those with good recovery. The median length of stay (days) for those with poor recovery was 8.0 (IQR 5.0-83.5) and 11 (7.0-14.0) for those with good recovery.

There were 2/7 (29%) male participants in the good recovery group as were 4/5 (80%) in the poor recovery group. The numbers (%) of participants with an abnormal first brain MRI scan were 5/6 (83%) and 2/5 (40%) in the good and poor recovery group respectively. The numbers (%) of participants with an abnormal first EEG were 3/6 (50%) and 4/4(100%) in the good and poor recovery groups respectively. One of five children (20%) in the poor recovery group had an abnormal first brain CT, no participant in the good recovery group had an abnormal first brain CT. Three of 5 (60%) participants in the poor recovery group required admission to PICU compared with 3/7 (43%) in the good recovery group. All participants in the poor recovery group (100%) and 5/7(71%) in the good recovery group had persisting symptoms at the time of discharge.

Table 5.12 Patient characteristics based on GOS-Peds categorisation at 6 months

	'Good recovery' GOSE of 2 or less (n= 7, 58%)	'Poor recovery' GOSE >2 (n=5, 42%)
Median age, years (IQR)	8.2 (1.8-13.5)	3.7 (IQR 1.8-7.7)
Sex, male (%)	2/7 (29)	4/5 (80)
Steroid use (yes)	1/7 (14)	1/5 (20)
Seizures at randomisation	3/7 (43)	4/5 (80)
Focal neurology at randomisation	3/7 (43)	2/5 (40)
MRI abnormality on admission	5/6 (83)	2/5 (40)
Brain CT scan abnormality on admission	0/4 (0)	1/5 (20)
EEG abnormality on admission	3/6 (50)	4/4 (100)
Pleocytosis (CSF WCC >4) on first LP	2/2 (100)	2/3 (67)
PICU admission	3/7 (43)	3/5 (60)
Length of hospital admission	11 (7.0-14.0)	8.0 (IQR 5.0-83.5)
Persisting symptom present at discharge	5/7 (71.4)	5/5 (100)

Key: PICU = paediatric intensive care unit, MRI = magnetic resonance imaging, CT=computerised tomography, EEG= electroencephalogram, CSF=cerebrospinal fluid, WCC=white cell count, LP=lumbar puncture, GOSE-Peds=Glasgow Outcome Score Extended-Pediatric version, IQR=interquartile range. Data are counts (%) unless otherwise specified.

GOSE-Peds at 12 months

GOSE-Peds scores were available for 7 participants at 12 months, 5/7 (71%) made good recovery while 2/7 (29%) made poor recovery (Table 5.10). The GOSE-Peds scores for 6 participants (86%) were unchanged from those obtained at 6 months. One participant had a GOSE-Peds score of 3 (Upper Moderate Recovery) at 6 months which improved to a score of 1 (Upper Good Recovery) at 12 months. One of the participants who made poor recovery at 12 months was the same male participant described above with a poor outcome LOS at 12 months. The second participant with a GOSE-Peds score of 6 (Lower severe disability) at 12 months was female, aged 2.6 years with a previous medical history of problems with speech and language. This participant presented 26 days from the onset of illness with seizures and a history of fever. On examination, she had no focal

neurology. An LP was not performed on admission. The first brain MRI and CT scans done on admission were normal and an abnormal EEG showing 'high amplitude slow waves, maximal over the posterior regions with frequent bursts of single short to runs of spikes and polyspikes and slow wave complexes over the frontocentral regions'. The participant was not on steroid treatment at the time of randomisation, did not require PICU admission, was admitted for a total of 8 days and had persisting symptoms at the time of discharge. A repeat research MRI scan done at 6 months after discharge remained normal whereas a repeat EEG done a month after the first had improved with no clear spike and wave activity. Comparison between the outcome groups based on the GOSE-Peds at 12 months is shown in Table 5.13.

Table 5.13 Patient characteristics based on GOSE-Peds categorisation at 12 months

	'Good recovery' GOSE-Peds of ≤2 (n= 5, 71%)	'Poor recovery' GOSE-Peds >2 (n=2, 29%)
Median age, years (IQR)	13.0 (1.4-14.7)	3.2 (2.7-3.7)
Sex, male (%)	3/5 (60)	1/ 2 (50)
Steroid use at randomisation (yes)	0/4 (0)	0/2 (50)
Seizures	4/5 (80)	2/2 (100)
Focal neurology on admission	1/5 (20)	1/ 2 (50)
MRI abnormality on first scan	1/4 (25)	1/ 2 (50)
Brain CT scan abnormality on first scan	0/4 (0)	1/2 (50)
EEG abnormality at randomisation	1/4 (25)	2/ 2 (100)
Pleocytosis (CSF WCC >4)	1/1 (100)	1/ 2 (50)
PICU admission	4/5 (80)	1/ 2 (50)
Length of hospital admission	8 (5-14)	82 (8-156)
Persisting symptom present at discharge	3/4 (75)	2/2 (100)

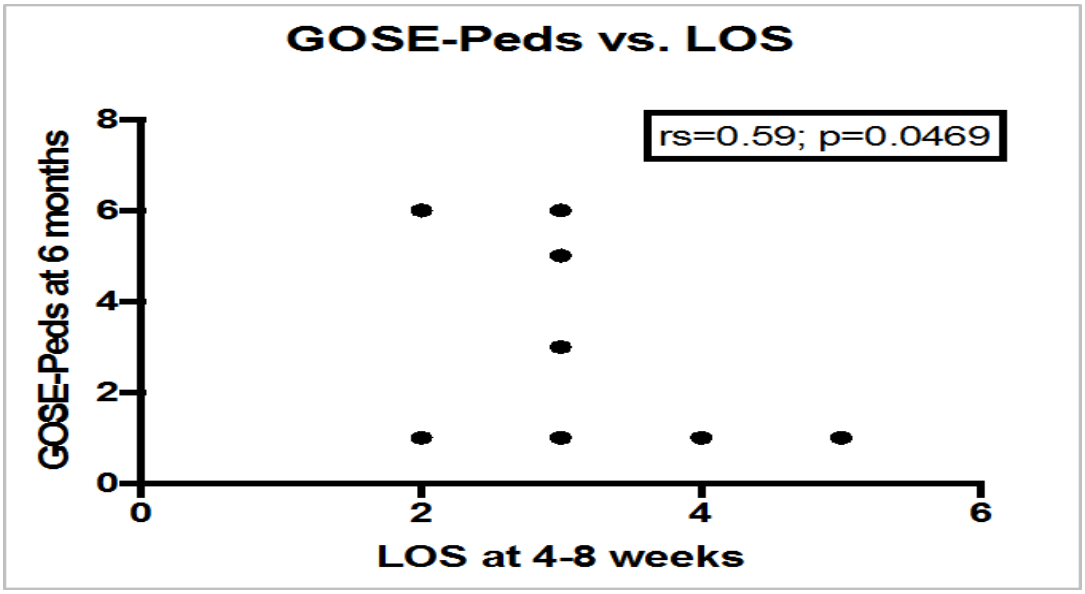
Key: PICU = paediatric intensive care unit, MRI = magnetic resonance imaging, EEG= electroencephalogram, GOSE-Peds = Glasgow Outcome Score Extended-Pediatric version, IQR=interquartile range, WCC=white cell count. Data are counts (%).

Correlation between GOSE Peds and LOS

Categorisation of participant outcomes (i.e. good or poor recovery) using the LOS at 4-8 weeks and GOSE-Peds score at 6 months were similar. An inverse correlation was

observed between the LOS at 4-8 weeks and the GOSE-Peds scores at 6 months (Spearman's rank correlation coefficient r_s =0.59; p=0.0469) (Fig 5.4).

Figure 5.4 Spearman correlation analysis plot showing inverse relationship between LOS at 4-8 weeks and GOSE-Peds at 6 months.



Note: 4 participants had same LOS and GOSE-Peds scores. LOS=Liverpool outcome score, GOSE-Peds=Glasgow Outcome Score Extended-Pediatric version

Premorbidity and outcomes

Two of the three participants who had premorbid conditions at presentation each had a GOSE-Peds score of 6 (Lower severe disability) at 6 and 12 months. The third had a GOSE-Peds score of 1 (Upper Good Recovery) at 6 months but the 12-month score was not available.

Adverse events

Each dose of the study treatment was well tolerated and no participant had to discontinue treatment sue to an adverse reaction. A total of 12 severe adverse events (SAEs) were reported for 4 participants, 8 of these occurred in one participant (Table 5.14). Nine of the events had resolved. One participant developed cortical blindness from the illness. None of the adverse events were judged to be related to the study treatment.

Table 5.14 List of serious adverse events (SAEs) for the IgNiTE cohort

Serious adverse event (SAE)	No. of participants reporting each SAE
Allergic reaction*	1
Bradycardias*	1
Prolonged seizure*	1
Surgery for PEG insertion, fundoplication, upper limb Botox and muscle and skin biopsies*	1
Sepsis, possible urinary tract infection as source*	1
Cortical blindness*	1
Clostridium difficile infection*	1
Bilateral achilles tenotomy*	1
Ventilation and intubation	1
Fever	1
Vomiting and lethargy	1

Key: * occurred in the same participant.

5.17 Comparison of clinical features in the IgNiTE cohort with other cohorts

Clinical features of participants in the IgNiTE study were compared with cohorts from two previous paediatric studies and the findings are detailed in Table 5.15.

Table 5.15 Comparison of clinical features in the IgNiTE cohort with other cohorts

	IgNiTE cohort	Kolski *et al* [4]	Fowler *et al* [252]
Type of study	Prospective	Prospective	Retrospective
Total number of children assessed	16	50	93
Mean age (range)	6.3 (16 weeks - 15.8years)	6.0 (6 weeks -17.8 years)	7.5 (5 weeks -17.7 years)
Fever	94%	80%	81%
Seizures	56%	78%	40%
Focal neurology	55%	78%	44%
CSF pleocytosis (definition used in study)	56% (> 4WCC)	59% (>5 WCC)	56% (≥ 6 WCC)
Elevated CSF protein level (criteria used in study)	30% (>0.4g/l	29% (>0.4g/l)	28% (>0.5g/l)
Abnormal EEG	75%	87%	90%
Abnormal neuroimaging	64% - MRI 9% - CT scan	60% - MRI CT scan- 37%	CT scan/MRI - 30%
Persisting symptoms at discharge	80%	67%	60%

Key: WBC=white cell count, CSF=cerebrospinal fluid, EEG=electroencephalogram, CT=computed tomography, MRI=magnetic resonance imaging

5.18 Discussion

This chapter describes a cohort of children recruited to the first ever RCT of IVIG for all-cause encephalitis and utilises the International Encephalitis Consortium definition for encephalitis. The findings of these interim analyses support existing knowledge of the poor outcomes associated with encephalitis. In this cohort, 67% made a poor recovery at 4-8 weeks after discharge from acute care, while 42% and 29-33% made poor recovery at 6 and 12 months post randomisation respectively. Particularly, 80% had persisting symptoms at discharge, mostly motor and cognitive difficulties. Although neurological and neuropsychological sequelae are more commonly reported in the literature, the findings of these analyses highlight other problems that can occur. Notably 64% of participants in this study were reported to have sleep difficulties at the time of hospital discharge, which is higher than 19% which was reported in a previous Israeli study of 47 children.[162] There are sparse reports suggesting an association between autoimmune encephalitis and sleep disorders.[388,389] Also, a German questionnaire study of meningitis reported sleep problems in 27-30% of patients when followed up 1-12 years after the illness.[390] However, in that study patients with viral meningoencephalitis and those <16 years were excluded. These data suggest the need for closer surveillance of children affected by encephalitis to allow a comprehensive assessment of the true impact of the disease.

In this cohort, 60% were admitted to PICU, similar to the observation in a North Indian study,[256] and 89% of those admitted in the IgNiTE cohort required invasive ventilation, highlighting the potential burden of the disease. Another important observation is that in 25-40% of cases who had serum autoantibody testing, a corresponding CSF test was not done. While the reason for this is not clear and could have been due to limited availability of CSF samples, this observation highlights a potential possibility that patients with AIE

could be missed since the sensitivity of autoantibody testing in serum has been shown to be lower than for CSF.[216,217]

The case mix presented in this Chapter showcase the spectrum of the illness and the differences in clinical presentation. Three children in this cohort had premorbid conditions, all affecting speech and language. Of these 67% made poor recovery (GOSE-Peds score of 6) at 12 months. Of note, in a retrospective cohort study of 68 Utah children with laboratory confirmed pneumococcal meningitis, 7 (10%) had premorbid neurological conditions. The authors concluded that the patients with a premorbid history of neurological conditions may have contributed to the relatively higher rates of neurological sequelae in their study of 64% compared to 40-60% reported in other paediatric studies.[391] Similarly, a Russian study of children with bacterial purulent meningitis showed a high incidence in patients with altered premorbid background.[392] The observation in this study puts forward the hypothesis of a possible association between premorbid health and outcomes following encephalitis, however no definite conclusions can be drawn from this observation given the small cohort size in this study. The findings in the IgNiTE cohort are considered in the context of those of two previous paediatric studies reporting on similar outcomes - a prospective Canadian study of 50 children,[4] and a retrospective Swedish study of 93 children.[252] A notable observation is the consistency in the proportion of participants with CSF pleocytosis or raised CSF protein count across all three studies, regardless of the definitions used. In this cohort, a cut off of 4 white cells in the CSF was used to define pleocytosis, whereas in the Canadian and Swedish studies, this was defined as a CSF WCC of >5 and ≥ 6 /mm^3 respectively. In the Swedish study, elevated CSF protein was defined as a level >0.5g/l whereas a cut off level of 0.4g/l was used in this study and the Canadian study.

Approximately 30% and 60% of patients had a raised CSF protein count and pleocytosis respectively across all three studies. The proportion of participants with an abnormal brain MRI scan in this, and the Canadian study, were similar. However, while only 9% of participants in the IgNiTE cohort had an abnormal brain CT scan on admission, this was 37% in the Canadian cohort. Neuroimaging results were presented as an aggregate in the Swedish study and it is unclear what proportion of participants had either an abnormal brain CT scan or MRI. CT scans are less sensitive than MRI scans at detecting subtle abnormalities especially early in the encephalitis illness. In this cohort, the first brain CT scan was performed on the same day as the admission in 79% of cases, which may explain the observed differences.

The data presented in this Chapter demonstrate the challenges experienced in the IgNiTE study, particularly relating to recruitment and site opening. A reflection on the possible reasons for the low recruitment, and challenges faced, is necessary and could be useful knowledge for the setup of future studies. RCTs are the gold standard for determining whether a cause-effect relationship exists between a treatment and outcome, and the scientific advantages of prospective RCTs are well established. Although a powerful tool in this regard, the set up and coordination of RCTs can be fraught with a variety of practical and ethical problems which need consideration in the trial development phase. For trials in conditions like encephalitis with a relatively low incidence, the involvement of multiple sites is crucial to achieving timely delivery of the study. While trial set up can be straightforward, particularly at large tertiary hospitals with a lot of experience in conducting RCTs, it can be a more challenging process for smaller hospitals with limited prior involvement in research. This is even more so for trials involving an investigational medicinal product (IMP). In IgNiTE, some unexpected staffing issues at certain sites

either increased their timeline to open the study, or in a few cases meant they had to withdraw from the study altogether. It had been envisaged that 21 months into the study, all 30 sites would have been open to recruitment but the significant delays with opening some sites meant that this was not achieved, and this possibly impacted on recruitment. One major challenge that was encountered in site set up was staff training. Research staff are required to be up-to-date with training on Good Clinical Practice (GCP) to provide some guarantee that they are adequately trained in aspects relevant to their proposed role in any study. For trials involving an IMP, it is becoming more common practice to incorporate staff training into set-up activities. For a time critical trial like IgNiTE (in terms of administering the study treatment), where for practical reasons such as the uncertainty around presentation of potential participants which may mean that a study trained research nurse was not available, it is necessary to involve clinical staff in delivering the study. The experience from the IgNiTE trial was that most ward staff nurses did not have GCP training done as part of their normal role, which limited their working on a clinical trial in line with ICH GCP guideline 2.8.[360] Therefore, in IgNiTE, a pragmatic approach that was taken was the use of staff training slides which included basic but relevant information on GCP, as well as study specific information pertinent to the role of a ward nurse or doctor working on the IgNiTE trial. The named research nurse at each site was trained on the slides by the lead nurse or doctor for the IgNiTE trial at the SIV. Downstream training was then administered by the site research nurse to clinical staff, following which a training log was to be completed as evidence for this. This approach had the advantage that training could be administered ad hoc if a potential participant was admitted to hospital and no trained staff was available. However, a reasonable number of staff still had to be trained upfront before the study could open at a site. This was necessary to demonstrate evidence that a site was ready to commence

recruitment. Due to the clinical demands and current workload in the NHS, it became increasingly difficult to achieve this upfront training for a reasonable number of ward staff. Since appropriate staff training is critical for IMP trials, this slowed down the process of set up at various sites, causing substantial delays between the site initiation visit and site opening as is highlighted in Table 5.2. One of the ways that was considered to mitigate this was to make the training an online, rather than face-to-face exercise. This was so that the clinical staff were able to complete the training at a convenient time, in some cases, even in between clinical shifts. It was felt by the site teams that this approach would be very helpful and thus for future studies, could be considered upfront in the trial set up stage and might help to reduce site setup time. Also, incorporating GCP into routine training for clinical staff will undoubtedly be beneficial.

Another aspect worth discussing is the process of assessment of a site's capacity and capability to run any trial. The experience with IgNiTE provides useful insight on this. Due to capacity issues, at one site for example, the CTP stopped taking on new studies half way through the setup process. At another site, it was only evident at the SIV that there were potential capacity issues which lingered on for over a year following SIV. These issues had not been resolved at the time of halting recruitment to the IgNiTE trial. These two examples bring to the fore that the assessment of capacity and capability not only applies to the front-line research staff directly involved in recruitment, but should extend to other departments within a hospital trust that are expected to be involved in the study. Thus the need a more detailed assessment which includes questions around the size of the research nursing team, availability of administrative support, support for the PI within the wider clinical team, expected site timelines for commencing recruitment, and

capacity of other research teams such as the CTP, to ensure a broader understanding on feasibility of running a trial at any site.

Other clinical and statistical aspects of IgNiTE require some deliberation. Prior to embarking on an RCT, consideration needs to be given to various aspects including the choice of entry criteria, the anticipated consent rate, the timing and dose of study treatment, and for placebo controlled IMP studies, the most ideal placebo to use. Furthermore, is the deliberation on an adequate sample size, and an a priori definition of what constitutes a clinically relevant treatment effect. To assess feasibility, in terms of achieving any proposed sample size, it is crucial to consider what the true incidence of the disease being studied. For calculating the sample size for a trial, the choices of an alpha of 0.5 and power of 80% are conventionally thought to be adequate.[393] However, for IgNiTE, an increase in the study power from 80% to 90% was mandated by the funder during the grant application stage, which led to an increase in the sample size. Published data at the time of designing the IgNiTE trial put an estimate on paediatric encephalitis admissions at approximately 2 per year in a district general hospital and 10 per year in a tertiary paediatric hospital. Thus, it was expected that in a 3.5-year period, approximately 1000 children (250 children per year) at the tertiary sites and 40 children (10 per year) at district general hospitals could be approached to participate in IgNiTE. The final sample size of 308 was based on the assumption of a 30% recruitment rate would have given 90% power to detect a 20% effect size, while accounting a 10% attrition rate in both treatment groups. Given the anticipated generous numbers of potentially eligible patients, it was projected that the recruitment target would have been achievable within 3.5 years. However, over the course of the study, it became apparent that paediatric encephalitis admissions fell far short of the original estimates, and recruitment at each site was much

lower than the anticipated target for most sites, highlighted in Table 5.3. As demonstrated in Figure 2.1 of this thesis, encephalitis admissions vary year-on-year. Whether the experience with IgNiTE reflects a stochastic effect and recruitment might have improved in subsequent years is yet to be unraveled. Nonetheless, this experience indicates the need to take into cognisance natural fluctuations in diseases and to ascertain the true incidence of a disease being investigated. Strategies such as increasing the duration of recruitment and involvement of more sites, both nationally and internationally could enhance deliverability of studies of rare diseases. However, the cost implications may be a deterrent for potential funders.

For an RCT like IgNiTE, it was essential to have robust entry criteria which not only provided guidance on what patients should be have been considered for the study, but also allowed some homogeneity in the cohort recruited. Having some entry criteria also defines the target population that would benefit from IVIG if a treatment effect was established. Robust entry criteria as was used in the IgNiTE study should increase both the sensitivity and specificity of making an accurate diagnosis of encephalitis. One point to consider is whether the entry criteria used in IgNiTE was too stringent and if more liberal entry criteria could have been used. The tradeoff between having strict and liberal entry criteria need to be recognised. While having strict criteria automatically selects a population that gives the best opportunity for a study treatment to shown to be efficacious, having liberal criteria reduces the likelihood of observing a treatment effect, since this approach would encourage admission of participants less likely to benefit from the treatment. Also having strict criteria might pose difficulties with generalising the findings of a study to the wider population.

For IgNiTE, at least two minor criteria had to be fulfilled, in addition to the major criteria for entry to the study. Given the variable presentation in encephalitis, arguably, the presence of an objective finding such as a brain MRI scan or EEG finding that is consistent with encephalitis might be considered confirmatory of the diagnosis as a standalone criterion. However, given the possibility of a false negative initial brain MRI scan and EEG, potential participants might be missed with this approach.

An additional challenge with IgNiTE was the time window for administering the first dose of the study treatment. This timeline was introduced since the focus of the study was the assessment of the role of early IVIG treatment in encephalitis, which was based on theoretical evidence that limiting the inflammatory response in encephalitis and doing this early on the illness, could improve outcomes. However, applying this time window might have had some negative impact on recruitment to the study. This is because symptoms and signs of encephalitis evolve over time and some children with encephalitis might not have met the study criteria within the time window. Also, the immediate period following admission and/or diagnosis is a sensitive one for the family and especially if the child is admitted to the intensive care unit. Therefore, a longer period of time is likely to be required by the family to consider information about the study to allow them to reach a decision regarding participation. In IgNiTE, at least 7% of patients who met the study inclusion criteria were excluded because administration of the first dose of study treatment was not going to be feasible within the time window. This is a significant number, considering the low recruitment. One proposal therefore was to relax the time window for administration of the first dose of study treatment which was done in IgNiTE, immediately prior to suspension of recruitment, therefore had not been effected. Although this strategy introduces more variability in time to treatment, the effect of early treatment could still be studied in a sensitivity analysis.

For any RCT, it is crucial that clinicians are in a state of both clinical and personal equipoise. For trials of rare diseases like IgNiTE, lack of clinical equipoise can negatively impact on trial deliverability. An interview study of clinicians involved in 6 RCT trials was conducted to investigate how clinicians conveyed equipoise during RCT.[394] This study identified the difficulty with clinicians being able to reconcile their own view about RCT treatments with the requirement to convey equipoise to patients and their parents/guardians. Clinicians in the study also identified that their personal views about trial treatment encroached on the ability to convey equipoise to patients. In IgNiTE, at least 9% of participants who met the inclusion criteria were excluded due to prior receipt or planned administration of IVIG as part of routine care. An additional 6 participants screened on the old log were excluded for the same reason although it was not clear from the logs whether those patients indeed did meet the study inclusion criteria. This observation suggests an increased willingness of clinicians to administer IVIG to children with encephalitis and possibly a lack of perceived equipoise in the use of IVIG in encephalitis. A strategy proposed to address this in IgNiTE was the election of trial ambassadors who were renowned specialists in the field to engage in a cross-country tour of the participating sites, with the view to re-emphasise the importance of the IgNiTE trial and to help address the issue of clinical equipoise. In addition, in-house meetings were held with the paediatric neurology teams at a couple of tertiary recruiting centers to help address this problem. For future trials, a questionnaire based evaluation of practice among paediatric neurologists might be useful to identify early on whether or not clinicians are in equipoise.

Another aspect of IgNiTE worth mentioning is the high rate of non-consent. Of the 52 eligible patients, consent to participate in the study was obtained for only 18, giving a consent rate of 35%. Although slightly higher than the envisaged 30% at the trial development stage, this is no doubt a challenge to recruitment. A root cause analysis was carried out in one of the IgNiTE sites to identify factors contributing to non-consent. It was felt that especially with those children admitted to intensive care, the high patient contact with several professionals during the period of care might introduce inconsistencies in the message being given to families, which could negatively impact on the informed consent process. A proposed strategy to mitigate this was for consent to be sought only by key individuals who had an established relationship with the family and/or an adequate grasp of the study e.g. the clinician directly caring for the child, the study principal investigator or co-investigator. Several ways of enhancing the informed consent process have been suggested.[395] These include branding the trial with key messages, training of local research personnel, promoting a culture of research, being familiar with the patient and family dynamics, being cognisant with local ICU culture (for patients admitted to ICU) and tailoring communication accordingly, and the involvement of bed side staff with whom the family would have established some relationship.[395] Although a balance needs to be achieved with the latter strategy especially since bedside staff may have limited knowledge of, minimal interest in, or harbor concerns about research, which could also have a negative impact on recruitment.

Some financial aspects are worth discussing. The use of placebo as the control arm in RCTs investigating medicinal products has become common place and is necessary to provide a clear picture regarding the efficacy of the treatment being studied. Double blinding in a clinical trial ensures that the preconceived views of participants and clinicians cannot systematically bias the assessment of outcomes.[396] It is thus crucial that

blinding is maintained in a placebo trial. For IgNiTE, the placebo that was used was constituted as a 0.9% saline and 0.1% human albumin mix. Addition of albumin was deemed necessary to make the placebo visually identical to IVIG. However, addition of albumin to saline resulted in a considerable reduction in shelf life from 3 years for 0.9% saline to 6 months for the final placebo product. The study treatment management strategy for IgNiTE was such that some study drug was held on site and these were replaced as they were used. Due to the short shelf life, placebo replacement was required on a 6 monthly basis. This led to considerable wastage of placebo as a result of the low recruitment, which led to an increase in cost. A revised model for study treatment management was considered in IgNiTE, such that only a small number of study treatment was held at central location, with none held locally, and only delivered to sites when needed. It was thought that although this could potentially be financially consuming from a delivery perspective, with the low recruitment, some cost saving overall was to be expected. The other concern with this approach was the potential for delays in delivery of study treatment from the central location. With the planned extension to the time window for administration of the study treatment, it was felt that the risk of not being able to recruit a participant due to non-availability of study treatment was low. Consideration was also given to the use of an alternative placebo such as 0.9% saline and the introduction of a masking step but the risk of unblinding with this approach and the impact on the integrity of the study was thought to outweigh the potential for cost saving. The importance of continued engagement of research staff cannot be overemphasised. With the rapid turnover of research staff, the possibility of multiple amendments to study documents, and the apparently low incidence of encephalitis, it was crucial to keep site engagement on the priority list. Several strategies were employed in IgNiTE to keep research staff engaged and motivated such as a fortnightly teleconference, organization

of training events, and the development of a newsletter that was sent out quarterly and provided updates on study progress and key changes.

Limitations

The findings presented in this chapter are results from an interim analysis of data from approximately 6% of the anticipated cohort. The small sample size which limits the usefulness of any statistical analysis, and missing data for some of the variables assessed are some drawbacks to making any inferences from the findings presented here. Validation of these data in a large sample size is necessary to reach any definite conclusions on the data presented. The analyses described here were performed on an intention to treat basis. Not all participants contributing data had a final diagnosis of encephalitis. Thus, a per protocol analysis excluding such participants would be useful to enhance comparison across studies of children with encephalitis.

Clearly one main limitation is the low recruitment which led to withdrawal of funding support. After 21 months from the commencement of recruitment to the IgNiTE trial start of the study, 18 participants had been randomised which was lower than the recruitment number that was anticipated to have been achieved in this time. This slow accrual led to withdrawal of funding support by the NIHR and recruitment was suspended in September 2017. A revised model for delivering the IgNiTE study that included removal of some of the secondary study endpoints such as the research MRI, neuropsychological assessment, and immunological tests was developed as a cost saving strategy. Also included in the revised model were an extension of the recruitment period and reduction of the sample size. The proposed revised model was submitted to the NIHR for re-consideration of funding support, and the outcome of this was pending at the time of submission of this thesis.

5.19 Conclusion

The high proportion of participants with neurological sequelae, in particular sleep and behavioural problems, at short, medium, and long term follow up signals the need for longer term follow up of these patients that is centered on a multidisciplinary approach. The challenges encountered in running a large multicenter RCT like IgNiTE are discussed and also the factors that possibly contributed to the low recruitment. For future large trials like IgNiTE, it is crucial that these factors are considered and where possible, addressed well in advance of trial commencement. Pilot studies have a considerable role in assessing study feasibility and should be considered. Ultimately, the IgNiTE study is crucial to reaching some conclusion regarding the role of IVIG in encephalitis, which as yet has not been sufficiently established. The findings of the IgNiTE trial could alter the way that children with encephalitis are managed, not only in the UK but worldwide.

6 Thesis discussion

This thesis investigated the epidemiology of childhood encephalitis in England and Wales and systematically reviewed available evidence from RCTs on the role of IVIG in childhood encephalitis. Also described in this thesis is first ever RCT of IVIG in all-cause childhood encephalitis, an interim analysis of data from the first cohort of enrolled participants, and a detailed review of, and reflection on the challenges of conducting a RCT of a rare disease like encephalitis. There were several major findings described in this thesis, which are discussed here.

Admissions for all-cause childhood encephalitis in England are increasing and the epidemiological profile of ADEM is changing

Presented in Chapter 2 of this thesis is a 33 year (1979-2011) review of childhood encephalitis hospital admissions in England. Through this work, the incidence of all-cause childhood encephalitis in England, which hitherto was unknown, was defined. It is also the first study to define both gender based and age group specific incidence rates for childhood encephalitis in England. An increasing trend in hospital admissions for all-cause encephalitis was observed between 1995 and 2011. Notably, the main driver of this increase was encephalitis of unknown aetiology. Thus, this finding could represent an increase in the number of yet to be identified pathogens that are implicated in encephalitis. In the context of emerging and reemerging infections, and the ability for these infections to cause devastating encephalitis epidemics, it is crucial that focus is directed to improving aetiological diagnosis in patients with encephalitis. For 40-60% of encephalitis cases, an aetiology is not identified. Knowledge of what pathogen is causing the disease is important to allow institution of specific treatment. It would also deter the unnecessary use of empiric treatments such as aciclovir and the side effects associated with this.

Broadening currently available molecular testing panels would seem a logical approach although this requires prior knowledge of what are the possible pathogens, which would be a limiting factor. Additionally, the growing focus on the use of newer technologies including next generation sequencing, and the use of proteomic and genomic based approaches to establish aetiological diagnosis provides some promise. Also identification of biomarkers associated with specific aetiologies would be useful but there are limitations such as availability of an ideal experimental model for each pathogen. Furthermore, given the finding from the epidemiological work in the thesis, the role of non-infectious triggers in encephalitis needs consideration. A recent study demonstrated an association between the use of pesticides and an encephalitis outbreak in Bangladesh.[397] The researchers observed through village analysis that visiting a lychee orchard between 24 hours (adjusted OR = 11.6 [1.02-109.8]) and 3 days before onset (aOR = 7.2 [1.4-37.6]), as well as having family members who worked in a lychee orchard (aOR = 7.2 [1.7-29.4]) were risk factors associated with an encephalitis illness. Also, visiting any garden while pesticides were being applied up to 3 days before illness (aOR=4.9 [1.0-19.4]) was correlated with acute encephalitis syndrome.

Furthermore, this thesis demonstrated a change in the epidemiological profile of ADEM. While ADEM admissions in infants were non-existent prior to 1990, a steady rise in ADEM admissions in infants was observed thereafter, showing a three yearly peak pattern. Although the increasing trend in ADEM admissions might represent better identification of cases with improved diagnostics, it is unlikely that this is solely accountable. The possibility that this represents a true increase in ADEM cases needs to be deliberated. Though the observed pattern of three yearly peaks in ADEM infant admissions suggests an infectious trigger, in the context of introduction of new vaccines into the UK immunisation schedule, the observed change in the epidemiological profile

warrants closer surveillance of adverse events routine immunisation. Surveillance work on this is ongoing through the Brighton Collaboration[1].

Implementation of the 2 dose MMR vaccination schedule has resulted in a drastic reduction in measles and mumps encephalitis admissions

The impact of MMR vaccination on encephalitis admissions in England had not previously been studied. The work conducted in Chapter 2 of this thesis demonstrated a significant reduction in mumps and measles encephalitis admissions that was aligned with the introduction of the MMR vaccine. Specifically, admission rates for measles encephalitis fell by 35-fold and 4-fold respectively following the introduction of the two dose combine MMR schedule, when compared with the period before and after introduction of the single dose MMR schedule. Similarly, mumps encephalitis admissions decreased by 30 and 60-fold respectively with the two dose MMR vaccination compared with before and after the single dose MMR vaccine was introduced. This highlights the role of vaccination as a key strategy in the prevention of encephalitis. The impact of vaccination on other forms of encephalitis including varicella and Japanese encephalitis has been demonstrated. VZV is considered a leading cause of encephalitis. Based on the work undertaken in this thesis, varicella encephalitis accounted for approximately 8% of all encephalitis admissions in England. Of the specific aetiologies, varicella encephalitis ranked second as the cause of encephalitis, with an incidence rate that was second only to ADEM, and higher than HSE. Notably, a Canadian study reported a case fatality rate from VZV encephalitis of 18%,[57] which goes against conventional assumption that varicella infections are benign. With these in mind, it is necessary to consider strategies

[1] A non-profit global vaccine safety research network launched in 2000 to facilitate the development, evaluation, and dissemination of information about the safety of human vaccines.

for the prevention of varicella encephalitis. In the UK, introduction of the varicella vaccination has been debated due to concerns that this will drive up the age at which those that are non-immune get the illness, and possibly increase the incidence of shingles. Work from this thesis contributes to the available evidence demonstrating the burden of VZV encephalitis in England that would be useful for policy makers. Additionally, these data provide a basis for comparison of post vaccination effect, should VZV vaccination be introduced in the UK.

The burden from severe encephalitis and predictors of mortality

Children with severe encephalitis represent a different disease cohort and the pathophysiological mechanisms are likely to differ somewhat from those in mild disease. Knowledge of factors associated with outcomes specific to this cohort was therefore necessary. No previous studies in the UK had reported on paediatric encephalitis PICU admissions or investigated factors associated with outcomes in this group of patients. The work presented in Chapter 3 of this thesis is the first ever report on a cohort of encephalitis patients admitted to PICU in England and Wales. This work described characteristics of 1031 children (0-17years) admitted to PICU in E&W over a 10 year period. Through this work, the PICU incidence rate for all-cause encephalitis and also that for specific aetiologies including ADEM, HSV, VZV and EV encephalitis in E&W were defined. The study also highlighted the potential burden from encephalitis. The case fatality rate in the PICU cohort was 8% and risk factors associated with mortality were suggested. Particularly, it was demonstrated that a higher proportion of children admitted to PICU with a diagnosis of encephalitis were ventilated compared with children with other diagnoses. Ventilator requirement increases the possibility of a longer stay in PICU, with the associated costs. It was also estimated in this thesis that the additional bed day cost

burden from PICU encephalitis admission is ~£800,000 year. These data bring to the fore the potential cost burden from encephalitis and are useful as preliminary information for policy makers in the UK NHS. Future work should be aimed at undertaking a detailed cost analysis of encephalitis hospital admissions, including admission to PICU. Some of this is already being explored as part of the UK Childhood Meningitis and Encephalitis Study. Although an adult study, the ongoing study of encephalitis ICU admissions across Europe with a planned sample size of 1000,[349] might be useful in validating the findings on predictors of mortality identified from the work conducted in this thesis. The IgNiTE trial may also provide some insight.

Improving outcomes for children with encephalitis and the role of immunoglobulin treatment

Despite several reports suggesting a beneficial role of IVIG in encephalitis, the results of a Cochrane systematic review reported in Chapter 4 of this thesis were inconclusive. Although the review suggested some benefit in patients with viral encephalitis, but the quality of the evidence was very low. The review highlighted two main aspects for future research.

First was the need for well designed, prospective trials of adequate sample sizes, to investigate the role of IVIG in encephalitis. To address this, a first ever RCT of IVIG in all-cause childhood encephalitis was initiated and this is described in Chapter 5 of this thesis. Results of an interim analysis of data from participants enrolled to this trial demonstrated that 80% of affected children had persisting symptoms at the time of hospital discharge, particularly motor difficulty (64%), problems with cognition (67%) and sleep difficulty (64%). These findings align with published literature describing outcomes from encephalitis. This thesis also provides first-hand information on the

challenges encountered with the set-up of, and recruitment to a multicenter trial of encephalitis. The reflections and suggestions presented in this thesis are undoubtedly useful information to be considered in the set-up of not only future encephalitis trials, but also other trials of any other rare diseases like encephalitis. Recruitment to the trial was suspended at the time of writing this thesis, pending an outcome of a funding review. If the outcome be successful, the study would be invaluable to providing a definite answer on the role of IVIG in childhood encephalitis.

Should a beneficial role of IVIG be established, a concern is the cost implication of its routine use in the NHS, and also uncertainty around continued availability. Hence it is important to understand mechanisms through which IVIG might work as a strategy to identifying other alternative cheaper treatment options, with guaranteed continued supply. I received a grant from Thrasher Research Fund to undertake an immunological project to investigate the role of regulatory T cells (T reg) in encephalitis. Within these broad aims of the project, I plan to investigate the role of IVIG on the T reg population in encephalitis. It is expected that data from this work would add to the body of evidence from several projects currently underway to understand the mechanisms of action of IVIG in encephalitis.

Secondly, the systematic review identified the need for defining an agreed outcome set for encephalitis trials to allow consistent comparisons across trials which is of utmost importance in the strive to improve encephalitis outcomes. Some work on this is being conducted by the Brain Infections Group in Liverpool, UK.

Conclusion

Ultimately, the epidemiological work undertaken in this thesis have added to the growing body of evidence describing hospital admission trends and incidence rates for childhood encephalitis, and have led to the identification of areas for future research. The IgNiTE trial is fundamental to addressing the yet unanswered question about the role of IVIG in encephalitis, and has the potential to revolutionise the way children with encephalitis are managed.

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8 Appendix

Search strategy used in identifying articles for the systematic review (Chapter 4)

Source	Search strategy
CENTRAL (the Cochrane Library) (2016, Issue 9)	#1 encephalitis:ti,ab,kw (Word variations were searched)
	#2 meningoencephalitis:ti,ab,kw (Word variations weresearched)
	#3 ((brain or cerebral) near/2 (infection* or infectious or inflamm* or swell*)):ti,ab,kw (Word variations weresearched)
	#4 #1 or #2 or #3
	#5 MeSH descriptor: [Immunoglobulins, Intravenous] explode all trees
	#6 ((intravenous or intra-venous) near/3 immunoglobulin*):ti,ab,kw (Word variations weresearched)
	#7 ivig or igiv:ti,ab,kw (Word variations have been searched)
	#8 immunoglobulin* or immunotherap*:ti (Word variations weresearched)
	#9 #5 or #6 or #7 or #8
	#10 #4 and #9
MEDLINE (PubMed) (1966 to 30 September 2016)	1 exp Encephalitis/
	2 encephalitis.ti,ab.
	3 meningoencephalitis.ti,ab.
	4 ((brain or cerebral) adj2 (infection* or infectious or
	inflamm* or swell*)).ti,ab.
	5 1 or 2 or 3 or 4
	6 adolescent/ or exp child/ or infant/ or infant, newborn/
	7 (child* or preschool* or pre-school* or schoolchild* or
	infant* or infancy or baby or babies or toddler* or teen*
	or adolescen* or youth*).ti,ab.
	8 6 or 7
	9 Immunoglobulins, Intravenous/
	10 ((intravenous or intra-venous) adj3
	immunoglobulin*).ti,ab.
	11 (ivig or igiv).ti,ab.
	12 (immunoglobulin* or immunotherap*).ti.
	13 9 or 10 or 11 or 12
	14 5 and 8 and 13
	15 limit 14 to "reviews (maximizes specificity)"
	16 randomised controlled trial.pt.
	17 controlled clinical trial.pt.
	18 randomized.ab.
	19 placebo.ab.
	20 drug therapy.fs.
	21 randomly.ab.
	22 trial.ab.
	23 groups.ab.
	24 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23
	25 exp animals/ not humans.sh.
	26 24 not 25
	27 14 and 26

Source	Search strategy
Embase (embase.com) (1974 to 30 September 2016)	1 exp *Encephalitis/
	2 encephalitis.ti,ab.
	3 meningoencephalitis.ti,ab.
	4 ((brain or cerebral) adj2 (infection* or infectious or
	inflamm* or swell*)).ti,ab.
	5 1 or 2 or 3 or 4
	6 adolescent/ or child/ or boy/ or girl/ or infant/ or preschool
	child/ or school child/ or toddler/
	7 (child* or preschool* or pre-school* or schoolchild* or
	infant* or infancy or baby or babies or toddler* or teen* or
	adolescen* or youth*).ti,ab.
	8 6 or 7
	9 exp *immunoglobulin/ and intravenous drug administration/
	10 exp *immunoglobulin/iv [Intravenous Drug Administration]
	11 ((intravenous or intra-venous) adj3 immunoglobulin*).ti,ab.
	12 (ivig or igiv).ti,ab.
	13 (immunoglobulin* or immunotherap*).ti.
	14 9 or 10 or 11 or 12 or 13
	15 5 and 8 and 14
	16 limit 15 to "reviews (maximizes specificity)"
	17 randomised controlled trial/
	18 controlled clinical trial/
	19 single blind procedure/ or double blind procedure/
	20 crossover procedure/
	21 random*.tw.
	22 placebo*.tw.
	23 ((singl* or doubl*) adj (blind* or mask*)).tw.
	24 (crossover or cross over or factorial* or latin square).tw.
	25 (assign* or allocat* or volunteer*).tw.
	26 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25
CINAHL (EBSCO host) (1981 to 30 September 2016)	S1 AB randomised controlled trial or controlled clinical trial or randomized or placebo or drug therapy or randomly or trial or groups
	S2 AB inflamm* or swell* or infection* or infectious or Immunoglobulins, Intravenous or intravenous or intra-venous or immunoglobulin* or ivig or igiv or immunoglobulin* or immunotherap*
	S3 MH Encephalitis OR AB (encephalitis or meningoencephalitis)
	S4 S2 OR S3
	S5 S1 AND S4
	S6 AB randomised controlled trial or controlled clinical trial or randomized or placebo or drug therapy or randomly or trial or groups
	Limiters - Age Groups: Infant, Newborn: birth-1 month, Infant: 1-23 months, Child, Preschool: 2-5 years, Child: 6-12 years, Adolescent: 13-18 years
ClinicalTrials.gov (www.clinicaltrials.gov)	(encephalitis OR meningoencephalitis) AND (immunoglobulin OR immunoglobulins OR ivig OR igiv OR immunotherapy)

Source	Search strategy
ICTRP Search Portal (apps.who.int/trialsearch)	(encephalitis OR meningoencephalitis) AND (immunoglobulin OR immunoglobulins OR ivig OR igiv OR immunotherapy)
Science Citation Index Expanded (SCI-EXPANDED) & Conference Proceedings Citation Index- Science (CPCI-S) (Web of Science Core Collection, Thomson Reuters) (1945 to 30 September 2016)	# 1 TOPIC: (encephalitis OR meningoencephalitis) OR TOPIC: (((brain or cerebral) NEAR/2 (infection* or infectious or inflamm* or swell*)))
	# 2 TOPIC: (child* or preschool* or pre-school* or schoolchild* or infant* or infancy or baby or babies or toddler* or teen* or adolescen* or youth*)
	# 3 TOPIC: (((intravenous or intra-venous) NEAR/3 immunoglobulin*)) OR TOPIC: (ivig or igiv) OR TITLE: (immunoglobulin* or immunotherap*)
	# 4 #3 AND #2 AND #1
	# 5 TOPIC: (random* or blind* or allocat* or assign* or trial* or placebo* or crossover* or cross-over*)
	# 6 #5 AND #4
Global Health Library (Virtual Health Library) (www.globalhealthlibrary.net/php/index.php)	(encephalitis OR meningoencephalitis) AND (immunoglobulin OR immunoglobulins OR ivig OR igiv OR immunotherapy)
Database of Abstracts of Reviews of Effects (DARE)	(encephalitis OR meningoencephalitis) AND (immunoglobulin OR immunoglobulins OR ivig OR igiv OR immunotherapy)