# Childhood Meningitis: current and previous UK epidemiology, clinical and laboratory characteristics and outcomes

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**Author Contributions**

The introduction literature review, chapter 1, is my own work and has not currently been submitted for publication. The general methods, chapter 2, includes methods for some of the work described below.

For both chapters 3 and 4, I was the first author of related publications in '*The Lancet Infectious Diseases Journal*'. The work included in chapter 3 is also co-authored by Dr Manish Sadarangani, Professor Andrew J Pollard, and Professor Michael J Goldacre. The work included in chapter 4 is also co-authored by Dr Mildred A Iro, Dr Manish Sadarangani, Mr Raphael Goldacre, Professor Andrew J Pollard, and Professor Michael J Goldacre. I interpreted the data, performed the literature reviews, drew the included figures and tables, wrote the first draft of papers for submission for publication, and completed revisions for the papers with assistance from the co-authors. The data analysis was performed by Mr Raphael Goldacre and the programming team at the Unit of Health-Care Epidemiology, Nuffield Department of Population Health, University of Oxford. The co-authors also contributed to the idea for and design of the study, data interpretation, and review of the work.

The work included in chapters 5, 6, 7, and 8 is based on a UK-wide multicentre study, the UK Childhood Meningitis and Encephalitis cohort Study (UK-ChiMES). I have been extensively involved in all aspects of this study since before study set-up including study design, study coordination, site initiations, data collection and monitoring, and I have done all the analyses presented in the thesis independently. The UK-ChiMES team at the

University of Oxford, and the Enceph-UK team at the University of Liverpool, have contributed to study coordination and data cleaning. Data was collected by individual research teams at each study hospital.

I designed and performed all analyses including statistical analyses, drew all tables and figures and wrote the text in chapters 5, 6, 7 and 8. I would like to acknowledge Jonathan Williman, University of Otago, for statistical advice.

I conceived and designed the study and performed the laboratory work independently that is included in chapter 7. The conclusions, chapter 9, is my own work.

**Abstract: Childhood Meningitis: current and previous UK epidemiology, clinical and laboratory characteristics and outcomes**

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Introduction: Following reductions in bacterial meningitis which occurred in the UK due to the implementation of vaccine programmes, contemporary knowledge was needed about the current aetiology of childhood meningitis in the context of historical changes in epidemiology, how to reliably distinguish bacterial and aseptic meningitis at presentation to hospital, and outcomes particularly following viral meningitis.

Methods: Annual hospital admission rates were analysed for bacterial and viral meningitis in children <15 years in England from the 1960s to 2011. Data from a prospective cohort study performed across 31 UK hospital sites from 2012-2016, recruiting children <16 years with confirmed or suspected meningitis was used to investigate current aetiology, clinical and laboratory features, clinical decision models to distinguish bacterial and aseptic meningitis, short term outcomes at 3 months post-discharge and health-related quality of life until 18 months post-discharge. A study of enteroviral (EV) PCR from non-CSF sites in a subset of participants with suspected or confirmed viral meningitis was also performed.

Results: Hospital admissions datasets demonstrated a reduction in bacterial meningitis of different aetiologies after the introduction of different vaccines, a reduction in viral meningitis admissions in children >1 year since the 1980s, but a recent increase in viral meningitis admissions in infants. Of 2754 prospectively recruited children with suspected meningitis, 892 had meningitis, of which 80.7% (720/892) were aseptic, 34.2% (305/892) were caused by enteroviruses and 31.3% (280/892) had no identified aetiology. Following analyses of clinical and laboratory features of meningitis of different aetiologies, a pre-existing bacterial meningitis score was assessed, and a new clinical decision model was developed to distinguish aseptic from bacterial meningitis at hospital presentation. In a subset of children, EV-PCR performed on non-CSF samples demonstrated that in EV meningitis all available stool samples were EV-PCR+, and 35% of children with aseptic meningitis of unknown aetiology had a stool EV-PCR+ result. Analysis of short term outcomes and health related quality of life indicated that although sequelae and lower quality of life scores occurred most often following bacterial meningitis, for enteroviral meningitis parental concern about sequalae and limited lower quality of life scores were reported compared with a control group.

Conclusion: These data could inform priorities for prevention and improve the care of children with meningitis.

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**Abbreviations**

| | |
|---|---|
| 4CMenB | multicomponent meningococcal capsular group B vaccine |
| ABC | Active Bacterial Core surveillance region, USA |
| AE | Adverse Event |
| ADEM | Acute disseminated encephalomyelitis |
| BBB | blood brain barrier |
| BMS | Bacterial Meningitis Score |
| BPSU | British Paediatric Surveillance Unit |
| CFR | case fatality rate |
| CMV | cytomegalovirus |
| CNS | central nervous system |
| CRF | case report form |
| CRP | C-reactive protein |
| CSF pleocytosis | A cerebrospinal fluid white blood cell count above a defined normal limit |
| CSF | cerebrospinal fluid |
| CT value | cycle threshold value |
| CV | coxsackievirus |
| DNA | deoxyribonucleic acid |
| E | echovirus |
| EBV | Epstein Barr Virus |
| ECDC | European Centre for Disease Prevention and Control |
| EDTA | ethylenediaminetetraacetic acid |
| EEG | electroencephalogram |
| EV | enterovirus |
| FRET | fluorescence resonance energy transfer |
| GAS | Group A Streptococcus |
| GBS | Group B Streptococcus |
| HES | Hospital Episode Statistics |
| HFMD | hand foot and mouth disease |
| HHV | human herpes virus |
| Hib | *Haemophilus influenzae* type b |
| HIPE | Hospital In-Patient Enquiry |
| HPeV | human parechovirus |
| HR-QOL | health related quality of life |
| HSCIC | Health and Social Care Information Centre |
| HSV | herpes simplex virus |
| ICD | International Classification of Diseases |
| ICU | intensive care unit |
| IM | intramuscular |

| | |
|---|---|
| IMD | invasive meningococcal disease |
| IPD | invasive pneumococcal disease |
| IQ | Intelligence Quotient |
| ITQOL | Infant Toddler Quality of Life Questionnaire |
| IV | intravenous |
| LCMV | lymphocytic choriomeningitis virus |
| LOS | hospital length of admission (in days) |
| LP | lumbar puncture |
| MenB/C/W/Y/X | meningococcal capsular group B or C or W or Y or X |
| MMR | measles mumps rubella vaccination |
| NESS | The National Enterovirus Surveillance System |
| NHS | National Health Service |
| NICE | National Institute for Health and Care Excellence |
| NICU | Neonatal intensive care unit |
| NPA | nasopharyngeal aspirate |
| NREVSS | The National Respiratory and Enteric Virus Surveillance System |
| NVT | non-vaccine type |
| OMP | outer membrane protein |
| ORLS | Oxford record linkage study |
| PCR (+, -) | polymerase chain reaction (positive or negative result) |
| PCV | pneumococcal conjugate vaccine |
| PHE | Public Health England |
| PMN | polymorphonuclear cells |
| QOL | quality of life |
| RBC | red blood cell count |
| RNA | ribonucleic acid |
| RT-PCR | real time-polymerase chain reaction |
| ROC curve | receiver operating characteristic curve |
| SAE | Serious Adverse Event |
| SD | standard deviation |
| SNHL | sensorineural hearing loss |
| SPSS | Statistical Package for the Social Sciences software |
| TB | *Mycobacterium tuberculosis* |
| UK | United Kingdom |
| UK-ChiMES | UK-Childhood Meningitis and Encephalitis cohort study |
| USA | United States of America |
| UTI | urinary tract infection |
| VT | vaccine type |
| VZV | varicella zoster virus |
| WBC | white blood cell count |
| WHO | World Health Organisation |

# 1. Chapter 1: Introduction

## 1.1. Historical aspects

### *1.1.1. Historical aspects for bacterial meningitis*

Historical changes in terminology for meningitis have paralleled advances in diagnostics. Clinical syndromes resembling meningitis can be found in historical documents including descriptions by Hippocrates around 400BC[1] and by Persian physicians around the tenth to twelfth centuries AD.[2] Disease characterized by delirium and fever was commonly termed 'phrenitis' until the early 1800s, which means brain fever.[2,3]

In Europe, an epidemic of a meningitis-like illness with a rash, defined as 'cerebral fever', was described by Gaspard Vieusseux in Geneva in 1805, and caused 33 deaths.[3-5] This was most likely the first description of an epidemic of meningococcal disease,[3,6] with studies indicating that the organism first became pathogenic in the early 1800s by acquiring genes required for synthesis of the polysaccharide capsule, and descriptions of epidemic meningococcal-like disease lacking from historical scripts.[6-8] Further descriptions of meningococcal meningitis-like epidemics followed including one in Medfield, Massachusetts in 1806.[3] In Africa, although the first reported outbreak of cerebrospinal meningitis occurred in 1840 in Algeria, epidemics of meningococcal meningitis occurring every two to three years have been described in sub-Saharan Africa since the early 1900s.[6]

From the early 1800s, the term 'meningitis' started to be used when increased interest in clinicopathological correlation and post-mortem examination indicated pathology in the

meninges.[3] The term 'meningitis' was used more widely after it was defined as 'inflammation of the arachnoid, or pia mater, or both' in John Abercrombie's textbook of neuropathology in 1828,[3] although the term 'cerebrospinal fever' is still found in literature from 1944.[5,9] Attempts were also made during the 19th century to further sub-classify meningitis or 'cerebrospinal fever', for example into 'cerebritis' for brain, and 'arachnitis' for meningeal manifestations.[3]

Increasingly, from the late nineteenth century, classification of types of meningitis were based on bacteriological findings following the discovery of the lumbar puncture and advances in bacteriology.[5,10] The lumbar puncture (LP) technique, attributed to Heinrich Quincke[3,10], was initially used to relieve raised intracranial pressure, and then to measure the chemical composition of cerebrospinal fluid (CSF) including cell counts, protein, glucose and bacteria, allowing organisms to be found before a patient died.[2,3] Important developments in the field of bacteriology in the late 19th century included the discoveries of *M. tuberculosis* by Koch, the pneumococcus by Sternberg and Pasteur, *N. meningitidis* by Viennese physician Weichselbaum, and *H. influenzae* by Pfeiffer.[3]

Clinical signs associated with meningitis were also described from the late 1800s including physicians, Kernig, and Brudzinski, defining meningeal signs still used today.[3,5,9] Treatment for meningitis in the early 1900s included antiserum for meningococcal disease, and then sulphonamides.[2,3] Mortality from bacterial meningitis was very high prior to the antibiotic era, with the discovery of penicillin in 1928.[3,11] In the UK, widespread introduction of bacterial meningitis vaccines commenced with the 1992 Hib conjugate vaccine programme.[12]

*1.1.2. Historical aspects for aseptic meningitis*

The term 'acute aseptic meningitis' was first proposed by Wallgren in 1924, who described occurrences of this clinical syndrome in France and Scandinavia,[13-16] although both epidemic poliomyelitis and mumps were already recognized.[15] Wallgren's criteria included an acute, short disease with meningeal signs and symptoms and a complete recovery, a CSF mononuclear pleocytosis with absence of bacterial or local para-meningeal infection, absence of a systemic infectious disease, and absence of community epidemic disease involving meningitis.[13-15] Early descriptions of aseptic meningitis also identified seasonality, with increased incidence in the late summer and autumn, and also higher rates of disease in young children.[13]

Although Wallgren initially defined 'aseptic meningitis' as a disease entity in itself, separate to disease caused by 'known' microorganisms, this concept was soon redefined as increasing numbers of microorganisms were identified.[13] Coxsackieviruses were discovered in 1948,[17,18] and following the introduction of 'tissue culture' to grow polioviruses in 1949[19] increasing numbers of viruses were isolated.[15,18] Several case series of 'viral' or 'aseptic' central nervous system (CNS) disease followed.[15] Despite increasing microbiological identification, for the majority of cases of aseptic meningitis a cause was not found.[13,15]

The introduction of polymerase chain reaction (PCR) since the 1990s has improved clinical diagnostics for aseptic meningitis, with early studies reporting high sensitivity (>90%) and specificity (almost 100%).[20-28] Prior to PCR, viral culture would take 2-8 days or longer, [16,23,29,30] and sensitivity was low, with early studies comparing PCR techniques to viral

culture showing that viral culture only detected around 28-76% of cases that were enteroviral PCR positive.[21,23,24,27,30-35] Aciclovir to treat herpes simplex virus (HSV) meningitis was discovered in the 1970s, and has been widely used since the 1980s.[36-38]

## 1.2. The pathogenesis and pathophysiology of meningitis

### *1.2.1. Pathogenesis and pathophysiology of bacterial meningitis*

Meningitis describes inflammation of the normally sterile meninges, most commonly the arachnoid and pia mater, and the subarachnoid space.[39,40] Bacterial meningitis usually occurs when bacteria that have colonised mucosal surfaces invade and replicate in the bloodstream.[40-43] Bacteria penetrate the blood-brain (BBB) or blood-CSF barrier to enter the subarachnoid space, by transcellular or paracellular routes or in infected phagocytes.[44,45] There is evidence that for some bacterial pathogens, meningitis is more likely with a greater degree of bacteraemia.[41,45] Many bacterial pathogens that cause meningitis have been shown to cross transcellularly by interacting and binding to host receptors on the endothelial cells that constitute the BBB.[44,45] Alternatively, direct invasion occurs from a contiguous source, for example a paranasal sinus or middle ear infection, or from a ventricular shunt or skull fracture.[39,40] Microorganisms in the CNS cause dysfunction and increased permeability of the BBB and leukocyte migration into the CNS, by mechanisms including release of inflammatory compounds during bacterial autolysis and replication, and by causing BBB endothelial cell apoptosis.[41,45] Bacterial compounds such as peptidoglycan and cell wall fragments are recognised by host cell pattern recognition receptors, including Toll-like Receptors, leading to release of host inflammatory cytokines and molecules.[42,43,46] These processes cause cerebral oedema,

increased intracranial pressure, and release of further inflammatory molecules, cytokines and free radicals from leukocytes and other host cells.[40-42] Vasculitis, focal ischaemia, central venous thrombosis, intracranial haemorrhage and hydrocephalus may also occur.[41,43] Neuronal cell death in bacterial meningitis is typically caused by necrotic cortical and apoptotic hippocampal injury, and is contributed to by both host and microbial factors.[40-42,47] Although antibiotics vastly reduce mortality, bacteriolytic antibiotics also increase the production of bacterial cell wall components which may contribute to further inflammatory processes.[42]

*1.2.2. Pathogenesis and pathophysiology of viral meningitis*

Although viruses cause most aseptic meningitis (for definitions *see section 1.7.1*), there also many other possible aetiologies, see *table 1.1*.[48-50] Viruses invade the CNS either from the blood supply by crossing the blood brain barrier, or by entering through peripheral or cranial nerves.[51,52] Enteroviruses (EV) usually spread between individuals by faecal-oral or respiratory routes.[53,54] Most viruses reach the CNS by haematogenous spread, initially entering by the respiratory system (for example mumps virus), gastrointestinal tract (for example enteroviruses), or skin (for example arboviruses).[55] Infection is then established and replication occurs in nearby lymphoid tissue.[55] If the virus adequately evades the immune response, a primary viraemia follows with virus circulating either in host cells, attached to host cells or freely.[51] Usually, viruses then replicate at a secondary site, for example the spleen or liver, before a secondary viraemia occurs involving larger amounts of circulating virus, adequate to invade the CNS.[51] The BBB or blood-CSF barrier is crossed by either directly infecting BBB endothelial cells,

being carried in infected lymphocytes, or by entering following BBB breakdown for example from inflammation due to systemic infection.[55] Viruses that enter the CNS by nerves include HSV, which travels through peripheral sensory neurons after infecting keratinocytes, and varicella zoster virus (VZV).[51,55] Travelling along nerves helps evade the host immune system.[51] Viruses including HSV-1 and influenza may also enter by olfactory neurons directly exposed to the nasal airway.[51,55]

Viruses that reach the CSF either remain in the meninges, or invade brain parenchyma.[51] Meningitis usually means infection of the meninges, whereas encephalitis or meningoencephalitis usually indicates that there is also involvement of brain parenchyma.[55] Once in the CNS, viruses replicate within host cells and spread either contiguously from cell to cell, by travelling through the extracellular space, along neurons or within migrating host lymphocytes or glial cells.[51] The cellular immune response to viruses means that lymphocytes accumulate in CNS.[55] Enteroviruses replicate within host cell cytoplasm after attaching to a host cell, opening a pore in the cell membrane and releasing viral RNA into the cytoplasm.[52] During enteroviral replication, host cell translation is shut off and cellular destruction occurs by apoptosis leading to tissue damage, presumed to be the main mechanism for pathogenicity, although cellular destruction also limits viral spread.[52] Other disease manifestations are proposed to be caused by different mechanisms, for example myocarditis and exanthems are considered to be caused by immune mechanisms.[54,56] Enteroviruses may continue to be shed in stool for several weeks, and from the upper respiratory tract for 1-2 weeks following infection.[26]

Few data are available concerning the pathology of viral meningitis, because it is usually a self-limiting, benign disease.[16] Data from post-mortem reports and studies in mice indicate inflammation and apoptosis around choroid plexus, hippocampus and cerebral cortex in disease caused by some coxsackieviruses, which are subtypes of enteroviruses.[16,52,55] In EV infection, findings of pathology including apoptosis in the hippocampus provides some rationale for sequelae including memory and behaviour changes.[52]

More is known about the pathology of viruses that infect the brain parenchyma. A study of seven post-mortem examinations following EV71 infection reported most inflammation in the spinal cord grey matter, brainstem, hypothalamus, subthalamic and dentate nuclei, and to a lesser extent the cerebral motor cortex, and suggested that EV71 may enter the CNS by the peripheral motor nervous system.[57] HSV typically causes encephalitis of the fronto-temporal lobes in older children and adults although other distributions have been described, and disease may be more diffuse in infants and young children.[58] Both HSV and VZV can establish latent infection in the trigeminal or dorsal root ganglia.[59]

**Table 1.1 Causes of aseptic meningitis**
*adapted from Kumar et al[48] and Lee et al[49](references[16,48-50])*

| **Infectious causes** | ***Examples*** |
|---|---|
| Viruses | Enteroviruses<br>Parechoviruses<br>Herpesviruses<br>herpes simplex virus 1 and 2, varicella zoster virus, cytomegalovirus, Epstein-Barr virus, human herpes virus-6<br>Respiratory viruses<br>adenovirus, rhinovirus, influenza viruses, coronaviruses<br>Arbovirus<br>Mumps virus<br>Measles virus<br>Rotavirus<br>Parvovirus B19<br>Lymphocytic choriomeningitis virus<br>Human immunodeficiency virus |
| Bacteria | Atypical bacteria, for example<br>*Mycoplasma pneumoniae, Mycobacterium tuberculosis, Brucella*<br>Spirochete bacteria, for example<br>*Borrelia burgdorferi*, *Leptospira*<br>Partially treated bacterial meningitis<br>Endocarditis<br>Para-meningeal infection |
| Fungi | *Cryptococcus neoformans, Candida* species |
| Parasites | *Toxoplasma gondii, helminths*, malaria |
| Rickettsiae | |
| **Non-infectious causes** | ***Examples*** |
| Post-infectious and post-vaccination | Rubella, varicella, pertussis vaccine, influence vaccine |
| Drugs | Nonsteroidal anti-inflammatory drugs, cotrimoxazole |
| Systemic disease | Collagen vascular disorders (including Kawasaki disease), sarcoidosis |
| Other | Malignancy for example leukaemia, brain or epidural abscess, urinary tract infection |

### 1.3. Enteroviral and parechoviral classification and disease manifestations

Enteroviruses are non-enveloped single positive-stranded icosahedral shaped RNA viruses from the Picornavirus family, with a genome size of approximately 7.2-8.5 kb.[52,60] The enteroviral RNA genome comprises a single open reading frame which encodes one long

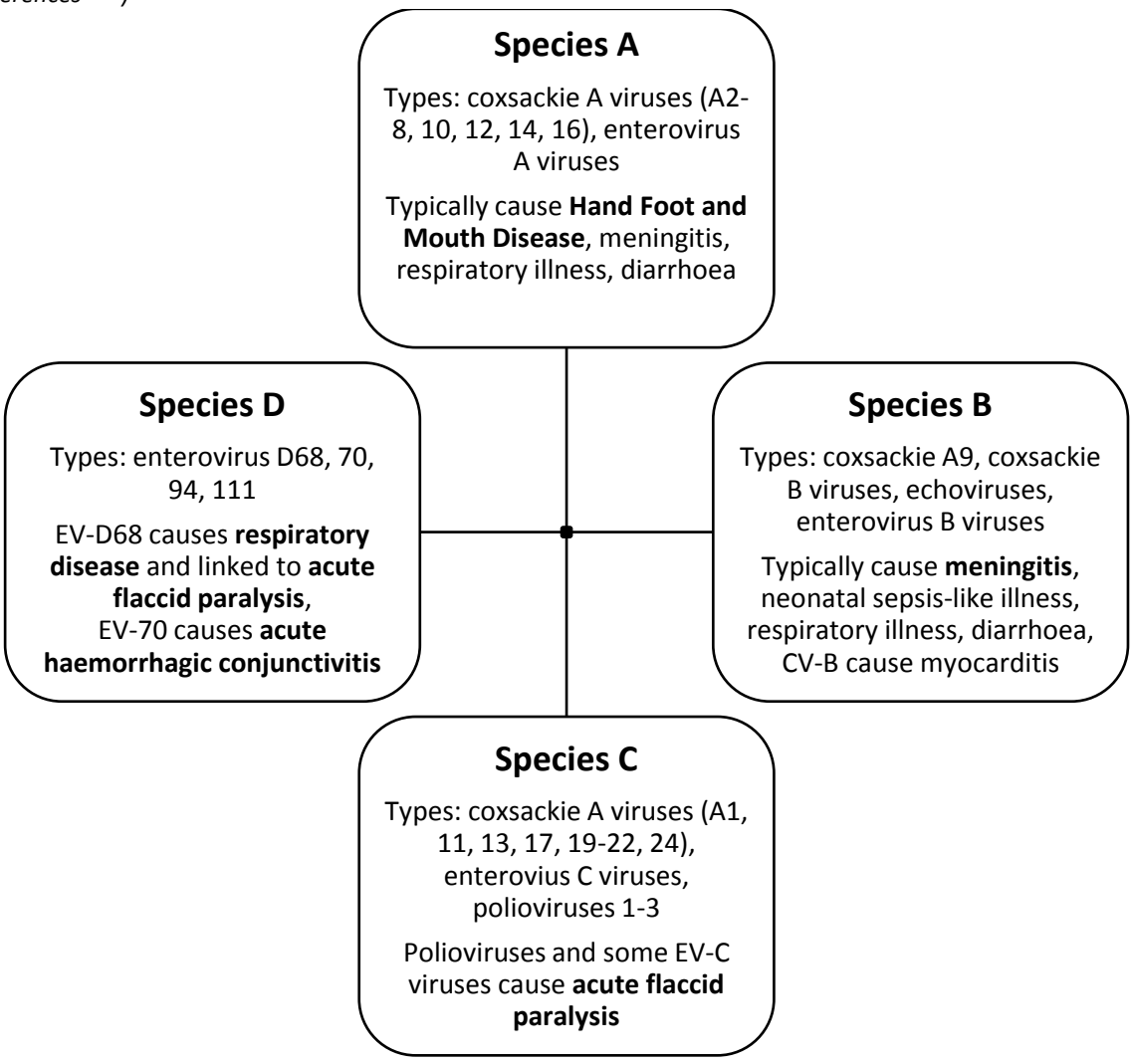
polyprotein, which following post-translational cleavage codes for four capsid proteins (VP1-4), and seven non-structural proteins, and is flanked by 5' and 3' untranslated regions and a 3' poly(A) tail.[52] The capsid is composed of four proteins, VP1-VP4.[52]

Currently, enteroviruses are classified based on at least 75% homology of their VP1 capsid protein nucleotide sequence, which correlates with previous methods of serotyping.[61,62] More than 110 enteroviral types infecting humans have now been identified, which are classified into four species (A-D) (*figure 1.1*).[53,54,60-62] Enteroviruses from species A typically cause hand foot and mouth disease (HFMD), and include enterovirus A and coxsackie A viruses.[53,60] Species B are most frequently isolated from CSF, and include coxsackieviruses B1-6, many echoviruses and several other serotypes.[53,60,63,64] Species C includes the three polioviruses and twenty other serotypes, some of which have also been associated with acute flaccid paralysis.[53,60] Species D includes enterovirus-D types including EV-D68 which causes respiratory disease and cases of acute flaccid paralysis, and EV-D70 which causes acute haemorrhagic conjunctivitis.[53,60]

The spectrum of disease caused by enteroviruses is broad, including mild upper respiratory tract infections, febrile illness with rash, respiratory disease (typically CV-A21, CV-B2-B5), diarrhoea (typically echoviruses), myopathy, eye disease including haemorrhagic conjunctivitis (typically EV-D70, CV-A24), hepatitis, aseptic meningitis, encephalitis, myocarditis and pericarditis (typically CV-B3 and B1), paralysis and neonatal sepsis-like illness.[52-54,60,63,65-67]

Enterovirus type A-71, and also coxsackievirus A-16, have caused outbreaks of HFMD during the past twenty years in Asia and the Pacific.[53,54,60] Although most cases cause mild disease characterised by fever, oral lesions and a maculopapular or vesicular rash on hands, soles of feet and other areas, some children develop neurological symptoms including aseptic meningitis, encephalitis, acute flaccid paralysis and acute transverse myelitis.[26,52] Neurological and neuropsychiatric sequelae may occur following EV A-71 CNS disease, and some cases are fatal.[53,54,60] Other enteroviruses also cause HFMD, including recent outbreaks of coxsackievirus A6 in the USA in Europe.[60]

**Figure 1.1 Enterovirus species causing disease**
*(references[53,60])*




Note: EV enterovirus, CV-B coxsackie B virus

Parechoviruses are also single stranded non-enveloped RNA viruses from the picornavirus family, and were recognised as having a distinctive genome to enteroviruses, and therefore a separate genus, since the early 1990s.[68] Parechoviruses have three capsid proteins, compared with enteroviruses which have four capsid proteins, due to lack of cleavage by parechoviruses of VP0 into VP4 and VP2.[69,70] Most disease is caused by parechovirus types 1-3, although at least 8 types cause disease, and up to 19 different types have been identified based on sequencing of the VP1 capsid protein.[69,71,72] Human parechoviruses cause a spectrum of disease ranging from mild gastrointestinal and respiratory disease to sepsis and more severe illness. [69,73,74] Most CNS disease is caused by type 3, which was first isolated in Japan in 1999.[69,75,76] One recent study reported that a quarter of infants with parechovirus infection required intensive care unit (ICU) admission.[74] Another recent study reported cerebral haemorrhage thought to be associated parechoviral infection in three neonates.[77] A Norwegian study found that 86% of 102 infants had at least one positive stool parechovirus PCR with monthly sampling over 24 months.[78]

### 1.4. Nasopharyngeal bacterial carriage

Bacteria must first colonise the nasopharynx for invasive disease to occur.[79-82] Bacteria frequently carried in the nasopharynx include *S. pneumoniae, H. influenzae*, *N. meningitidis, Moraxella catarrhalis*, alpha-haemolytic streptococci and *Staphylococcus aureus*.[80] Unlike polysaccharide vaccines that do not produce a T-cell dependent immune response, and do not appear to effect carriage, immunisation with conjugate vaccines reduces nasopharyngeal carriage.[80,83] This reduction in carriage is responsible for herd

immunity, for example in adults following infant pneumococcal conjugate vaccination (PCV), and in unimmunised age groups following infant and teenage MenC vaccination.[84-87] Herd immunity occurs when non-immune people are indirectly protected from disease, because of increased frequency in a population of immune people who are protected directly, for example by vaccination.[85-87]

Studies of meningococcal carriage have demonstrated that although carriage of meningococci is required for invasive disease, the meningococci that are carried are diverse, most are not hyperinvasive strains, and most are unencapsulated.[79,81,82,88] European studies reported that meningococcal carriage increases during childhood and peaks at age 20-24 years.[79] A meta-analysis of carriage studies in Europe and countries with similar meningococcal epidemiology reported carriage rates of 4.5% in infants, 7.7% in 10-year olds, 23.7% in 19-year olds and then reducing to 12.1% at age 30, and 7.8% at age 50 years.[85] However, individual studies varied with a Dutch study reporting rates as low as 3% in older teenagers,[89] but a German study reporting rates of >40% in 25 year olds.[88] Factors shown to affect meningococcal carriage rates include smoking, presence of respiratory infections, social behaviour, number and closeness of contacts, social economic status, and time to plating of swabs. [79,90,91] An English retrospective database study also identified maternal smoking to be associated with meningococcal meningitis, and having older siblings to be associated with *Haemophilus influenzae* meningitis.[92]

Carriage studies in the United Kingdom (UK) demonstrated a reduction in nasopharyngeal carriage of meningococcal capsular group C (MenC) following the 1999 introduction of the MenC vaccination programme, which has also led to indirect protection from disease

in unvaccinated people by herd immunity.[85-87] Studies in teenagers aged 15-19 years showed that carriage of capsular group C meningococci reduced from 1999 to 2001 (rate ratio 2001:1999 of 0.19), although overall carriage of meningococci overall increased slightly.[86,87] A recent UK carriage study of young people aged 10-25 years was performed to inform vaccine implementation strategies for the 4CMenB vaccine.[90] During 2011-2012 carriage rates of all meningococci increased from around 4% at age 10-12 years to around 25% at age 19-25 years, with the majority non-groupable.[90] The most frequent capsular groups isolated were groups B (6.5% overall) and Y (5.5% overall).[90] Although it is unknown whether 4CMenB reduces carriage, this study suggested vaccination should occur in early adolescence before the increase in carriage.[90]

Reported carriage rates for *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* vary between studies and countries, however the first episode of carriage with one or more of these organisms often occurs in infancy, and carriage rates are generally lower in adults.[80,93-95] Factors that affect carriage of these organisms include vaccines, disease state, interaction between children and interaction between adults and children, presence of otitis media, and viral respiratory tract infections.[80,94-96]

Carriage studies in the UK following PCV7 and PCV10 vaccine introduction found that although the prevalence of pneumococcal carriage overall has remained stable in children aged <5 years at around 50% pre-and post-vaccine introduction, there have been dramatic changes in pneumococcal carriage serotypes.[84,97] One study reported that in children <5 years, post-PCV7 non-vaccine type (NVT) carriage increased from 15% to 45% and vaccine type (VT) reduced from 31% to 4%.[84] A further study post-PCV 13 reported

ongoing reductions in carriage of PCV-7 serotypes in children <5 years, in addition to a reduction in carriage of the additional six PCV-13 serotypes from 9.9% in 2008/09 to 0.4% in 2012/13.[97] In older unvaccinated age groups, serotype replacement in carriage also occurred, for example in young people aged 5-20 years, studies reported overall pneumococcal carriage rates of 21-28% pre-and post-PCV7 and -13 vaccines, with carriage of PCV-7 serotypes reducing from 10% to 0.9% from 2001/02 to 2012/13, PCV-13 serotypes not in PCV-7 increasing post-PCV-7 and then reducing post PCV-13 from 5.3% to 1.8% from 2008/09 to 2012/13, and carriage of all NVT strains increasing from 8.5% to 19.6% from 2001/02 to 2012/13.[84,97] Serotypes that are currently frequently carried cause less invasive disease than the previously carried PCV-13 vaccine types.[97] Studies have also demonstrated effects of vaccination on carriage of other bacteria, for example, showing an increase in *H. influenzae* (especially non-typeable) and *S. aureus* carriage in some age groups following PCV7 vaccination.[80,98,99]

## 1.5. The epidemiology of bacterial meningitis

### *1.5.1. The epidemiology of bacterial meningitis in the UK*

Bacterial meningitis is an acute, severe disease and remains a cause of substantial morbidity and mortality in children.[100] Meningitis and encephalitis are estimated to cause 4-5% of childhood deaths globally from age one month to four years.[101]

The vast majority of previously published bacterial meningitis epidemiology in England is derived from microbiologically confirmed cases reported to Public Health England (PHE).[102] These data may underreport incidence and cases that are not microbiologically

confirmed may be missed, although hospitals and laboratories are expected to notify PHE on identification of organisms that frequently cause bacterial meningitis.[102]

Previous studies reported that the majority of neonatal (aged <3 months) bacterial meningitis in the UK was caused by Group B Streptococcus (GBS) and *E. coli*.[103-105] *Neisseria meningitidis* and *Streptococcus pneumoniae* also caused disease, most commonly after the first month of life.[103-105] *Listeria monocytogenes* caused disease usually within the first month of life.[103,105] Other causes included *Staphylococcus aureus*, other Gram positive and negative bacteria, and enterococci.[103,105] Nosocomial infection in hospitalised premature infants also occurred, commonly with coagulase negative staphylococci.[100,103-105] Previous studies in the post-Hib vaccine era, reported that most bacterial meningitis in children aged >3 months in the UK was caused by *N. meningitidis* and *S. pneumoniae*, with *N. meningitidis* causing the greatest proportion of cases.[104,106] Other bacterial causes included *Haemophilus influenzae* type b, a frequent cause of disease prior to widespread Hib vaccination,[12,107] *S. aureus* and *E. coli*.[100,104,106]

A study that investigated the incidence of all-cause bacterial meningitis from laboratory surveillance data in England from 2004 to 2011 reported 3.3 cases per 100 000 children aged <15 years per year, with 8.7 cases per 100 000 children <5 years, and 72 cases per 100 000 infants <3 months.[104] This study showed no significant overall change in childhood bacterial meningitis rates during the included years, although there was an increase in rates in infants aged <3 months caused mostly by increased GBS disease and a decline in incidence in infants aged 3-11 months because of a reduction in disease caused by *N. meningitidis* and *S. pneumoniae*.[104] Of children aged 5-14 years, there was no

overall change in rates, because although *N. meningitidis* cases declined, there was a slight increase in other Gram positive and Gram negative bacterial cases.[104]

Previously reported case-fatality rates (CFRs) for bacterial meningitis in the UK and Europe range between 5-10% (*N. meningitidis*) and 5-17% (*S. pneumoniae*).[108-115] CFRs for *S. pneumoniae* meningitis in Africa are as high as 73%.[113,116,117] A global systematic review reported a median risk of long-term disabling sequelae in survivors of bacterial meningitis of around 20%, with such sequelae most likely after pneumococcal meningitis.[118]

*1.5.2. The epidemiology of meningococcal disease in the UK*

Worldwide, almost all invasive meningococcal disease (IMD) is caused by six of twelve capsular groups (A, B, C, W, X, Y) with regional variation in predominant groups.[119-121] Meningococcal classification is determined by the polysaccharide capsule composition (capsular group), and other genes including the outer membrane protein (OMP) PorB variant, OMP PorA variable region, and the lipopolysaccharide structure.[81,122] Meningococcal strains are also further classified according to sequence type. Sequence typing is based on allelic variation in seven housekeeping genes.[123,124] Strains with sequence types that are closely related share a common lineage and are grouped as the same clonal complex.[82] A clonal complex may express different capsular groups, for example, clonal complex 11 strains cause hypervirulent disease and have previously expressed capsular groups B, C and more recently capsular group W.[125]

In the UK, disease incidence peaks at age <2 years with a second small peak at 15-19 years.[113,126] Meningococci exhibit seasonality with most cases occurring in winter.[113,126] Most invasive meningococcal disease presents as meningitis with septicaemia (60%) or meningitis (15%), although isolated septicaemia also frequently occurs (25%).[106]

The majority of childhood disease in the UK is caused by meningococcal capsular group B (MenB), with smaller contributions from capsular groups W, Y and C.[110,126] Of children aged <15 years, 93% of disease was caused by MenB in England and Wales from 2006/07 to 2009/10,[110] which reduced to 82% in England in 2014/15.[117] The reduction in proportion of cases caused by MenB also occurred in infants from 94% from 2006/07-2009/10 in England and Wales,[110] to 80% in 2014/15 in England.[117]

The all-age incidence of laboratory confirmed cases of all IMD reduced in the UK from 2/100 000 in 2006/07-2010/11 to 1.36/100 000 in 2012.[110,113] The incidence of meningococcal disease in infants reduced from 45 per 100 000 in 2006/07 to 31 per 100 000 in 2010/11 in England and Wales[110] and was 19 per 100 000 in 2014/15 in England.[117] From 2006/7-2010/11, in England and Wales, the incidence was 13/100 000 in children aged 1-4 years, 2.6/100 000 in children aged 5-9 years, 1.2/100 000 in children aged 10-14 years, and 3.2/100 000 in children aged 15-19 years.[110]

Overall, PHE data reporting laboratory confirmed cases indicated that IMD incidence reduced by 73% from 2000/01 to 2013/14 in England and Wales.[127] In children, particularly infants, the latter years of this decline was driven by a reduction in MenB cases since 2009/10, before MenB vaccination was available.[117,12679,92] However, the

greatest decline in all meningococcal disease occurred earlier from 1999/2000 to 2005/06 and was caused by the 1999 introduction of MenC conjugate vaccine in the UK.[127,128] MenC disease reduced by 97% in the eight years following MenC vaccine introduction.[127,128]

Notably, provisional data from PHE for the 2015/16 year showed a 27% increase in all IMD cases from 636 in 2013/14 to 805 in 2015/16, with 55% caused by MenB, 26% by MenW, 13% by MenY, and 5% by MenC.[129] The 4CMenB vaccine was introduced into the routine infant schedule to protect against MenB in September 2015 with a catch-up programme for infants born from May 2015.[130,131]

The increase in IMD since 2013/14 was caused mostly by an increase in MenW,[129,132] a gradual increase in MenY, and a smaller increase in MenB.[129] At all ages, there were 34 cases of MenW reported to PHE in 2011/ 12, with an increase to 176 cases in 2014/15,[117,133] and 210 cases reported in 2015/16 provisional data.[129] This increase in MenW disease initially occurred in adults, but increasing rates were then seen at all ages.[127] The recent increase in MenW disease is mostly caused by strains which belong to sequence type 11 clonal complex (cc11) and express the W capsule.[127] Disease has occurred in healthy people of all ages with many requiring intensive care (37%), and a high reported case fatality rate of 10-12%.[117,127] From 2000/01 to 2003/04 an outbreak of MenW disease also occurred in the UK associated with people who had travelled to the Hajj.[125,134] The Hajj-associated MenW strain also belonged to cc11, however the recent outbreaks are genetically distinct from the Hajj strain.[125,134] In response to the increase in

MenW disease, in the UK, the adolescent MenC conjugate vaccine programme was replaced by a MenACWY conjugate vaccine programme from August 2015.[132,134 134]

*1.5.3 The epidemiology of meningococcal disease in Europe, USA, Australasia and Sub-Saharan Africa*

The reported incidence of invasive meningococcal disease in the rest of Europe and the USA has been lower than in the UK.[113,119119] In Europe (including the UK), in 2012, the overall incidence of IMD at all ages was 0.68/ 100 000, which was a reduction from 0.95/100 000 in 2008.[113] Infants have the greatest burden of disease with 12.3 cases per 100 000 population in 2012.[113] The highest rates occurred in Lithuania, the UK and Ireland.[113] In Europe, most disease in children was caused by capsular group B, followed by MenC with some contribution from MenY and MenW, and a recent all-age increase in MenY.[113]

In the USA, predominant capsular groups causing disease are B, C and Y with some contribution from group W.[135] The incidence of IMD in the USA in 2014 was 0.16/100 000 all ages.[135] A reduction IMD was reported during the past two decades, with reported incidence reducing by 64% from 1998-2007.[119,136] MenY disease increased from the 1990s in the USA and caused 37% of disease during 1997-2002, although this proportion has now reduced.[136] The incidence of IMD in infants from 2006-2012 was 2.74 per 100 000 infants.[137] During this period, MenB caused 64%, MenC caused 12%, and MenY caused 16% of infant cases.[137] Two small outbreaks of MenB disease at universities in New Jersey and California in 2013 were managed with the 4CMenB vaccine.[112,138]

In Australia and New Zealand, MenB caused most disease from early 2000s to 2012, with some contribution from capsular groups C, and rarely W and Y.[112,119] Rates of IMD in Australia are around 1/100 000.[112,119] New Zealand experienced an epidemic of a single clone of MenB disease from 1991, with rates reaching 17/100 000 in 2000 and much higher in infants.[112,119,139] Rates of MenB decreased during the early to mid-2000s, with a reduction in epidemic strain incidence occurring at a similar time to the 2004 introduction of a strain-specific outer membrane vesicle based vaccine, and incidence is currently around 1/100 000.[112,119,139,140]

Sub-Saharan Africa has the highest rates of IMD in the world, with epidemic patterns of disease described since the early 1960s, across an area including 21 countries.[141,142] [120] These epidemics can cause disease rates as high as 100/100 000 per year or 1000/ 100 000 per week during an outbreak.[120] In sub-Saharan Africa most epidemics occur in the dry season, and it has been theorised that this occurs because lower night time temperatures cause people to congregate in small areas.[143] MenA caused most epidemic disease prior to 2009, although more recently MenW caused outbreaks in 2010 and 2011 particularly in Niger, and MenC (which was previously rare in Africa) caused disease in 2013-14 in Nigeria and in 2015 in Niger. [81,119,120,144-146] MenX has caused outbreaks in Niger, Uganda, Kenya, Togo and Burkina Faso during 2006-10.[120] A monovalent serogroup A conjugate vaccine (MenAfriVac) programme was implemented in sub-Saharan Africa commencing in 2010, and rates of MenA decreased at a similar time to vaccine introduction, likely related both to natural variation in clonal waves and the vaccine effect.[119,120]

*1.5.4. The epidemiology of invasive pneumococcal disease in the UK*

*S. pneumoniae* causes a spectrum of invasive disease including meningitis, pneumonia, bacteraemia and sepsis.[113,147] A small proportion of the >90 pneumococcal serotypes identified cause most childhood disease.[113,147] Serotypes are determined by serological properties of capsular polysaccharide.[147] The highest rates of invasive pneumococcal disease (IPD) occur in adults ≥65 years and then children <2 years old.[148,149] In Europe, disease rates are highest in the winter months.[113] Prior to pneumococcal conjugate vaccination programmes, in the year 2000 the global incidence of pneumococcal meningitis in children <5 years was 17/100 000 with a CFR of 60%.[116] The highest incidence was in Africa (38/100 000, CFR 73%) and lowest in Europe (6/100 000, CFR 38%).[116] Two recent UK studies reported CFRs for pneumococcal meningitis of 11% at age 3-59 months from 2006-10,[111] and 17% for children aged <5 years from 2004-09.[109]

The introduction of pneumococcal conjugate vaccine programmes in the UK has reduced IPD, both in vaccinated children and adults, through herd immunity resulting from reduction in carriage.[149] The 7-valent pneumococcal conjugate vaccine, PCV7, was introduced in September 2006 at 2, 4 and 13 months with a catch up programme for children up to two years, and was replaced by the 13-valent vaccine from April 2010.[149,150]

The reduction in IPD which occurred in England and Wales following routine infant conjugate pneumococcal vaccination was partially offset by serotype replacement, with

an increase in observed IPD caused by serotypes that were generally uncommon in the UK prior to these vaccination programmes.[109,148,150] From 2000-06 (pre-PCV 7) to 2009-10 (post-PCV 7), IPD reduced overall in laboratory surveillance data by 56% in children <2 years old, and by 19% in adults >65 years old due to herd immunity.[149] VT disease reduced by 98% in children <2 years and 81% in adults >65 years.[149]

Further reductions in IPD occurred following PCV13 introduction.[150] Comparing data on microbiologically-confirmed cases for 2008-10 (pre-PCV 13) to 2013-14 (post PCV-13), at all-ages IPD decreased by 32%.[150] In children <2 years overall IPD reduced by 46%, PCV13 serotypes not in PCV7 reduced by 89%, and PCV7 serotypes reduced by a further 76%.[150] However, in children <5 years, non-PCV13 type disease increased significantly during these years, with an overall increase in non-PCV13 type IPD from 10·8 per 100 000 in 2012/13 to 12·0 per 100 000 in 2013/14 in children <2 years, and from 3·6 to 4·1 per 100 000 from 2012/13 to 2013/14 in children aged 2–4 years.[150] A significant increase in non-PCV13 serotypes also occurred in adults aged >45 years pre and post PCV-13 implementation.[150]

The overall incidence of reported laboratory confirmed cases reduced from the pre-pneumococcal conjugate vaccine era, 2000-2006, to the post- vaccination years, 2013-14, in children aged <2 years from 51.8/100 000 to 12.1/100 000 (77% reduction), but NVT disease increased from 5.3 to 10.2 per 100 000.[150] IPD reduced at age 2-4 years from 15.8/100 000 to 4.8/100 000 (74% reduction) with an increase in NVT disease from 1.2 to 3.5/ 100 000, and IPD reduced at age 5-14 years from 5/100 000 to 1.1/100 000 (77% reduction) with no significant change in NVT disease.[150] At all ages there was a 56%

reduction in IPD.[150] Concerns have been raised recently in the UK about continued cases of IPD caused by PCV-13 serotypes 3 and 19A,[151] and notably the study reporting rates of IPD in England and Wales from 2000/01 to 2013/14 indicated fluctuations in incidence for serotype 3.[150]

Considering childhood pneumococcal meningitis incidence, there are fewer available data than for IPD incidence overall. Studies of laboratory confirmed cases in children <5 years old reported an overall reduction in pneumococcal meningitis of 44% from 2000-06 to 2008-10, with incidence reducing from 3.18/100 000/year to 1.44/100 000/year and VT disease reducing by 95%, but NVT disease increasing.[109,149] At all ages in England and Wales the incidence of laboratory confirmed pneumococcal meningitis was 0.26/100 00 from 2004-2011 with no significant change during these years.[104] Recent unpublished data indicate that pneumococcal meningitis rates in children aged <5 years have continued to reduce from approximately 4/100 000 in 2000/01-2005/06 to 1.2/100 000 in 2015/16, with 44 cases reported in 2015/16, the vast majority of which were not PCV-13 vaccine type.[152]

*1.5.5. The epidemiology of invasive pneumococcal disease in Europe and the USA*

European Centre for Disease Prevention and Control (ECDC) data reported an overall IPD rate in Europe of 4.3/100 000 in 2012, with the UK rate that year reported as 8.2/100 000.[113] Considering children, in Europe, in 2012, the incidence of IPD was 5.1/100 000 aged <5 years and 10.9/100 000 infants.[113] The countries with the highest incidence of

IPD were Denmark, Sweden, Finland and Norway, with rates around 13 to 16 per 100 000.[113] The incidence of meningitis was not reported separately.[113]

*S. pneumoniae* is the most common cause of bacterial meningitis in the USA.[153] Comparable or greater reductions in IPD were reported in the USA following the introductions of PCV7 in 2000, and PCV13 in 2010.[148,154]

Rates of laboratory confirmed cases of IPD in the USA are available from the Active Bacterial Core (ABC) surveillance region, which currently includes ten sites covering approximately 30 million people, although earlier data include only eight sites.[155] These data showed an overall all-age reduction in IPD following PCV7 introduction, from 24.3 cases per 100 000 people in 1998-99 to 17.3/100 000 in 2001 (29% reduction).[155] The largest decline occurred in children aged <2 years of age with a reduction from 188 cases per 100 000 in 1998/99 to 59/100 000 in 2001 (69% reduction).[155] From 1998-99 to 2004-05, the number of laboratory confirmed cases of pneumococcal meningitis reduced at all ages from 1.13/100 000 to 0.79/100 000, in children <2 years from 10.16/100 000 to 3.66/100 000 (64% reduction), in children 2-4 years from 0.95 to 0.87/100 000 (8% reduction), although in children aged 5-14 years no change was reported and incidence in 2004-05 was 0.29/100 000.[148] A further study from this dataset, reported that following PCV13 introduction, in 2012/13, the incidence of IPD was 64% lower in children <5 years, and 53% lower in children aged 5-17 years, than would have been predicted if PCV7 had been continued alone.[156] Overall rates of IPD in ABC region in 2014 were 8.7/100 000 at all ages, 15.1/100 000 in infants aged <1 year, 10.1/100 000 in children aged 1-2 years, 6.2/100 000 in children aged 2-4 years, and 1.3/100 000 in children aged 5-17 years.[157]

A case based study of children at eight hospitals in the USA also reported a 42% reduction in rates of laboratory confirmed IPD, comparing 2007-09 to 2011 pre- and post-PCV13, although this study showed no reduction in rates of meningitis.[154]

*1.5.6. The epidemiology of meningitis caused by Haemophilus influenzae type b*

*H. influenzae* type b currently causes very few cases of childhood meningitis in the UK.[104] However, it was the most common cause of childhood bacterial meningitis prior to the *Haemophilus influenzae* type b (Hib) conjugate vaccination programme, which resulted in virtual elimination of the disease.[12,104,107] *H. influenzae* strains are either encapsulated and categorized into 6 serotypes (a–f) according to their capsular polysaccharide, or unencapsulated and referred to as non-typeable.[113,158] Worldwide, *H. influenzae* type b has caused the most severe invasive disease,[113,158] and is a cause of epiglottitis, pneumonia, bacteraemia and sepsis, in addition to meningitis.[12,113] Disease incidence is highest in early childhood with a second peak also seen in older adults, and highest rates of disease occur in winter.[113]

Following the 1992 introduction of the Hib vaccine programme in the UK, laboratory surveillance studies indicated a 98% reduction in invasive Hib disease by 1998.[12,107] However, a small increase in invasive Hib disease cases was seen in laboratory surveillance data from 1999 and resolved with the introduction of preschool booster programme for children aged 6 months to 4 years from 2003, and routine 12 month booster from 2006.[107,159]

All European countries introduced Hib infant vaccination by 2009 and from 2008-12, the European invasive Hib disease rate at all ages was <0.1/100 000.[113] However the World Health Organisation (WHO) estimated that in 2014, global coverage with Hib vaccine was only 56%, and that Hib was still responsible for 2% of childhood mortality <5 years worldwide. [160,161] In 2012, ECDC data reported that the incidence of all *H. influenzae* disease in Europe was 0.49/100 000 at all ages and 4.2/100 000 for infants, with most cases non-typeable, and the UK all-age rate was 1.16/100 000.[113,162] A study of laboratory confirmed cases of bacterial meningitis in England and Wales, reported an incidence of 0.03/100 000 for *H. influenzae* meningitis in 2011 at all ages.[104] In 2014, there were only twelve laboratory reports to PHE of invasive Hib disease at all ages, with only 3 reports in children aged <15 years.[163]

### 1.6. The epidemiology of neonatal bacterial meningitis

A recent study of laboratory confirmed bacterial meningitis cases from 2004 to 2011 in England,[104] and a one year population-based active surveillance study in England and Wales from 2010-2011,[103] reported that 30-50% of neonatal (aged <3months) bacterial meningitis was caused by Group B Streptococcus (GBS), 11-14% by *E.coli,* 8-11% by *N. meningitidis* and 7-9% by *S. pneumoniae*.[103,104] Infrequent reported causes included *Staphylococcus aureus,*[103,104] *Listeria monocytogenes*, other gram positive bacteria including *Streptococcus pyogenes* and other streptococcal species, *Enterococcus faecalis*, coagulase negative staphylococci, and other gram negative bacteria including *Klebsiella pneumoniae* and *Haemophilus influenzae*.[104]

During the past twenty years, the British Paediatric Surveillance Unit (BPSU) have conducted studies of neonatal meningitis incidence during three defined periods, 1985-87, 1996-97 and 2010-11.[103,164,165] The studies required paediatricians in England and Wales to report episodes of neonatal meningitis in a card system, and have indicated that the incidence of neonatal bacterial meningitis overall during the past 20 years remained stable at approximately 0.2/1000 live births.[103,164,165] CFRs for all cause neonatal meningitis declined between 1985-87 and 1996-97 from 20% to 7%, although in 1996-97 the CFR for GBS was 12 % and for *E. coli* was 15%.[164,165] In 2010-11, the CFR for bacterial meningitis was 8% overall, but highest for pneumococcal meningitis (19%), and higher for preterm compared with term infants.[103]

GBS typically causes meningitis, sepsis and pneumonia in neonates, but also causes invasive disease in adults particularly with comorbid illnesses.[166,167] A recent study of laboratory confirmed invasive GBS infections in England and Wales reported an increase in GBS invasive disease from 1991 to 2010 at all ages.[167] Early onset GBS disease (defined as onset <7 days of life) increased from 0.28 to 0.41/1000 live births, and late onset neonatal disease (7-90 days) increased from 0.11 to 0.29/1000 live births from 1991 to 2010.[167] However, the greatest increase in GBS disease occurred in people aged ≥75 years.[167] The study of laboratory confirmed bacterial meningitis cases in England from 2004 to 2011 also reported an increase in neonatal (aged <3months) bacterial meningitis of 7.4% per year caused mostly by an increase in GBS meningitis, and an overall increase in the incidence of GBS meningitis at all ages from 0.05/100 000 to 0.08/100 000.[104] A study reporting population-based surveillance for invasive GBS infections in some

European countries including the UK, Australia and Canada, found an increase in incidence from 2000-2010 driven by increased rates in older people aged >60 years, but in contrast to the English study there was no significant changes in rates for neonates.[166]

## 1.7. The epidemiology of aseptic meningitis

### *1.7.1. Causes of aseptic meningitis*

Most childhood meningitis in developed countries is currently aseptic and presumed to be caused by viruses.[12,111,128,149,168,169] Aseptic meningitis has been previously defined as a raised CSF white blood cell count (WBC) and absence of an identified bacterial pathogen on CSF culture or Gram stain,[49,170,171] although there is some variation in definitions with one proposed definition also including clinical evidence of acute meningitis and different levels of diagnostic certainty if there was antibiotic pre-treatment,[170] and different clinical studies also excluding meningitis with either positive blood or CSF bacterial cultures.[172,173]

Previous studies from Europe and the USA including cases between 1996-2012, have reported that between 4-19% of meningitis is bacterial (*table 1.2.*).[168,174-177] Although study methods vary, recent studies have reported that for between 30-76% of aseptic meningitis cases, no cause is identified from the results of laboratory tests, although the majority are presumed to have a viral aetiology.[172,177-179]

| Table 1.2 Studies from Europe and the USA reporting proportions of bacterial and aseptic meningitis | | | | | | | |
|---|---|---|---|---|---|---|---|
| Study design | Country | Inclusion criteria | Exclusion criteria | Years | Total participants | Percentage bacterial | Reference |
| Retrospective | Belgium | Hospitalized children,<br>CSF WBC >8/ µL,<br>Aged 1month-18 years | Purpura, sepsis, immunodeficiency, neurosurgery, Lyme meningitis, traumatic lumbar puncture, antibiotic pre-treatment* | 1996-2008 | 174 | 14.9% | *Tuerlinckx et al.*[176] |
| Retrospective | Belgium | Hospitalized children,<br>CSF WBC >5/ µL,<br>Aged 1month-15 years | Immunosuppression, Tuberculosis or Lyme meningitis, antibiotic pre-treatment* | 1999-2003 | 277 | 10.5% | *Pierart et al.*[175] |
| Retrospective | France | Consecutive hospitalized patients with aseptic or bacterial meningitis,<br>CSF WBC ≥7/µL,<br>Aged 1month-16 years | Neurosurgical disease, immunodeficiency, traumatic lumbar puncture, antibiotic pre-treatment, patients referred from other hospitals* | 2000-2004 | 155 | 5.8% | *Dubos et al.*[174] |
| Retrospective | USA | Patients presenting to emergency department with discharge code of meningitis and LP performed in emergency department,<br>CSF WBC ≥10/µL or CSF positive culture for bacterial pathogen,<br>Aged 1month-19 years | Critical illness (altered mental state, cerebral herniation, need for respiratory of blood pressure support), purpura, ventricular shunt device, recent neurosurgery, immunosuppression, other bacterial infection requiring admission, Lyme disease, antibiotic pre-treatment | 2001-2004 | 3295 | 3.7% | *Nigrovic et al.*[168] |
| Prospective | UK | Hospitalized patients with meningitis or possible meningitis defined by CSF WBC ≥20/µL <29 days, or >5/µL 29 days-15 years, or pathogen in CSF, or discharge diagnosis of meningitis,<br>Aged 0-15 years | Not stated | 2011-2012 | 70 | 18.6% | *Sadaranani et al.*[177] (pilot study) |

*These studies also excluded children with specific missing clinical information for evaluation of different clinical decision rules

The causes of aseptic meningitis are summarised in *table 1.1*. Enteroviruses are responsible for most aseptic meningitis when a pathogen is identified, with studies reporting that between 54-88% of aseptic meningitis with an identified pathogen is caused by enteroviruses.[172,178-180] Other viral causes of meningitis include parechoviruses, herpes viruses including HSV 1 and 2, VZV, cytomegalovirus, Epstein-Barr virus and human herpes virus-6 and -7 (HHV-6 and -7); respiratory viruses including the adenoviruses, influenza viruses and rhinoviruses; arboviruses, human immunodeficiency virus, mumps virus, lymphocytic choriomeningitis virus, parvovirus B19 and rotavirus.[16,48,49,73,171,181] HSV and VZV are often reported to be next most common cause of viral meningitis after enteroviruses, although previous studies have not always considered parechoviruses.[178,180] Prior to measles mumps rubella (MMR) vaccination, which was introduced in UK in 1988, mumps was a common cause of viral meningitis.[182-184] Although viral meningitis is usually self-limiting, most children are admitted to hospital and receive intravenous antibiotics while a bacterial aetiology excluded.[172,185,186]

Non-viral aetiologies of aseptic meningitis include partially treated bacterial meningitis, atypical bacterial infections including *Mycobacterium tuberculosis, Mycoplasma pneumoniae and Brucella*, atypical spirochete bacteria including *Borrelia burgdorferi* and *Leptospirosis*, fungal, parasitic and Rickettsial infections.[48,49,171,181] Non-infectious causes include drugs, collagen vascular disease, malignancy, meningitis following vaccination or infection, and other systemic disease.[48,49,171,172,178,181]

*1.7.2. The epidemiology of viral meningitis in the UK*

There are few previous studies reporting the epidemiology of viral meningitis in the UK. One recent study investigated the incidence of laboratory confirmed cases of viral meningoencephalitis reported to PHE on a voluntary basis from 2004-2013, with one third of included cases occurring in children.[180] This study found an increase in viral meningoencephalitis incidence from 0.6/100 000 in 2004 to 3.9/100 000 in 2013 at all ages in England and Wales.[180] Considering children, the reported laboratory confirmed viral meningoencephalitis incidence in 2013 was 329/100 000 in infants <3 months, and 0.8/100 000 in children 5-14 years.[180] Enteroviruses caused most cases with a known microbiological diagnosis, accounting for 92% of cases at age <3 months, and 50% of cases 1-14 years.[180] In 2013, HSV was the next most common cause in children <15 years with incidence peaking in infants <3 months at 8.9/100 000, and VZV was the third most common cause.[180] The proportion of cases caused by HSV and VZV increased with age.[180]

An earlier study in the Oxfordshire region, in which 2233 consecutive CSF samples from adults and children were prospectively tested for viral PCRs (EV, HSV, EBV, VZV), found that 6.6% of samples were PCR+, with enteroviruses accounting for 54% of positive results, and HSV or VZV accounting for 31% of positive results.[20] A retrospective study including births in the Oxfordshire area from 1979-1999 reported that both male sex and low birth weight were associated with enteroviral meningitis.[92]

*1.7.3. The epidemiology of viral meningitis in other countries*

Several studies have reported hospital admission rates for viral meningitis in the USA using International Classification of Disease (ICD) coding.[186-188] One study found that from

1988-99 at all ages, 50% of meningitis associated hospital admissions were caused by viral meningitis.[187] This study reported admission rates in infants <1 year of 213/100 000 for viral and 61/100 000 for unspecified meningitis, in children aged 1-4 years of 14/ 100 000 for viral and 7/ 100 000 for unspecified meningitis, and in children aged 5-19 years of 14/ 100 000 for viral and 2/ 100 000 for unspecified meningitis.[187] Another USA study reported that in adults and children from 1993-2008, 91% of emergency department visits for meningitis were for viral or unspecified meningitis.[188] This study also reported 31 meningitis visits to the emergency department per 100 000 population in children <18 years with no change in the number of visits during 1993-2008.[188] A further recent study included children presenting to the emergency department at 41 USA paediatric centres with viral meningitis defined by ICD codes and discharge diagnoses, and required performance of a lumbar puncture.[186] This study found a reduction in emergency department visits from 2005 (0.98 cases per 1000 visits) to 2011 (0.25 cases per 1000 visits).[186]

Studies from Europe have also reported viral meningitis incidence from hospitalisation data.[183,189,190] A Finnish birth cohort study including 12000 children born in 1966 reported a viral meningitis incidence of 219/100 000 per year in infants, and 28/100 000 children aged <14 years using hospital admissions and discharge data.[183] A population based cohort study in Denmark from 1977-2001 reported peaks in hospitalisation rates for viral meningitis based on ICD coding of 59 children per 100 000/year after birth, 39/100 000 at age <6 months, 16/100 000 in five-year old children, and between approximately 4 and 16 per 100 000 in children aged 1-14 years.[189] The cohort study from Denmark also found an overall reduction in viral meningitis hospitalisations for children

from 1977 to 2011 and peaks in disease incidence every 3 to 5 years, and higher hospitalisation rates were associated with males compared with females, living in urban areas compared with rural areas, and living in a household with more children.[189] A study in one tertiary medical centre in Greece estimated the incidence of aseptic meningitis in children from discharge diagnoses at 17/100 000.[190]

*1.7.4. The epidemiology of enteroviral meningitis in the UK and other countries*

Most enteroviral infections occur in young children aged <5 years, with the highest incidence in infants.[67,191] Enteroviral infections exhibit seasonality with more cases occurring in the summer and autumn in temperate climates.[171,172,180,191] Enteroviral infections are common, demonstrated in a recent study which reported that in children aged 2-36 months with fever without a focus, 16% were enterovirus positive by nasopharyngeal or blood sample.[192]

Enteroviral infections can be either endemic or epidemic, for example epidemics of aseptic meningitis have occurred caused by echoviruses 30, 13, 6, 9 and coxsackie B5,[49,54] including outbreaks of meningitis caused by echovirus 30 in Europe in recent years.[191,193-195] Other enteroviruses cause endemic disease, for example coxsackievirus B3 has caused endemic disease in the USA for several years.[49,54] Epidemics of enterovirus 71 have occurred during the past 20 years in several countries including in Asia, Europe and the Pacific, although endemic disease also occurs.[49,54]

Most enteroviral surveillance programmes worldwide rely on notifications or samples being submitted to surveillance laboratories, which has limitations because molecular

diagnostic techniques have vastly improved over time and may not be consistent between laboratories, not all specimens are sent to laboratories that perform typing, notifications are often voluntary, and clinical information about cases is often not available.[53,66,67,191,196] Therefore it is likely that many cases are missed and results indicating predominant strains may be inaccurate.For example in a UK study from 2000-2011, only 28% of samples were typed,[67] and in a study from France only 69% were fully typed.[66] Therefore it is likely that the actual incidence of EV infection is much higher than is reported by surveillance and laboratory confirmed case studies.[52,54]

A study of microbiological surveillance data including confirmed CSF cultures reported to Public Health England from 1975 to 1994 identified EV positive CSF cultures in 2/100 000 infants aged <1 year.[197] At all ages, 70% of CSF enteroviral serotypes cultured were EV-A9, and echoviruses 7, 9, 11, 19, and 30.[197] This study found a decrease in all EV infection reports from 3153 reports in 1975 to 777 reports in 1994 at all ages.[197]

A recent study of all enteroviral infections, including CSF, respiratory, stool and blood samples, reported voluntarily to Public Health England from 2000-2011 at all ages, found an overall incidence of 7/100 000 in children <15 years, and 1.4/100 000 in adults.[67] An increase in EV disease was seen in 2000-2001, with 10.6 reported cases per 100 000 children <15 years in 2001, decreasing to 3.1/100 000 children in 2006, and then gradually increasing to 11.5/100 000 children in 2011, with a much higher rate reported in infants of 238/100 000 in 2011.[67] The 2000-2001 peak was also reported by other studies of laboratory confirmed viral meningitis cases in England and Wales and was caused by echovirus 13 and 30 outbreaks.[195,198,199] In the PHE surveillance study from

2000-2011, of the 28% of samples with typing performed, 55% were echoviruses (most commonly echovirus 18, 30, 11, 9, 25, and 71), 23% coxsackie B (most commonly B2, B3, B4), 5% were coxsackie A viruses, 0.9% EV 70 and 0.6% EV71 (0.6%). [67]

A study in Edinburgh from 2005-2010 reported that the most common enteroviruses isolated from CSF at all ages were echovirus 9, 6, 30 and coxsackie virus A9.[200] Consistent with previous studies which have reported that echovirus 11 and coxsackie B viruses often cause disease in neonates,[66] in the Edinburgh study, coxsackie B viruses and echoviruses 11, 16 and 25 were associated with neonatal CSF infection.[200] A further study by the same group in Scotland, reported that of 336 CSF samples collected during the whole period 2005-2012, the most frequently isolates EV types at all ages were E9, CAV9, CBV5, E6, E11 and E30.[201] The study also found that in CSF samples of young children, coxsackie A viruses and EV71 were detected occasionally [201]

A surveillance study was recently completed in the UK and Ireland from June 2014-June 2015, which identified 710 neonates <90 days with EV or parechoviral meningitis, of which 95% were EV.[202]

Enteroviral surveillance studies from other European countries have also been reported.[63,64,66,196] A surveillance study from the Netherlands from 1996-2011 reported an increase in the percentage of stool samples that were EV positive from 6.5% in 2007 to 10.8% to 2011, and parechovirus positive from 0.3% in 2007 to 2.5% in 2011.[196] Similar to other studies from Europe and the USA,[66,191] the most frequent EV types isolated were echoviruses 11, 6, 30, 7, and 13, and coxsackieviruses B4 and B5.[196] In a French

surveillance study from 2000-2004, the most frequent EV types found in CSF, stool, respiratory and blood samples were echoviruses 30, 13, 6, 11, 9, and 7, and coxsackieviruses B5, B4, B1, and B2, with 79% of all samples from children <15 years.[66] Similar to the UK,[67,195,198,199] this study also showed a 2000 outbreak of echoviruses 30, 13 and 6.[66] A small study from Barcelona reported that the most commonly isolated enterovirus in neonates was echovirus 5, followed by echoviruses 11, 21, 25 and coxsackievirus B4, with coxsackievirus B4 more likely to cause meningitis.[64] A small study in Bonn, Germany found that of 327 CSF samples in children, 4.3% were enterovirus positive, 0.6% were parechovirus positive, and all typed EV samples were species B.[63]

There are two national passive enterovirus surveillance systems in the USA.[203] The National Enterovirus Surveillance System (NESS) has monitored trends in enteroviral and parechoviral infections since 1961 including some clinical information, using data provided on a voluntary basis from participating laboratories.[203] The National Respiratory and Enteric Virus Surveillance System (NREVSS) has also collected reports of enteroviral infections from participating laboratories in the USA since 2007, without clinical data.[203] From 1970 to 2005, between 11 and 27 laboratories in the USA reported an average of 1467 infections per year to NESS, of which approximately half were CSF samples.[191,204] During 1970 to 2005, at all ages, the most common EV types isolated were echoviruses 9, 11, 30, 6 and coxsackie B5, with 44% of reports occurring in infants.[191,204] Some parechovirus infections were reported, with the vast majority occurring in infants.[191,204] During 2009-2013, 2724 EV specimens (32% CSF) were reported to NESS with coxsackievirus A6 and parechovirus 3 most commonly detected, and more than half of all

specimens from children <5 years.[205] The NREVSS reported 20 364 positive enteroviral specimens between 2009-2013 from 93 participating laboratories.[205]

An outbreak of enterovirus D68 disease causing severe respiratory disease and some cases of acute flaccid paralysis started in the USA during 2014 and then spread worldwide including Canada, Europe and Asia.[53,206] Further cases have been reported in Europe in 2016.[207,208] During the 2014 D68 outbreak in the USA, 1% mortality was reported, and many children required admission to intensive care units.[206] The increase in D68 was considered to be a true increase in disease, not an increase in detected cases by improved molecular diagnostics.[53] A recent outbreak of EV-A71 disease has also been described in 2016 in Catalonia, Spain, causing brainstem encephalitis and aseptic meningitis in children.[209]

Some recent studies suggest an increase or high frequency of enteroviral and parechoviral infections particularly in young infants.[53,200,210] A true increase in disease could be caused by changes in the biological properties of viruses, or changes in host immunity, for example a study from Finland showed an increase in the proportion of pregnant women without antibodies to coxsackievirus B4, suggesting that newborn infants would lack those antibodies.[200,210] Interestingly, maternal enteroviral infection during pregnancy has been associated with future risk of developing type 1 diabetes in children.[211]

Hand foot and mouth disease (HFMD), typically due to EV71 and coxsackie A16, usually causes a mild flu-like illnesses, although in some cases aseptic meningitis, encephalitis

and paralysis can occur.[212] EV71 caused disease with neurological complications in Eastern Europe during the 1970s.[213] More recently, large outbreaks have been recorded in Asia and the Western Pacific since the late 1990s including in Malaysia, Taiwan , Singapore, China, Hong Kong, Japan, Republic of Korea, Vietnam and Cambodia, although the disease also occurs globally including in North America, Europe and Africa.[53,214]

In China, between 2008-2012, there were more than 10 million cases of HFMD causing 3000 deaths at all ages.[215] [212] From 2010-12 surveillance data from China indicated an incidence of 1.2/1000 people per year at all ages, and 38/1000 at ages 12-23 months, with most cases occurring between ages 6 months and five years.[214,215] The overall CFR was 0.03%, and cardiopulmonary or neurological complications occurred in 1.1% with young children most susceptible to complications.[214,215] EV-A71 caused most cases including most severe cases, followed by coxsackie A16.[214,215] Disease peaked in spring and early summer, with a second autumn peak.[215]

*1.7.5. Parechoviral meningitis*

Parechoviral infections often presents with a sepsis-like syndrome in young infants, frequently aged <3 months. [200,201,216,217] Disease peaks in spring and summer, with some studies demonstrating biennial peaks in reported parechoviral infections.[200,201,216,217] Parechovirus type 3 causes the vast majority of CNS disease,[200,201,216,217] whereas parechovirus types 1 and 2 more commonly cause mild gastroenteritis in children.[217]

There remain few studies reporting the incidence of parechovirus meningitis. The two studies from Edinburgh of enteroviral and parechoviral infections from 2005-2012 in

adults and children, reported that parechovirus was only isolated from CSF of young infants aged <3 months, and found a marked biennial pattern with peaks in even numbered years (2006, 2008, 2010, 2012) compared with enteroviral infections which occurred throughout all years.[73,200] Of 68 CSF parechoviral positive samples, 66 were type 3.[200,201] A further retrospective study across three London hospitals described clinical features and outcomes of fifty infants who had parechoviral infections between 2008-2012.[217]

An outbreak of parechovirus type 3 infection in 118 infants was described in New South Wales, Australia from 2013-14.[74] A study of parechovirus serum seropositivity in adults and children in the Netherlands and Finland reported that almost all adults were seropositive for parechovirus types 1 and 2, and that in Finnish cohort seropositivity for parechovirus type 1 at age one year was 27%, and at age five years was 83%, and seropositivity for parechovirus type 2 at age one year was 56%, and at age five years was 91%.[218] However, seropositivity for parechovirus type 3 in children from both the Finnish and Dutch cohorts remained low (<3%).[218] Another study in Finland reported that by age two years, parechovirus was detected at least once in stool samples for 39% of included children, with type 1 most common (93%), and shedding lasting up to 93 days.[219]

*1.7.6. HSV meningoencephalitis*

Laboratory surveillance data for England and Wales from 2004 to 2013, reported 156 laboratory confirmed cases of HSV-1 or 2 meningoencephalitis in children <15 years overall in England and Wales, with incidence highest incidence in infants <3 months.[180] HSV disease demonstrates a bimodal age distribution, with disease incidence peaking in

infants and older people ≥60 years.[59,180] HSV meningoencephalitis has associated high morbidity and mortality, with up to 30% mortality in treated cases, and 70% mortality if untreated.[59] Typically, meningoencephalitis causes pathology in the fronto-temporal lobes, and can present with focal neurological findings and personality change.[21,181] EEG studies typically show periodic sharp and slow wave complexes in the fronto-temporal regions.[59] HSV infection may also present as myelitis.[181]

Neonatal HSV infections are usually acquired from the mother during delivery.[59] The three typical disease patterns described are skin, eye, mouth disease (accounting for approximately 80% of cases), disseminated infection and isolated CNS disease.[59] Recurrent lymphocytic meningitis, termed 'Mollaret's meningitis', is usually attributed to HSV-2 infection, but occurs rarely in children.[59]

*1.7.7. VZV meningoencephalitis*

VZV was the third most common cause of viral meningoencephalitis in children in the laboratory study from 2004-2013 in England and Wales.[180] Neurological manifestations of VZV infection in children include acute cerebellar ataxia, aseptic meningitis or meningoencephalitis, Guillain Barre syndrome (which may cause cranial nerve palsies), isolated seizures, and rarely myelitis, optic neuritis, Reye's syndrome and stroke.[59,220] Acute cerebellar ataxia, which may be an immune-mediated phenomenon, is reported to occur in approximately 1/4000 cases of VZV infection, and typically causes gait ataxia, tremor, vomiting, headache, and sometimes CSF pleocytosis, approximately one week following the onset of rash.[59] VZV pathology may include a cerebral vasculitis, which rarely can cause stroke.[59,181]

*1.7.8. Mumps meningitis*

Mumps meningitis is estimated to occur in 10-30% of mumps infections, and is more common than mumps encephalitis.[171,181] Before routine MMR vaccination was introduced in the UK in 1988, mumps was probably the most frequent cause of viral meningitis in the UK, with peaks in disease occurring every 2-3 years.[171,182-184] A UK study from 1993 assessed the risk of aseptic meningitis following MMR vaccination, which typically occurs 2-4 weeks following vaccination, at around 1 per 11 000 doses.[221]

*1.7.9. Other viral meningitis*

Aseptic meningitis is occasionally caused by HHV-6, HHV-7, EBV and CMV.[16] HHV-6 and 7 may be associated with febrile seizures and encephalitis.[16,222] However, HHV-6 has also been reported to persist in CSF of children without symptoms.[223] Meningoencephalitis caused by herpesviruses including VZV, EBV, CMV, HHV-6 may be associated with immunocompromise.[181] Lymphocytic choriomeningitis virus (LCMV) causes meningitis which can be also associated with pharyngitis and myalgia, is usually transmitted by house mice, and peaks in autumn.[181]

*1.7.10. Arboviral meningitis*

Arboviruses are transmitted by vectors such as mosquito or ticks.[224] Arbovirus infection is usually asymptomatic or causes a mild febrile illness, but can cause severe invasive disease typically with meningitis, encephalitis or acute flaccid paralysis.[224] In North America, mosquito transmitted viruses are more common, for example West Nile virus and St Louis virus, whereas disease spread by ticks is more frequent in Europe and

Asia.[181] Japanese encephalitis, transmitted by mosquitos, is endemic in Southeast Asia.[181] In the USA, West Nile Virus (mosquito-borne) was the most common arbovirus infection in 2014, occurring more commonly in adults than children.[224] West Nile virus is also endemic in Asia, Europe and Africa, but not the UK.[181]

*1.7.11. Aseptic meningitis caused by atypical organisms*

Meningitis caused by *Mycobacterium tuberculosis* usually presents as a subacute illness with non-specific symptoms, and hydrocephalus is a common complication.[225] In one South African study *M. tuberculosis* was the most commonly reported cause of meningitis.[226] *Mycoplasma pneumoniae* can cause neurological manifestations.[227] One recent study reported neurological symptoms in 22/89 children in hospital with *M. pneumoniae* infections, with encephalitis specified more frequently than meningitis.[228] *Brucella* species are zoonosis, which can cause neurological manifestations including aseptic meningitis and encephalitis, and should be considered in children living in endemic areas.[229]

Spirochetes causing meningitis include *Leptospira and Borrelia burgdorferi.* [230,231175,233] *Leptospira* are also zoonosis, and require consideration in endemic counties.[230,231] In a recent study from Laos, *Leptospira* caused 12% of bacterial or fungal meningitis or meningoencephalitis, and were sometimes associated with peripheral neurological abnormalities.[230,231] *Borrelia burgdorferi*, transmitted by ticks in North America and Western Europe, causes Lyme disease and can cause neurological manifestations including aseptic meningitis and peripheral facial nerve palsy.[179,232]

Fungal meningitis usually occurs in immunocompromised children, and causes include *Candida albicans*[104] and *Cryptococcus neoformans*.[233] Parasitic causes of meningitis include *Toxoplasma gondii, helminths* and malaria.[233] *Rickettsia* species should also be considered in endemic regions, for example, in a recent study from Laos, *Rickettsia* were identified as a frequent cause of meningitis or encephalitis, and were associated with a longer period of fever compared with other bacterial meningitis.[230,231]

### 1.8. Vaccines for meningitis

Conjugate vaccines are immunogenic in young children aged <2 years.[169,234] A conjugate vaccine was introduced into the routine infant vaccination schedule with a catch-up programme in the UK in October 1992 for *Haemophilus influenzae* type b.[12,107,159,169,235] A capsular group C meningococcal conjugate vaccine was introduced in September 1999 into the UK infant vaccination schedule with a catch up campaign following an increase in MenC disease.[110,128,169] A 7-valent pneumococcal conjugate vaccine, PCV 7, was introduced in September 2006 at 2, 4 and 13 months also with a catch up programme, and was subsequently replaced by the 13-valent vaccine, PCV 13, from April 2010.[149,169,236]

The first broadly protective vaccine against capsular group B meningococcal disease (4CMenB), based on subcapsular proteins which are not capsular group specific, was introduced into the routine infant UK vaccine schedule in September 2015 for infants born from July, with a catch-up programme for infants born from May.[237,238] Another bivalent MenB vaccine, based on recombinant protein antigens, was licenced in the USA from October 2014 for young people aged 10-25 years, and was also licenced in Europe

from March 2017 for people aged >10 years.[239-241] Development of a vaccine against capsular group B was delayed because its polysaccharide capsule is immunologically identical to neural-cell adhesion molecules, causing immunological tolerance.[242] Outer membrane vesicle vaccines were previously used to control strain specific epidemics of MenB disease in some parts of world, and the New Zealand strain specific OMV vaccine is included in the 4CMenB vaccine.[237,243]

Following the recent introduction of infant 4CMenB vaccination, recent UK data presented in poster form at the European Society of Paediatric Infectious Diseases meeting in 2017 indicated that for a cohort of children aged one year, there were six cases of MenB in children eligible for 4CMenB vaccination compared with 12 cases for non-eligible children, and compared with an average of 18 cases per year in the eligible cohort during four previous years.[130] An earlier published study also reported that for eligible children following vaccine implementation from September 2015-June 2016, there were 37 MenB cases (21 of whom had received a single dose, 10 two doses) compared with an average of 74 cases per year for same cohort during the previous four years.[131]

A conjugate MenACWY vaccine was also introduced in the UK in August 2015 for adolescents aged 13-18 years, with a catch-up campaign for university leavers in response to a year-on-year increase in MenW disease.[132,238] [132,134] This replaced MenC conjugate vaccine that was previously given at age 13-14 years, and to university students.[134] One study has reported reduced MenW cases in the eligible age group.[132]

Routine MMR vaccination, a live attenuated vaccine, was introduced in the UK in 1988 protecting against mumps and measles, which can both be associated with meningitis.[182] Polio vaccination was introduced in 1955, and the current routine childhood vaccine schedule includes inactivate polio vaccine given as part of a combination vaccination.[244] Live-attenuated VZV vaccines are available as either a single antigen varicella vaccination or a combined MMRV vaccination, but are not part of the routine UK schedule.[245]

Due to high rates of EV-71 disease in China, two inactivated enterovirus-71 whole cell vaccines were licenced in China in December 2015, and a third vaccine has completed phase 3 clinical trials.[214,246-249] However, these vaccines do not confer cross-protection across other enteroviruses and many HFMD cases are not caused by enterovirus-71.[214,246-248] There is ongoing research into multivalent or combination vaccines to protect against other enterovirus types.[214,246-248]

Vaccines are also available to protect against Japanese encephalitis, tick-borne encephalitis, rabies and influenza.[181] Clinical trials are ongoing for Group B Streptococcus vaccination in pregnant women, for both a capsular polysaccharide conjugate vaccine and a protein vaccine,[250] and HSV vaccine candidates are in development.[251]

## 1.9. Clinical and laboratory features of meningitis

### *1.9.1. Clinical features of meningitis*

Typical clinical features of bacterial meningitis include headache, fever, photophobia, nausea, vomiting, rash, lethargy, irritability, altered mental state or impaired consciousness.[44,100,252] Purpuric rash usually suggests meningococcaemia.[100,252,253] Infants may present with less specific findings including irritability, poor feeding, fever, lethargy, hypothermia, vomiting, diarrhoea, respiratory symptoms, seizures, and bulging fontanelle.[44,105,172,254] A retrospective study including 802 children in Canada with aseptic meningitis from 1998-1999 found that headache, nausea, vomiting, meningism or photophobia occurred in 99% of children ≥5years, compared with 55% <5years, but non-specific symptoms including rash, diarrhoea and cough occurred more frequently in younger children compared with older children.[172] Due to non-specific clinical findings in young infants with meningitis, a lumbar puncture should usually be performed in this age group if there are signs of sepsis.[100,105,254,255] A large Australasian study reported that 8% of neonates who had a LP as part of an evaluation for sepsis, had meningitis.[255]

Notably, concerns have previously been discussed in the UK about the safety of performing LPs particularly in meningococcal disease.[256-258]A recent study in adults highlighted delays in LPs being performed for adults with suspected CNS infections, contributed to both unnecessary antibiotics for some cases, and delayed management for others.[259]

Signs of meningeal irritation occur more commonly in older children including nuchal rigidity, Kernig's sign, which is positive if hip flexion followed by knee extension causes

back pain, and Brudzunski's sign, which is described as positive if passive neck flexion results in hip flexion.[44] A USA study published in 1992 including 172 children indicated that at least one meningeal sign was present in 72% of bacterial and 17% of aseptic meningitis in infants, and 93% of bacterial and 85% of aseptic meningitis in children aged >1 year.[260] However, another study including 326 children presenting with meningeal signs to an emergency department in the Netherlands between 1988 and 1998 found that only 30% of children with meningeal signs had bacterial meningitis, and 13% had aseptic meningitis.[261]

Complications of bacterial meningitis include brain cortical necrosis, vasculitis or venous thrombosis which may cause focal neurological signs, raised intracranial pressure, hydrocephalus, seizures, subdural effusions, inflammation of the cochlear duct or auditory nerve, joint involvement (typically in meningococcal or Hib meningitis), and adrenal haemorrhage (typically in meningococcaemia); and complications relating to sepsis including circulatory shock, limb gangrene, syndrome of inappropriate ADH and disseminated intravascular coagulation.[44,100,253]

Viral meningitis may also present with headache, fever, photophobia, neck stiffness, nausea and vomiting, although often less specific signs are seen in young infants like fever and irritability.[171,252] Seizures, signs of raised intracranial pressure, focal neurology and abnormal mental state can also occur.[262-264] Enteroviral meningitis may be associated with a generalised maculopapular rash, or localised vesicular rash, for example in HFMD.[171]

*1.9.2. Clinical features distinguishing bacterial and viral meningitis*

There are few data defining clinical features and laboratory parameters of viral and aseptic meningitis at presentation to hospital, or how these compare with bacterial meningitis.[265-267] A retrospective study from Belgium including 21 children with bacterial and 71 children with aseptic meningitis reported more seizures, petechiae, higher blood CRP and WBC count, lower platelets, higher CSF lactate, WBC count, neutrophils and protein, and lower CSF glucose in bacterial compared with aseptic meningitis, whereas clinical findings of headaches, neck stiffness, nausea and vomiting were more common in aseptic meningitis.[268] A USA study published in 1992 including 52 children with bacterial and 119 children with aseptic meningitis recruited prospectively reported that toxic appearance and lethargy or coma were more frequent in bacterial compared with aseptic meningitis, in infants all meningeal signs and a bulging fontanelle were more frequent in bacterial than aseptic meningitis, and in children aged >12 months positive Brudzunski's and Kernig's signs (but not nuchal rigidity) were more frequent in bacterial than aseptic meningitis.[260] A prospective UK study across three hospital from 2011-12, including 70 children with meningitis, reported that infants aged <3 months with bacterial meningitis compared with aseptic meningitis presented with more respiratory distress and reduced GCS, but there were no significant differences in children aged ≥3 months.[177]

*1.9.3. Clinical features of meningitis caused by different viral aetiologies*

Few studies have compared clinical features of meningitis with different viral aetiologies.[76,263,269] A retrospective study from the USA compared clinical features of 388 infants with enterovirus (n=54), parechovirus (n=66), or neither in their CSF.[76] Infants with CSF parechovirus infection, all of whom were aged <6 months, compared with CSF

enteroviral infection had lower peripheral WBC and lymphocyte counts, higher maximum temperatures, longer duration of fever and longer duration of hospitalisation.[76] Eight infants with parechoviral meningitis required ICU care compared with one infant with enteroviral meningitis.[76] Notably, only 2% of infants with parechoviral meningitis had a raised CSF WBC count compared with 38% of infants with EV meningitis.[76] A small study from Spain including young infants aged <1 month, reported that 4 out of 9 infants with parechoviral infection compared with 3 out of 32 enteroviral infection were admitted to the ICU, and that more infants with EV infection had a raised CSF WBC and CSF protein compared with parechoviral infection.[269]

A retrospective study from Canada including 117 adults and children compared with signs of meningitis in participants who were CSF enterovirus PCR positive (EV+) to those who were CSF enterovirus negative, and reported more history of fever, photophobia and shorter onset of illness for EV+ compared with EV- patients, but found no difference in CSF parameters.[263]

Parechoviral infections in young infants commonly present with a sepsis-like illness, with previously described features including fever, poor feeding, and neurological symptoms for example lethargy, irritability and seizures.[74,217] A retrospective study at three London hospitals between 2008-2012 included fifty infants with parechoviral infections, 75% of which were detected meningitis.[217] This study reported that half of infants required admission to the ICU, and 19/50 infants required ventilator support.[217] Infants with parechovirus detected in blood only were more likely to be admitted to ICU, but only those with parechovirus detected in CSF had seizures.[217] In the retrospective study of

118 infants with parechoviral infection during an outbreak in Australia in 2013, in which parechovirus was detected in CSF in 75 cases, clinical features frequently reported were tachycardia, fever, irritability, tachypnoea, erythematous rash, poor feeding and poor perfusion, and 63% of infants required a fluid bolus and 25% required admission to the ICU.[74]

*1.9.4. CSF findings in meningitis*

UK National Institute for Health and Care Excellence (NICE) guidance on bacterial meningitis and meningococcal septicaemia suggests the following normal values for CSF in children: CSF appearance clear and colourless, opening pressure 10-100mm$H_2O$ (aged <8 years) and 60-200mm$H_2O$ (aged >8 years), total protein 0.15-0.45 g/L, glucose concentration approximately 60% plasma of plasma concentration (2.78-4.44 mmol/L), and CSF WBC count <21 cells/μL for neonates <28 days, and <6/μL in older infants and children.[252] A normal WBC cut-off in neonates <2months of <15 cells/μL has also been used previously.[170,270]

Studies have indicated that normal CSF WBC and protein levels are higher in neonates compared with older infants and children (*table 1.3.*), but the normal range for CSF glucose or CSF:serum glucose ratio is similar in neonates, older infants and children.[270-273] A recent study from the USA reported LP results in more than 1000 infants who had an LP to rule out meningitis, and who did not have a bacterial infection or positive CSF viral PCR.[272] This study reported mean CSF WBC counts of 8.63/μL at age <8 days (90$^{th}$ percentile 26/μL), 4.33/μL (90$^{th}$ centile 8/μL) at age 4 weeks, with a reduction to 2.22/μL (90$^{th}$ centile 7/μL) at 8 weeks.[272] Mean CSF protein was 1.06g/L at age <8 days, 0.62g/L at

age 4 weeks, and 0.54g/L at 8 weeks.[272] For CSF glucose, in 258 infants aged 0-8 weeks, mean glucose was 2.6mmol/L.[272] Another recent study including 380 infants without an illness which would cause CSF pleocytosis, reported a CSF WBC 95$^{th}$ percentile in infants aged ≤28 days of 19/μL (median 3/μL, mean 9.2/μL), and in infants aged 29 to 56 days of 9/μL (median 2/μL, mean 3.1/ μL).[273] It has been generally considered that higher CSF WBC and protein levels occur in neonates because the blood brain barrier is immature and more permeable to proteins.[271,274] In contrast, one study proposed some evidence that the normal CSF WBC for neonates is the same as at other ages and should be <5/μL.[274]

Older studies have also been used to understand normal CSF findings in neonates, although they were performed before routine PCR use, which may mean that some cases were undiagnosed viral meningitis.[270,271] In a study published in 1996, which reported CSF results in 108 neonates without meningitis, the mean protein was 0.808 g/L at age <1 week, 0.69g/L at age <2 weeks, and 0.642 ±0.242 g/L overall at age ≤30 days.[270] The mean WBC was 7.3/μL at age ≤30 days, but 15.3/μL in the first week.[270] A 1992 study also reported CSF results in 75 infants without meningitis and found a mean CSF WBC count of 11/μL at 0-4 weeks and 7.1/μL at 4-8 weeks, and mean CSF protein of 0.84g/L at 0-4 weeks and 0.59g/L at 4-8 weeks.[271]

| Table 1.3 Studies reporting CSF WBC counts and protein in neonates without meningitis | | | | |
|---|---|---|---|---|
| Country, Years | Age <1 week | Age <2 weeks | Age 4 weeks | Age 8 weeks |
| CSF WBC (/µL), mean (upper centile), n | | | | |
| USA, 1995-6, 2001-2, and 2008-9[272] | 8.63/µL (90th 26/µL), n=118 | | 4.33/µL (90th 8/µL), n=150 | 2.22/µL (90th 7/µL), n=36 |
| USA, 2005-2007[273] | 9.2/µL, (95th 19/ µL), aged <29 days, n=142 | | | mean 3.1/µL (95th 9/µL), aged 29-56 days, n=238 |
| USA, 1993-4[270] | 15.3/µL, n=17 | | | |
| | 7.3/µL, aged ≤30 days, n=108 | | | |
| USA, published 1992[271] | 11/µL, aged 0-4 weeks, n=35 | | | 7.1/µL, aged 4-8 weeks, n=40 |
| CSF Protein (g/L), mean, n | | | | |
| USA, 2001-2 and 2008-9[272] | 1.06g/L, n=54 | | 0.62g/L, n=74 | 0.54g/L, n=28 |
| USA, 1993-4[270] | 0.81 g/L, n=17 | 0.69g/L, n=33 | | |
| | 0.64g/L, aged ≤30 days, n=108 | | | |
| USA, published 1992[271] | 0.84g/L, aged 0-4 weeks, n=35 | | | 0.59g/L, aged 4-8 weeks, n=40 |

Studies have also shown that bacterial meningitis can occur in neonates without a CSF WBC above an accepted normal cut-off, suggesting the CSF WBC is less sensitive in neonates than in older infants and children, with two different studies reporting that 13% and 17% of neonates with bacterial meningitis to not have a CSF WBC>22/µL.[271,275] The finding of enterovirus in the CSF, particularly of young infants, in the absence of CSF pleocytosis is well described.[276-280]

Typically reported CSF findings for meningitis caused by different organisms are reported in *table 1.4*.[44,252]

| **Table 1.4 Typical CSF findings in meningitis caused by different organisms** *(references [44,252])* | | | | |
|---|---|---|---|---|
| Meningitis aetiology | Opening pressure | CSF WBC | CSF:plasma glucose ratio | CSF protein |
| Bacterial meningitis | raised | >1000/µL | <0.6 | >1g/L |
| Viral meningitis | normal | 5-500/µL | ≥0.6 | 0.5-1g/L |
| *M. tuberculosis* meningitis | raised | 100-500/µL | <0.6 | >1g/L |

In clinical practise, CSF WBC is sometimes adjusted for the presence of red blood cells if a traumatic tap is obtained, because peripheral WBCs will be contained in blood which have not come from CSF. Often an adjustment of 1 leukocyte to 500 RBC is made, based on typical WBC:RBC ratios in peripheral blood.[281] One study found this adjustment inaccurate.[281] Other studies have suggested making adjustment in traumatic taps by calculating an observed: predicted CSF WBC ratio based on the peripheral WBC (CSF WBC (predicted) = CSF RBC X(blood WBC/blood RBC)).[282] NICE guidance suggest that use of the unadjusted CSF WBC is safest.[252]

Although several studies document that CSF WBC and CSF protein are significantly higher in bacterial compared with viral meningitis, and some studies also report that CSF glucose is lower in bacterial compared with aseptic meningitis, no consistent cut-off levels have been identified that accurately differentiate bacterial and aseptic meningitis.[283-287] One study reported that an elevated CSF protein was not sensitive nor specific for a CSF EV+ result at all ages.[277] Another study reported significant differences in CSF lactate levels in viral and bacterial meningitis, although this is not routinely tested.[268]

Usually aseptic meningitis is reported to be associated with CSF lymphocytosis, and bacterial meningitis with a predominance of polymorphonuclear cells (PMNs).[181,285] It is frequently suggested that PMNs may predominate early in aseptic meningitis, for example in the first 24 hours,[35] however one study has also reported that PMNs may persist beyond the first 24 hours, suggesting PMN or mononuclear predominance may not discriminate well between aseptic and bacterial meningitis.[285] A recent study

reported CSF neutrophil to lymphocyte ratio as a predictor of bacterial or viral meningitis, which performed better for people aged >14 years compared with children.[288]

It has been widely reported that EV meningitis or EV infection of the CNS in the absence of a raised CSF WBC count occurs most commonly in young infants aged <1-3 months.[172,276-280] Other factors associated with absence of a raised CSF WBC count in EV infection of the CNS are not well described, although limited studies have found an association with a lower peripheral WBC count, and earlier lumbar puncture.[278,280] It has been suggested that lack of pleocytosis in young infants may be due to immunological immaturity, for example due to a poorly developed chemokine response for leukocyte recruitment.[277]

Studies have also demonstrated that infants who have parechovirus detected in their CSF, frequently do not have a raised CSF WBC count.[74,217] In a London study, 95% of CSF parechoviral positive infants had a normal CSF WBC, 79% had a normal CSF protein, and only small proportion of all included infants had raised or lowered peripheral WBC counts or a raised CRP, but clotting abnormalities occurred more frequently.[217] In an Australian study including 118 infants with parechoviral infection, 75 of whom had parechovirus detected in CSF, only four infants had a raised CSF WBC count and only six had a raised blood CRP.[74]

*1.9.5. Blood test findings in meningitis*

Studies have reported variable findings for serum white blood cell counts to predict bacterial or aseptic meningitis, but generally report that although mean blood WBC

counts are higher in bacterial than aseptic meningitis, sensitivity for bacterial meningitis is low when a blood WBC cut-off is defined.[268,283,284,286] One small study reported a significant difference in blood WBC counts, but not blood neutrophils or lymphocytes.[268] Other studies have not shown strong evidence for the predictive value of blood WBC.[283,284]

A systematic review published in 1998 of studies investigating CRP to distinguish bacterial meningitis from aseptic meningitis or other illnesses reported a high probability of not having bacterial meningitis if CRP test was negative at univariable analysis, but less evidence for the predictive probability of positive test.[289] A more recent small paediatric study also reported low CRP to be sensitive for predicting viral meningitis, but high CRP was not specific for bacterial meningitis.[268] Other studies have also reported CRP as a good predictor of bacterial compared with viral meningitis, but with better sensitivity than specificity.[283,284] A further retrospective study in 1999 found higher sensitivity and specificity for CRP than for blood WBC.[286]

Although not routinely tested, studies have investigated serum procalcitonin[283,284,290] to distinguish viral and bacterial meningitis, with a recent meta-analysis reported high sensitivity and specificity.[290]

*1.9.6. Risk factors for meningitis*

Congenital or acquired immunosuppression may predispose children to specific bacterial or viral causes of meningitis, for example terminal complement pathway defects increase risk for *N. meningitidis* infection, and hyposplenism increases risk for encapsulated

bacterial infections.[177,291] Crowding, poor living conditions, tobacco smoke exposure, lack of immunisation, concurrent respiratory tract infection, season and young age are also factors that may predispose to bacterial meningitis.[100,253,292] Direct bacterial invasion can also occur from a contagious source of infection, neurosurgical procedures or anatomical defects.[100]

**1.10 Clinical decision rules**

There have been several clinical decision rules developed which attempt to predict children presenting to hospital with bacterial meningitis.[168,293-296] The Bacterial Meningitis Score (BMS) predicts children aged at least 1 month with a raised CSF WBC count to be at 'very low risk' of bacterial meningitis compared with aseptic meningitis if they have none of the following five features: positive Gram stain, CSF protein ≥80mg/dL, peripheral neutrophil count ≥10 000 cells/μL, seizure at or before presentation, CSF neutrophil count ≥1000 cells/μL.[295]

The BMS was developed in a retrospective cohort of 696 children aged 29 days-19 years admitted to a USA hospital between 1992-2000 with a CSF WBC >7/μL, with participants initially identified by searching ICD hospital coding.[173] Of included participants, 18% had bacterial meningitis defined by a positive CSF culture, or raised CSF WBC and bacterial pathogen present in blood.[173] Patients with aseptic meningitis who were pre-treated with systemic antibiotics and children who would require admission to hospital regardless of bacterial meningitis risk were excluded.[173] The score was developed by comparing clinical and laboratory features in a univariate analysis, selected features that were continuous variables were then categorised by selecting cut-offs in receiver operating characteristic

(ROC) curves, entered into a multivariable logistic regression analysis, and then a score based rule was identified using the beta-coefficients in the logistic regression analysis.[173]

The BMS was externally validated in a multicentre retrospective study across 20 USA studies from 2001-2004, including 3295 children 29 days-19 years with a CSF WBC ≥10/μL, or positive CSF bacterial culture.[168] Children were also initially identified by searching ICD coding.[168] This study excluded children who would require admission to hospital regardless of bacterial meningitis risk, for example with critical illness, and excluded children who were pre-treated with antibiotics.[168] In this cohort, 3.7% of participants had bacterial and 96.3% had aseptic meningitis.[168] The sensitivity of the BMS for predicting bacterial meningitis was 98.3%, with a negative predictive value of 99.9% and specificity 61.5%.[168] During this study, both the Hib and PCV7 vaccines were part of immunisation programme in the USA.[168] A study published in 2007 also investigated modifying the BMS by reducing the score depending on whether more patients were admitted with aseptic meningitis on the day of presentation compared with different numbers of previous days, however the results indicated a small reduction in sensitivity for predicting bacterial meningitis with the adjusted rule.[297]

Subsequently the BMS has been evaluated in several studies, and a meta-analysis was published in 2012 including eight non-UK validation studies, most of which were retrospective.[168,173-176,294,295,298,299] For 5312 children aged >1 month included in the BMS meta-analysis, the combined sensitivity was 99.3%, negative predictive value was 99.7%, and specificity was 62.1%.[295] Overall, there were nine children who had bacterial meningitis, but were classified as 'very low risk', of whom three had purpura or petechiae

and three were aged <2 months.[295] The remaining three misclassified children had meningococcal meningitis with no petechiae or purpura and were aged >2 months.[295]

Other proposed clinical decision rules for identifying bacterial meningitis include a rule investigated in studies from the Netherlands.[296,300] The rule was derived in a retrospective cohort of 360 children aged 1 month to <15 years presenting with meningeal signs at one hospital in the Netherlands between 1988-1998.[300] A complex scoring system was developed including six clinical findings, which were duration of symptoms, vomiting, cyanosis, altered level of consciousness, meningeal irritation, petechiae and blood CRP, and CSF neutrophil count and CSF:serum glucose ratio, with different scores provided for different ranges of laboratory results.[300] Studies identified cut-off scores at which bacterial meningitis not observed including a total of 586 children, of which 360 were in the derivation study and 226 were recruited in a prospective cohort.[296]

A further previous rule called the 'meningitest' was developed in France, and suggested treating as bacterial meningitis if one of the following features were present: seizure, purpura, toxic appearance, procalcitonin ≥0.5ng/mL, positive Gram stain or CSF protein ≥50mg/dL.[294,301] The 'meningitest' was 100% sensitive in both the derivation study including 111 children, and validation study including 198 children aged 29 days to 18 years with a CSF WBC ≥7/µL, although specificity in the validation study was 36%.[301 294]

A systematic review of bacterial meningitis clinical prediction rules,[293] and a study that directly compared the BMS and 'meningitest' in 198 children,[294] reported that the BMS

had highest sensitivity and specificity.[293,294] The study directly comparing the BMS and 'meningitest' reported that although both rules were 100% sensitive, the BMS had higher specificity compared with the 'meningitest' (56% vs 36%).[294] The BMS has not been validated in the UK, and none of the previously proposed rules were investigated in the UK population.[293,294]

## 1.11. The use of PCR in meningitis diagnosis

### *1.11.1. Basic Principles of PCR*

The basic principles of PCR are well described. The PCR procedure involves several temperature cycling steps, in which double stranded DNA is denatured at a high temperature around 95°C, the temperature is then lowered to around 54-60°C to allow primers to anneal to the complementary DNA sequence, and then raised to around 72-80°C to allow synthesis of a new DNA strand with DNA polymerase.[302,303] An initial reverse-transcriptase step is required for RNA.[303]

Prior to real-time PCR (RT-PCR), DNA product was detected after amplification so quantification was limited, because response curves saturate at the same level irrespective of the original amount of target present.[303] In real-time PCR, first described by *Higuchi et al*,[304] quantity of product can be monitored during the growth phase of amplification.[303] Fluorescent reporters are used to detect DNA in real-time PCR.[305] The degree of fluorescence depends on the amount of double stranded DNA present so can be used for quantification.[303] A cycle threshold (CT) value refers to the number of cycles

required to reach any determined fluorescence threshold, and can be compared between samples within the same experiment for quantification.[303]

Non-specific probes include SYBR Green dye which becomes fluorescent when it binds any double stranded DNA.[303] Sequence specific probes are based on nucleic acids or synthetic analogues of nucleic acids.[303] Some sequence specific probes, including Taqman probes,[306] consist of an oligonucleotide probe with a fluorophore and quencher molecule attached at different ends.[303,306] When the Taqman probe is intact the quencher molecule remains in close proximity to the fluorophore and prevents the fluorophore from emitting fluorescence by fluorescence resonance energy transfer (FRET).[303,307] When the probe binds the target DNA sequence, Taq polymerase then cleaves the fluorophore from the probe releasing it from proximity to the quencher molecule causing fluorescence, which is measured by the real-time PCR instrument.[303,306304] With each cycle, more reporter dye molecules are cleaved, and the intensity of fluorescence increases.[303]

*1.11.2. Diagnosis of meningitis by PCR*

Studies have reported that PCR of CSF or blood is more sensitive than Gram stain or culture and has high specificity for diagnosis of bacterial meningitis caused by *N. meningitidis, S. pneumoniae,* and *H. influenzae*.[308,309] The majority of laboratory confirmed meningococcal infections in England in recent years were diagnosed by PCR only or PCR and culture.[310] In the 2015/16 epidemiological year, 39.8% of all meningococcal infections reported to PHE were diagnosed by PCR only, and altogether 61.6% by PCR or PCR and culture.[114]

Enterovirus reverse transcriptase-PCR has been available since the 1990s allowing rapid diagnosis with results obtainable the same day.[22,24] A recent study continues to demonstrate that EV-PCR is more sensitive than culture, reporting that only 39% of EV infections detected by PCR were also detected by culture, and reporting lower detection rates by culture compared with PCR for stool and nasopharyngeal samples.[311] EV-PCR usually targets the highly conserved 5'non-coding region of the RNA genome.[33,201]

A recent study from England and Wales showed the increase in proportion of EV infections diagnosed by PCR in adults and children in recent years, from 36% of all laboratory confirmed EV infections reported to Public Health England in 2000, to 92% in 2011.[67] A corresponding reduction in diagnoses made by culture, microscopy or serology also occurred.[67]

Many children with viral meningitis are admitted to hospital and receive intravenous (IV) antibiotics while a diagnosis of bacterial meningitis is excluded. The study of >7000 children seen in the emergency department with viral meningitis identified by ICD coding in the USA from 2005-2011, reported that 85% of children received a parenteral antibiotic and 91% were admitted to hospital.[186] In a Canadian study from 1998-99 which included 802 children ≤18 years with aseptic meningitis, almost three quarters of children also received an antibiotic.[172] Similarly, all 46 infants in a study from New South Wales, Australia with enteroviral meningoencephalitis received at least one dose of antibiotic.[312]

Many studies have assessed the impact of enteroviral PCR testing or more rapid availability of EV PCR results on length of stay (LOS), antibiotic use and hospital

costs.[22,28,185,257,279,313-319] Several studies have shown a reduction in LOS, antibiotic use or hospital costs for children who have EV-PCR performed and are EV+ compared with children who are EV-.[257,315,316,318,319] A study from the USA showed a reduction in LOS from 67 to 42 hours, and a reduction in days (3.5 days to 2 days) of antibiotics, in children diagnosed with viral meningitis who were EV+ compared with EV-.[319] Another retrospective USA study of 442 infants aged <90 days who had CSF EV-PCR performed between 2000-2006 during enteroviral season only, and did not have bacterial or HSV infection, reported that a CSF EV+ result was associated with a 1.54-day decrease in the length of stay in multivariate analysis, and a 33.7% shorter duration of antibiotic use.[317] A French study attributed reduction in LOS between two time periods when EV RT-PCR testing was available to greater physician responsiveness.[313,314] During the earlier time period of the studies, availability of earlier EV-PCR results reduced length of antibiotics by 2.3 days, but not LOS.[313] A further recently published USA study demonstrated a shorter LOS of 2.4 days for CSF-EV+ compared with 2 days for CSF-EV- young infants aged ≤60 days, and interestingly also reported that no CSF-EV+ infants ≤60 days had bacterial meningitis.[320]

Several studies have also shown reduction in LOS, hospital costs or antibiotic use in children with reduced EV-PCR test turnaround time.[22,185,279,317,321] A USA study including 441 children reported that a reduction in EV-PCR test turnaround time from 53 to 13 hours resulted in reduced LOS for EV-PCR+ children from 44 to 28 hours, and also reduced length of antibiotics.[321] The retrospective USA study including 442 infants, 154 of whom were CSF EV-PCR+ also found that increasing PCR test turnaround time to >24 hours increased LOS by 13.6% in EV+ patients.[317]

A recent multiplex-PCR assay was developed to detect fourteen bacterial, viral or fungal pathogens in CSF, with studies reporting high sensitivity and specificity.[322,323]

**1.12. Outcomes following meningitis**

Outcomes following bacterial meningitis include deafness, epilepsy, motor impairment and other neuropsychological and cognitive sequelae.[118,324-326327,328] A meta-analysis of 132 studies from 1980-2008 reported that following post-neonatal bacterial meningitis, the risk of any long term sequelae was 20% globally, and the risk of major sequelae was 13% globally but 9% in Europe.[118] Major sequelae were defined as cognitive deficit (intelligence quotient (IQ) <70), bilateral hearing loss, motor deficit, seizures, visual impairment and hydrocephalus, with hearing loss the most frequent.[118] Minor sequelae were behavioural and learning difficulties, unilateral hearing loss, diplopia and hypotonia.[118] The risk of major long-term sequelae globally following meningitis caused by *S. pneumoniae* was 25%, *H. influenzae* type B was 9.5%, and *N. meningitidis* was 7%.[118] Risk of sequelae was higher in younger children and infants compared with older children, and 20% of children had more than one sequelae.[118]

A recent UK case control study of 245 children who survived group B meningococcal disease found that 9% had a major disabling deficit at mean age 6.5 years, and approximately one third had at least one deficit in physical, cognitive or psychological function.[326] Deficits in memory and executive functioning were also common.[326] Major deficits were defined as intellectual disability (IQ <70), seizures, bilateral sensorineural hearing loss, disabling motor impairment, and significant visual loss, or communication

disability.[326] A prospective Canadian study including 419 children with invasive meningococcal disease reported complications at discharge for 21% of children, including 13% with neurological and 11% with non-neurological complications.[327] Complications included hearing loss, motor deficit, visual disturbance, seizures, amputation, skin scarring and renal dysfunction.[327] Presence of seizures, shock, bruising and low platelet counts were associated with developing complications in children.[327]

A recent USA study including 90 children who survived GBS meningitis as infants, excluding preterm infants, found that at mean age 6.8 years, 25% of children had mild to moderate impairments, and 19% had severe long term neurodevelopmental sequelae.[328] Severe impairment was defined as results of cognitive testing >2 standard deviations (SD) below the mean, or other severe neurologic or functional impairment including blindness, bilateral sensorineural hearing loss, cerebral palsy, motor deficits, or significant delays in development and learning.[328]

The Avon Cambridge Longitudinal study assessed outcomes in 31 children who had meningitis with no serious neurological sequelae.[329] Meningitis was defined by parent self-report, with no indication of whether cases were viral or bacterial.[329] The study showed some neurocognitive, education and psychological differences for meningitis exposed children compared with non-meningitis exposed children.[329] A recently published systematic review of outcomes following bacterial or viral meningitis including 39 studies, reported mean IQ scores 5.5 points lower and more developmental delays than controls in bacterial meningitis, but no significant difference in IQ or developmental delay compared with controls in viral meningitis.[330]

There are few studies, limited also by sample size, reporting outcomes following viral meningitis for children.[74,262,264,331-336] Results from these studies vary, with most literature reporting no permanent neurological sequelae,[74,262,264,330-332] but others reporting ongoing neuropsychological, motor, developmental or cognitive sequalae including language delay, particularly if meningitis occurred as a young infant.[333-336] Outcomes in children with immunodeficiencies may be worse, and can include chronic infection.[181] Mortality is rare in aseptic or enteroviral meningitis,[172] however mortality is described in enteroviral infections caused by EV71 (0.03%),[215] and in some coxsackievirus infections.[52,331] No mortality was described in a recent case series of 118 infants with parechovirus infection,[74] although one child (1/50) died in an English study.[217]

Two small studies have reported developmental delay following parechoviral meningitis.[217,336] A study in London reported that following parechoviral infection, 6/19 infants with available data had developmental delay at follow-up, including one child with cerebral palsy and visual impairment, one child with no words at age 18 months and one with generalised hypotonia.[217] A recent prospective Australian study reported developmental concerns for 5/8 children at age 12 months, who had confirmed parechoviral encephalitis as young infants.[336] However an Australian case series of 118 infants with parechovirus infection reported that only four infants had motor delay or abnormal tone at age 3-6 months.[74]

Neurological sequelae and cognitive impairment are common in survivors of HSV encephalitis, with one recent study from France suggesting complete neurological

recovery in only half of survivors.[59,337] A Canadian study reported residual neurological sequelae for 9 out of 39 children one year following hospital admission for a central nervous system complication of VZV infection.[220]

### 1.13. Aims of Thesis

Long term trends in childhood bacterial and viral meningitis in the UK have not previously been reported. The current aetiology of all-cause childhood meningitis is not well defined. Rapid identification of children who have viral meningitis and exclusion of a bacterial cause by improved diagnostics and validated clinical decision rules would decrease unnecessary hospital admissions and intravenous antibiotic management.[168,185,186,314] More knowledge is required to assist prompt identification and improve understanding of enteroviral meningitis. Health related quality of life following viral meningitis has not previously been described, and few studies report outcomes following viral meningitis. Further detailed knowledge is also required about outcomes following bacterial meningitis, as well as viral meningitis, to inform clinical guidelines and priorities for prevention.

The overall aim of this thesis is to investigate the current and previous epidemiology of childhood meningitis in the UK, analyse clinical and laboratory characteristics of meningitis of different aetiologies, and assess outcomes following childhood meningitis.

The specific aims of this thesis are:

*Chapter 3*

To report trends in hospital admission rates for meningitis and septicaemia caused by *Haemophilus influenzae*, *Neisseria meningitidis,* and *Streptococcus pneumoniae* in England from 1968 to 2011.

*Chapter 4*

To report trends in hospital admission rates for viral meningitis in children in England from 1968 to 2011.

*Chapter 5*

1. To analyse the current aetiology of childhood meningitis using data collected prospectively from children presenting with suspected meningitis to hospitals across the UK, from December 2012 to June 2016.
2. To assess antibiotic pre-treatment and the proportion of children receiving antibiotic management, length of hospital admission, and the proportion of children investigated by PCR testing for specific groups of children with meningitis of different aetiologies.

*Chapter 6*

1. To describe clinical and laboratory features of meningitis of different aetiologies or suspected meningitis in infants and children.
2. To validate the Bacterial Meningitis Score[168] in the UK population.
3. To develop a new clinical decision rule to distinguish bacterial and aseptic meningitis, for participants recruited prospectively to the UK-ChiMES study.

*Chapter 7*

1. To perform EV-PCR on stool, serum and respiratory samples, and investigate the proportion of EV-PCR+ samples, in children with EV meningitis or aseptic meningitis of unknown cause.

2. To investigate the time following onset of symptoms that positive or negative EV-PCR results are obtained for CSF and non-CSF samples in childhood EV meningitis or aseptic meningitis.
3. To compare age, clinical and laboratory features in childhood EV meningitis with CSF pleocytosis to without CSF pleocytosis.
4. To compare clinical and laboratory features in children with EV meningitis, to children with aseptic meningitis of unknown cause who have a EV-PCR+ stool, respiratory or serum sample.

*Chapter 8*

To assess outcomes following bacterial and aseptic meningitis, or a non-meningitis illness in children aged <16 years recruited to the UK-ChiMES study including:

1. Neurological sequelae and other complications at discharge and 3 months following discharge from hospital.
2. Health-related quality of life assessed by Infant Toddler Quality of Life Questionnaire[338] at hospital discharge, 6, 12 and 18 months following discharge in children aged <2 years.

## 2. Chapter 2: General methods for retrospective analysis of hospital admission rates, and the prospective UK-Childhood Meningitis and Encephalitis cohort Study (UK-ChiMES)

This chapter includes general methods for the analysis of retrospective hospital admission rates for children in England, and for the UK-Childhood Meningitis and Encephalitis cohort Study. Specific methods including data analysis and laboratory methods are described separately within *chapters 5, 6, 7* and *8*.

### 2.1. Methods for analysis of retrospective hospital admission rates in children in England

*These methods have been published in the Lancet Infectious Diseases journal, and relate to the accepted manuscripts, as per Elsevier copyright agreements (2014 and 2016) for personal use in a thesis:*

*1. Martin NG, Sadarangani M, Pollard AJ, Goldacre MJ. Hospital admission rates for meningitis and septicaemia caused by Haemophilus influenzae, Neisseria meningitidis, and Streptococcus pneumoniae in children in England over five decades: a population-based observational study. Lancet Infect Dis. 2014 May;14(5):397-405. doi: 10.1016/S1473-3099(14)70027-1.*[169]

*2. Martin NG, Iro MA, Sadarangani M, Goldacre R, Pollard AJ, Goldacre MJ. Hospital admissions for viral meningitis in children in England over five decades: a population-based observational study. Lancet Infect Dis. 2016 Nov;16(11):1279-1287. doi: 10.1016/S1473-3099(16)30201-8.*[339]

*2.1.1. Datasets*

Datasets were analysed that included routinely collected administrative statistics on hospital care. From 1968-1985, hospital admission statistics in England were collected in the Hospital In-Patient Enquiry (HIPE). The HIPE was a 10% sample of every hospital admission in the English National Health Service (NHS). From 1989, hospital admission statistics in England have been collected in the Hospital Episode Statistics (HES) dataset on a 100% basis covering all NHS day cases and inpatients. HIPE (1968-1985) and HES (1989-2011) data were combined. The 10% sample in HIPE was scaled by multiplying by ten.

For most of the included years, the English data were based on numbers of episodes of care only, rather than numbers of people in receipt of care. However, from 1999, data were linkable allowing the same patient to be counted only once through multiple admissions or transfers between hospitals. Linkage was performed using encrypted values of the individuals' NHS number (which is unique for each person), and by encrypted HES patient ID numbers. Prior to 1999, NHS numbers were often missing and therefore data were not linked in the national dataset. From 1999, both episode-based (numbers of admissions) and person-based (numbers of people receiving care) rates were reported. The HES data were provided, as individual-level records for each episode of care, by the Health and Social Care Information Centre (HSCIC) and were linked by the Oxford Record Linkage Study (ORLS) team. English national hospital statistics were not collected from 1986-1988.

Data from the Oxford Record Linkage Study (ORLS) were also analysed, which included all NHS hospital admissions in the former Oxford Regional Health Authority area from 1963-2011. Successive admissions for the same individuals were always linked in the ORLS dataset. From 1963-1998, individuals were matched by identifiers including encrypted full names, addresses, date of birth and NHS numbers when available, and individuals were then assigned a regional personal number for further analyses. From 1999, the ORLS dataset was the regional subset of English national HES. The ORLS dataset was a continuous dataset from 1963.

The national HIPE and regional ORLS datasets were collected independently of each other in the period covered by both. Therefore, it was useful to determine whether findings in one supported findings in the other. The ORLS data were presented in the figures for annual trends, with the all-England data. Tables included only the all-England data. Unless otherwise stated, data reported were for all-England.

*2.2.2. Analysis*

Annual age-specific and age-standardised admission rates were analysed for meningitis and septicaemia caused by *N. meningitidis*, *S. pneumoniae,* and *H. influenzae,* and viral meningitis, defined by any relevant International Classification of Disease (ICD) code in children aged <15 years (*table 2.1. and 2.2.*). Admission rates for viral meningitis caused by mumps virus, measles virus, enteroviruses, varicella zoster virus (VZV), and herpes simplex virus (HSV) were also analysed separately (*table 2.2.*). For infants <1 year with viral meningitis, age in days was available within the HES dataset from 1989.

A hospital admission included day cases (a hospital admission without an overnight stay), or overnight inpatient admissions, but did not include emergency department visits without admission. Cases were included if the diagnosis was recorded in any diagnostic position. Admission rates and their 95% confidence intervals were calculated using the direct method of standardisation (in five-year age groups, confined to people aged under 15 years), and the European standard population.[169,339,340] Population denominators from the Office for National Statistics were used.[341]

The terminology and ICD codes for viral meningitis were complicated, because of changes and some inconsistencies in terminology and coding between ICD revisions (*table 2.2.*). Cases coded as encephalitis were not included.

The median length of hospital admission was calculated for all viral meningitis, and the subset of children with enteroviral meningitis, in five-calendar-year groups from 1968-2011, for infants aged <1 year and children aged 1-14 years.

Annual age-standardised admission rates for *S. pneumoniae* meningitis and septicaemia combined in adults aged ≥65 years from 1999-2011 were also analysed to assess any impact of herd immunity driven by the infant PCV7 vaccine programme.

**Table 2.1 Table showing International Classification of Disease (ICD) codes used to identify hospital admission rates for meningitis and septicaemia caused by Haemophilus influenzae, Neisseria meningitidis and Streptococcus pneumoniae in children from 1963 to 2011** ***(reference[169])***

| ICD Revision | Years | ICD Disease Classification and Code | | | | |
|---|---|---|---|---|---|---|
| | | **Meningococcal infection** | **Pneumococcal meningitis** | **Pneumococcal septicaemia** | **Haemophilus meningitis** | **Haemophilus septicaemia** |
| **7** | **1963-1967** | 057 | 340·1 | 053·2 | 340·0 | Not coded |
| **8** | **1968-1978** | 036 | 320·1 | 038·2 | 320·0 | Not coded |
| **9** | **1979-1994** | 036 | 320·1 | 038·2 | 320·0 | Not coded |
| **10** | **1994-2011** | A39 | G00·1 | A40·3 | G00·0 | A41·3 |

**Table 2.2 International Classification of Disease (ICD) codes used to identify hospital admission rates for viral meningitis in children from 1968 to 2011**
***(reference[339])***

| | **ICD revision 10 1995-2011** | **ICD revision 9 1979-1994** | **ICD revision 8 1968-1978** |
|---|---|---|---|
| **Viral meningitis of all causes** | A87·0 enteroviral meningitis<br>A87·1 adenoviral meningitis<br>A87·2 lymphocytic choriomeningitis<br>A87·8 other viral meningitis<br>A87·9 viral meningitis, unspecified<br>G02·0 meningitis in viral diseases classified elsewhere<br>G03·0 nonpyogenic meningitis<br>B00·3 herpesvirus meningitis<br>B01·0 varicella meningitis<br>B02·1 herpes zoster meningitis<br>B05·1 measles meningitis<br>B26·1 mumps meningitis | 047 meningitis due to enterovirus (47·0 coxsackie, 47·1 ECHO, 47·8 other, 47·9 unspecified)<br>049·0 lymphocytic choriomeningitis<br>049·1 meningitis due to adenovirus<br>053·0 herpes zoster with meningitis<br>072·1 mumps meningitis<br>321·1 meningitis due to coxsackie virus<br>321·2 meningitis due to ECHO virus<br>321·3 meningitis due to herpes zoster<br>321·4 meningitis due to herpes simplex<br>321·5 meningitis due to mumps<br>321·6 meningitis due to lymphocytic choriomeningitis<br>321·7 meningitis due to other and unspecified viruses<br>322·0 nonpyogenic meningitis | 045 aseptic meningitis due to enterovirus (045·0 coxsackie virus, 045·1 ECHO virus, 045·9 aseptic meningitis, unspecified)<br>079·2 lymphocytic choriomeningitis |
| **Unspecified viral meningitis** | A87·9 viral meningitis, unspecified<br>G03·0 non-pyogenic meningitis | 047·9 viral meningitis unspecified<br>321·7 meningitis due to other and unspecified viruses<br>322·0 nonpyogenic meningitis | 045·9 aseptic meningitis, unspecified |
| **Enteroviral meningitis including unspecified codes** | A87·0 enteroviral meningitis | 047 meningitis due to enterovirus (47·0 coxsackie, 47·1 ECHO, 47·8 other, 47·9 unspecified) | 045 aseptic meningitis due to enterovirus<br>045·0 coxsackie virus<br>045·1 ECHO virus<br>045·9 aseptic meningitis, unspecified (due to enterovirus) |
| **Enteroviral meningitis – specific codes only** | A87·0 enteroviral meningitis | 047 meningitis due to enterovirus (47·0 coxsackie, 47·1 ECHO, 47·8 other) | 045 aseptic meninigits due to enterovirus<br>045·0 coxsackie virus<br>045·1 ECHO virus |
| **Meningitis caused by mumps virus** | B26·1 mumps meningitis | 072·1 mumps meningitis<br>321·5 meningitis due to mumps | no code |
| **Meningitis caused by VZV** | B01·0 varicella meningitis<br>B02·1 herpes zoster meningitis | 321·3 meningitis due to herpes zoster<br>053·0 herpes zoster with meningitis | no code |
| **Meningitis caused by HSV** | B00·3 herpesvirus meningitis | 321·4 herpes simplex meningitis | no code |

## 2.2. The UK-Childhood Meningitis and Encephalitis cohort Study (UK-ChiMES): summary of general methods

### *2.2.1. General information and site initiation*

From December 2012-June 2016, 3003 children with suspected or confirmed meningitis or encephalitis were recruited to the prospective UK-ChiMES study from 31 hospital sites across England, Wales and Scotland. The UK-ChiMES study was a collaboration for recruitment of participants between the UK-Childhood Meningitis Study, Department of Paediatrics, University of Oxford, to study childhood meningitis, and the Liverpool Brain Infections Group, University of Liverpool, to study encephalitis, as reflected in the inclusion criteria.

Site initiation visits were conducted face-to-face at each hospital site.

*2.2.2. Inclusion criteria*

The inclusion criteria for the UK-ChiMES study were:

Child aged <16 years in hospital

AND

either A. Suspected meningitis or encephalitis fulfilling ≥1 of criteria 1-5

or B. Lumbar puncture (LP) performed as part of an evaluation for infection

Criteria 1-5

1. Either:
    a. LP was performed or
    b. LP clinically indicated but was deferred
2. Acute or sub-acute (<4 weeks) alteration in consciousness, cognition, personality or behaviour persisting for more than 24 hours

    AND any two of:

    a. Fever (≥ 38◦C) or acute/sub-acute prodromal illness
    b. Seizures (new onset)
    c. Focal neurological signs (acute or sub-acute onset)
    d. CSF pleocytosis (WBC >4 cells/μL)
    e. Neuroimaging or EEG compatible with encephalitis
3. Clinical suspicion of encephalitis of any cause
4. Clinical suspicion of encephalitis but above investigations not done
5. Clinical suspicion of meningitis or encephalitis and the patient died

The exclusion criteria were:

1. Patients with confirmed non-infectious or non-inflammatory CNS disorders due to hypoxic, ischemic, vascular, toxic and metabolic causes
2. Patients with pre-existing indwelling ventricular devices, for example external ventricular drains and ventriculoperitoneal shunts

*2.2.3. Enrolment*

Participants were identified either by the research team at the hospital site identifying eligible participants from reviewing new hospital admissions records, or by clinicians or laboratory staff identifying participants. Patient information sheets were provided for parents and children. Written consent was obtained from the parent or legal guardian, because all participants were aged under 16 years. Assent forms for children were also available. Once consent had been given, enrolment was completed directly on to a password protected database using OpenClinica.

*2.2.4. Participant withdrawals from study*

Participants could withdraw from the study at any time. Data up to the time of withdrawal was included in analysis unless the participant requested that data not be used.

*2.2.5. Mortality and adverse events*

All deaths were reported to the coordinating centre in Oxford using a notification of death form within two days of the site study team becoming aware of the event.

In this non-interventional study, related and unexpected serious adverse events were to be reported to the coordinating centre within 24 hours, but none were reported. Adverse events (AE) and serious adverse events (SAE) including death (SAE), hospital readmission (SAE), persistent or significant disability (SAE) and treatment on an emergency outpatient basis (AE), were expected as part of routine clinical care and were reported as part of the outcomes analysis.

### *2.2.6. Clinical data collection*

Data from patient notes and an interview with parents during hospital admission, were collected either directly onto an electronic Case Report Form (CRF) using the OpenClinica database, or collected onto a paper CRF and then transcribed onto the electronic CRF. Data collected included past medical history, clinical features, hospital laboratory tests results, radiology results and management (*table 2.3.*).

| **Table 2.3 Clinical data collected in the UK-ChiMES study** | |
|---|---|
| *Data collected if available including:* | |
| Demographic data | Date of birth, ethnicity<br>Dates of onset of symptoms, hospital admission, hospital discharge, intensive care unit admission and discharge.<br>Mortality |
| Past medical history | Previous neurosurgery, head trauma, local anatomical defects, concerns about development, other past medical history, regular medications, vaccination history and travel history. |
| Clinical features | Symptoms on admission and duration of each symptom.<br>Examination findings on first admission to hospital including temperature, heart rate, blood pressure, central capillary refill time, respiratory rate, Glasgow Coma Scale score, and general, cardiovascular, respiratory, gastrointestinal and neurological findings. |
| Hospital site laboratory test results | Date, time and all available results of initial, first successful and subsequent lumbar punctures, including PCRs.<br>Date and results of full blood count, renal function test, electrolytes, inflammatory markers and any other blood test results.<br>Date and results of autoimmune tests, blood cultures, PCRs and serology.<br>Results of other microbiology samples including PCR results of bacterial and viral throat swabs, urine, skin or oral lesion samples. |
| Radiology results* | Dates of any radiology imaging obtained, and key chest xray abnormalities. |
| Management | Medications during or prior to admission. |
| Outcomes at discharge | Overall diagnosis<br>General and neurological sequelae at discharge. |
| Research samples | Dates and availability of study samples, either leftover from clinical samples, or taken specifically for study. |

Note: *Neuroimaging and neurophysiology reports were required to be forwarded to the coordinating centres following the study.

### *2.2.7. Laboratory sample collection*

Scavenged samples were collected from routine laboratory tests already performed as part of clinical care if available, and additional samples for research were obtained if consent was given, summarized in *table 2.4.* A guide was provided to hospital sites about procedures for taking and processing samples.

| Table 2.4 Research samples collected for the UK-ChiMES study | | |
|---|---|---|
| **Sample type** | **Left over from routine tests** | **Taken for research (after consent)** |
| CSF | ✓ | ✓ |
| Blood | | |
| - serum* | ✓ | ✓ |
| - blood clot | ✓ | ✓ |
| - Paxgene tube[342] | | ✓ |
| - EDTA | ✓ | ✓ |
| NPA* | ✓ | ✓ |
| Throat swab[343]* | ✓ | ✓ |
| Stool* | ✓ | ✓ |
| Saliva (Oragene kit[344] for DNA) | | ✓ |

Note: *Sample used as part of thesis work. NPA = nasopharyngeal aspirate.

*2.2.8. Outcome data collection*

Outcomes at discharge were recorded on the electronic OpenClinica CRF. Outcomes at three months after discharge were collected by a phone call from research nurses at sites and recorded on the electronic CRF. Outcomes included neurological and other sequelae, results of hearing tests, and details about any clinic appointments or hospital visits.

Quality of life and neuropsychological questionnaires were posted to participants' parents at discharge, 6, 12 and 18 months post discharge (*table 2.5.*). Paper questionnaires returned by post were entered into the OpenClinica database by coordinating centres. The Infant-Toddler Quality of Life Questionnaire[338,345] was analysed in this thesis.

| Table 2.5 Neuropsychological and Quality of Life Questionnaires for UK-ChiMES study | | | | |
|---|---|---|---|---|
| Age | <2 years | 2-4 years | 5-16 years | Time to complete |
| Quality of life questionnaires | ITQOL*†[338,345] (parent) | PedsQL-4.0†[346,347] (parent) | PedsQL-4.0†[346,347] (child and parent) | 5-10 minutes |
| Neuropsychological questionnaires | Infant characteristic questionnaire[348] | Preschool behavior checklist[349] | SDQ†[350] (5-10 years parent only, 11-17 years child and parent) | 5-10 minutes |

Notes: *Questionnaire analysed as part of thesis work. †ITQOL=Infant toddler quality of life questionnaire, PedsQL=Paediatric Quality of Life Inventory TM, SDQ=Strength and disabilities questionnaire.

### *2.2.9. Data monitoring*

A study monitor visited each hospital site after five participants were recruited to the study. During the visit, five CRFs were compared with source data, which were clinical notes and laboratory results. All remaining CRFs were monitored remotely through the electronic OpenClinica database. All consent forms were faxed to the coordinating centre and monitored.

### *2.2.10. Data analysis and laboratory methods*

Data analysis and laboratory methods are described in the relevant results chapters (*chapter 5 section 5.2., chapter 6 section 6.2., chapter 7 section 7.2., chapter 8 section 8.2.*).

## 3. Chapter 3: Hospital admission rates for meningitis and septicaemia caused by *Haemophilus influenzae, Neisseria meningitidis* and *Streptococcus pneumoniae* in children in England over five decades: a population-based observational study

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### 3.1. Introduction

*Haemophilus influenzae*, *Neisseria meningitidis,* and *Streptococcus pneumoniae* can cause severe childhood disease, associated with substantial mortality and significant long-term morbidity. Case fatality rates in developed countries for childhood bacterial meningitis caused by these pathogens remain at 5-10%.[100,351] The median risk of long term disabling sequelae in survivors of bacterial meningitis is approximately 20% with the highest rates following pneumococcal meningitis.[118,324]

Unlike polysaccharide vaccines, conjugate vaccines are highly effective in children aged younger than 2 years, induce immunological memory responses, and protection can be boosted by subsequent doses.[234,352] A *H. influenzae* type b (Hib) conjugate vaccine was introduced into the routine infant vaccination schedule in the United Kingdom (UK) in October 1992.[12,107,159,235] *N. meningitidis* causes septicaemia and meningitis.[252] A capsular group C meningococcal conjugate vaccine was introduced in September 1999 as three infant doses along with a catch up campaign to 19 years of age following an increase in MenC disease, the second most common serogroup causing disease in the UK at the

time.[110,128] *S. pneumoniae* causes a wide spectrum of invasive disease including meningitis, septicaemia, pneumonia, and bone and joint infection.[113] The 7-valent pneumococcal conjugate vaccine, PCV 7, was introduced in September 2006 at 2, 4 and 13 months also with a catch up programme to 2 years of age, and was replaced by the 13-valent pneumococcal vaccine from April 2010.[149,236,353]

A substantial reduction in invasive bacterial disease caused by *Haemophilus influenzae*, *Neisseria meningitidis,* and *Streptococcus pneumoniae* in the UK was reported in microbiological surveillance data since the introduction of effective conjugate vaccines.[12,111,128] However, long-term trends in hospital admission rates were not previously reported.

The aim of this study was to report trends in hospital admission rates for meningitis and septicaemia caused by *Haemophilus influenzae*, *Neisseria meningitidis,* and *Streptococcus pneumoniae* in England from the 1960s to 2011.

### 3.2. Methods

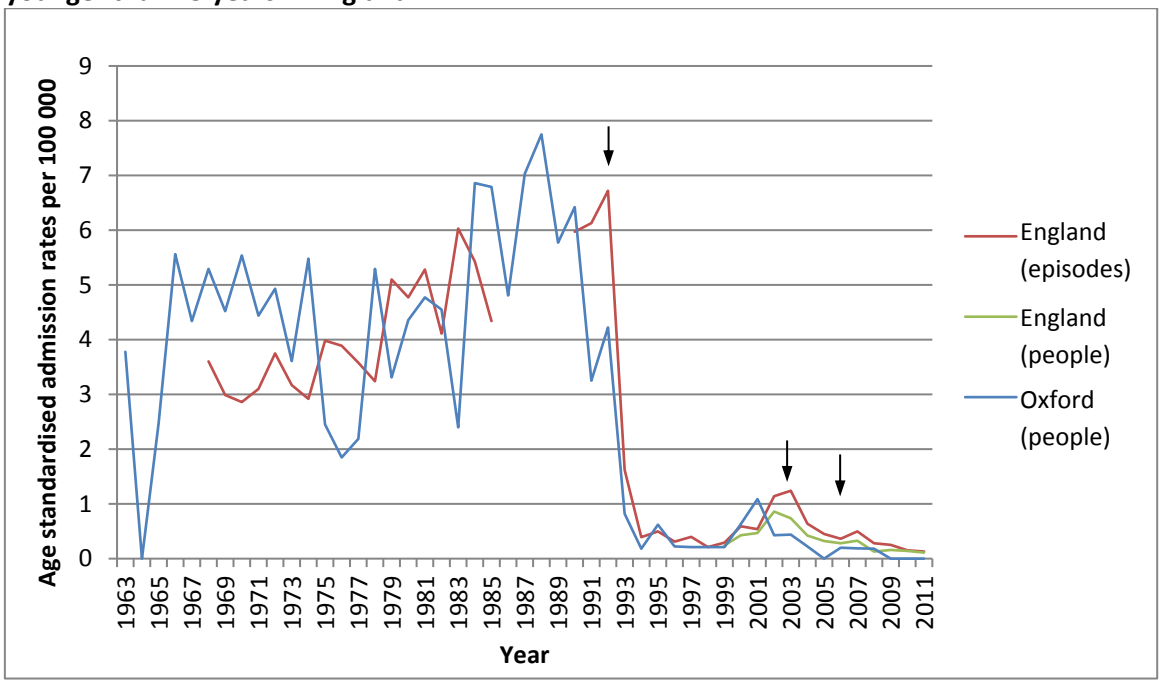
The methods used in this chapter are 'Methods for analysis of retrospective epidemiology from hospital admission datasets' (*chapter 2, section 2.1*).

### 3.3. Results

*3.3.1. Haemophilus influenzae*

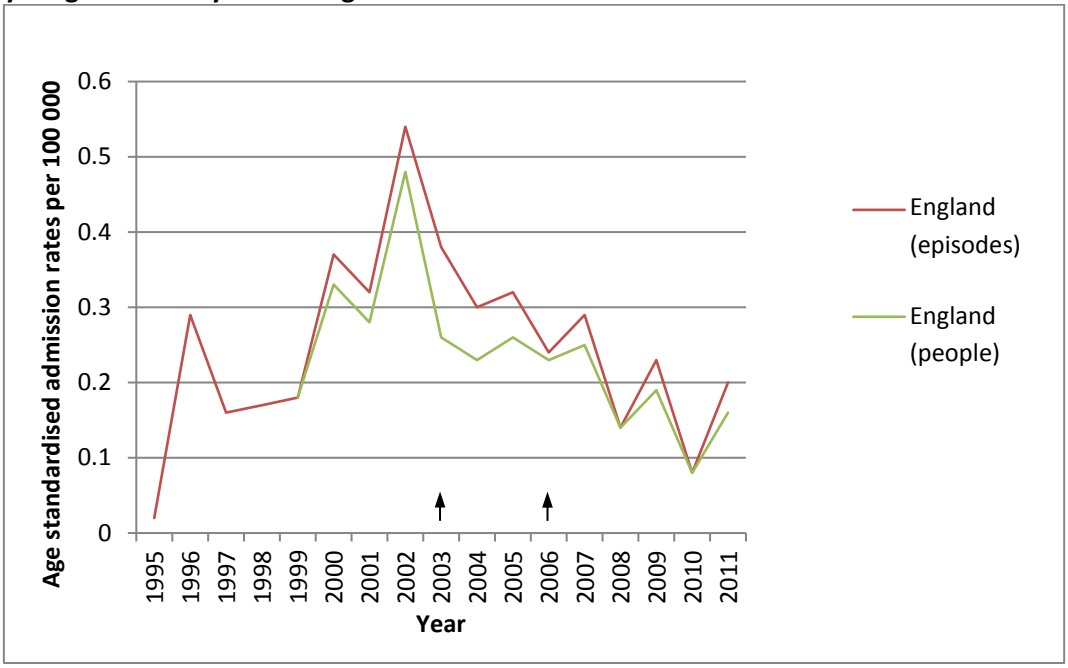
Annual age-standardised episode-based rates of *Haemophilus influenzae* (any type) meningitis across England varied between 2·86 and 6·72 episodes per 100 000 children per year between 1968 and 1992, with a trend to a gradual increase, reaching a peak of 6·72/100 000 children (95% confidence interval 6.18-7.26) in 1992 (figure 3.1). Following the introduction of routine Hib vaccine in 1992, there was a 94% decline to 0·39 (0.26-0.52) admissions per 100 000 children per year within 2 years. There was a small rise in admissions in the early 2000s, peaking at 1·24/100 000 (0·99-1·48) children in 2003, which reduced again to 0·28/100 000 (0·17-0·39) by 2008. Admission rates for *H. influenzae* (any type) septicaemia remained low from 1995-2011 (*figure 3.2*). The all-England and the Oxford admission rates were very similar (*figures 3.1 and 3.2*).

**Figure 3.1 Hospital admission rates for Haemophilus influenzae meningitis in children aged younger than 15 years in England**




Notes: Arrows indicate (1) Hib vaccine introduced in October 1992; (2) catch up Hib booster vaccine for children aged 6 months to 4 years introduced in 2003; (3) routine 12-month Hib booster vaccine in the second year of life introduced in 2006. Routine collection of English national hospital statistics stopped in 1985, and usable data did not become available again until 1990. Linked person-based data for England became available from 1999.

**Figure 3.2 Hospital admission rates for Haemophilus influenzae septicaemia in children aged younger than 15 years in England**



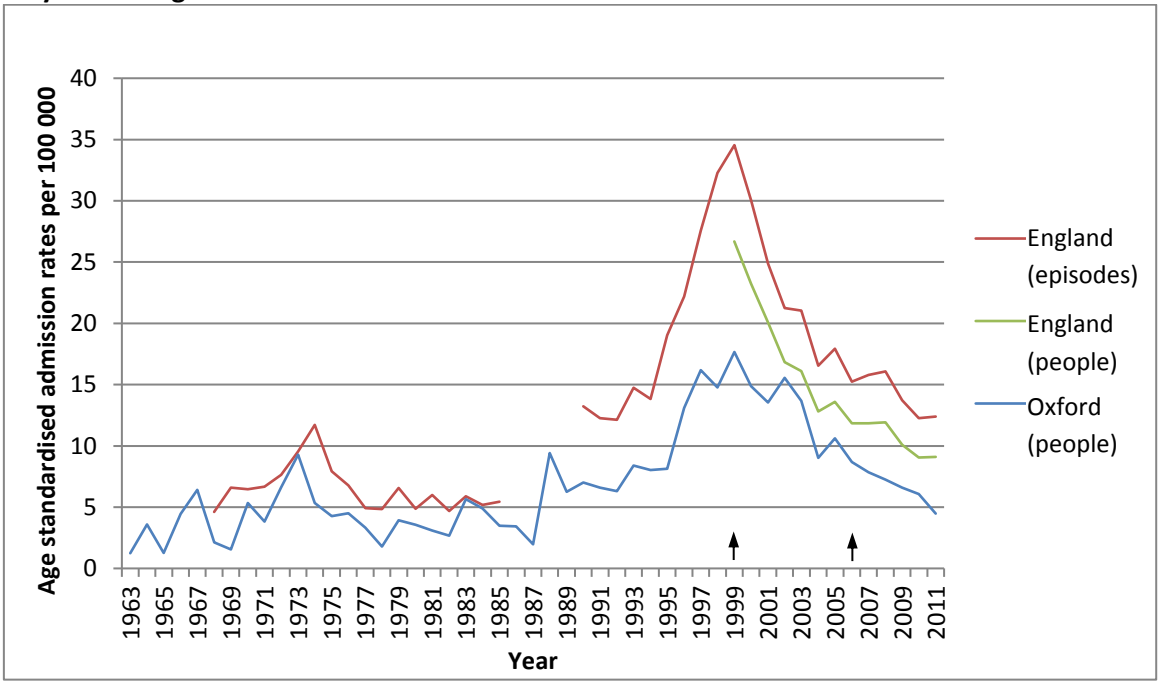

Note: Arrows indicate (1) catch up Hib booster vaccine for children aged 6 months to 4 years introduced in 2003; (2) routine 12 month Hib booster vaccine in the second year of life introduced in 2006.

*3.3.2. Neisseria meningitidis*

Prior to 1985, the episode-based admission rate for meningococcal disease in England was relatively stable around 5/100 000 children per year, with a small rise during the mid-1970s (*figure 3.3*). There was an increase in admission rates during the 1990s, reaching a peak in 1999 at 34·54/100 000 (95% CI 33·30-35·78) children per year. Hospital admission rates fell after the MenC vaccine was introduced and were 12·40/100 000 (11·68-13·12) children in 2011. Person-based admission rates declined by 66%, from 26·68 (25·59-27·77) children per 100 000 in 1999 to 9·10 (8·48-9·71) children per 100 000 in 2011. The comparison between episode-based and person-based rates, for example 12·4 and 9·1, respectively in 2011, demonstrates the extent of multiple-counting of the same people as revealed by record linkage. The pattern of admission rates in all-England and Oxford were similar albeit that the rates were a little lower in the latter (*figure 3.3*). Hospital admission

rates in England are higher than previously published incidence from laboratory surveillance data in England and Wales (*table 3.1*).

**Figure 3.3 Hospital admission rates for meningococcal infection in children aged younger than 15 years in England**




Note: Arrows indicate (1) MenC vaccine introduced in September 1999; (2) MenC booster vaccine at age 12 months introduced in 2006.*Routine collection of English national hospital statistics stopped in 1985, and usable data did not become available again until 1990. Linked person-based data for England became available from 1999.

**Table 3.1 Comparison of rates of meningococcal infection per 100 0000 children under 15 years old from linked person-based hospital admission data in England and laboratory surveillance data in England and Wales**
***(references[110])*†***

| | <1 year | | 1 to 4 years | | 5 to 9 years | | 10 to 14 years | |
|---|---|---|---|---|---|---|---|---|
| **Year** | **Hospital admissions** | **Laboratory cases** | **Hospital admissions** | **Laboratory cases** | **Hospital admissions** | **Laboratory cases** | **Hospital admissions** | **Laboratory cases** |
| **2006** | 58·86 | 44·8 | 18·37 | 13·8 | 5·04 | 3 | 1·95 | 1 |
| **2007** | 58·19 | 44·5 | 19·76 | 15·3 | 3·98 | 3 | 1·91 | 1·1 |
| **2008** | 61·58 | 42·3 | 18·89 | 13·4 | 3·62 | 2·5 | 2·49 | 1·5 |
| **2009** | 46·69 | 31·6 | 16.00 | 9·3 | 4·26 | 2 | 2·19 | 1·2 |
| **2010** | 49·90 | 30·5 | 13·31 | 11·6 | 3·51 | 2·4 | 1·34 | 1·4 |
| **2011** | 41·38 | | 14·95 | | 3·78 | | 1·70 | |

Notes: *Data from *Ladhani et al*[110]

† Data are epidemiological years 2006/07, 2007/08, 2007/08, 2008/09, 2010/11

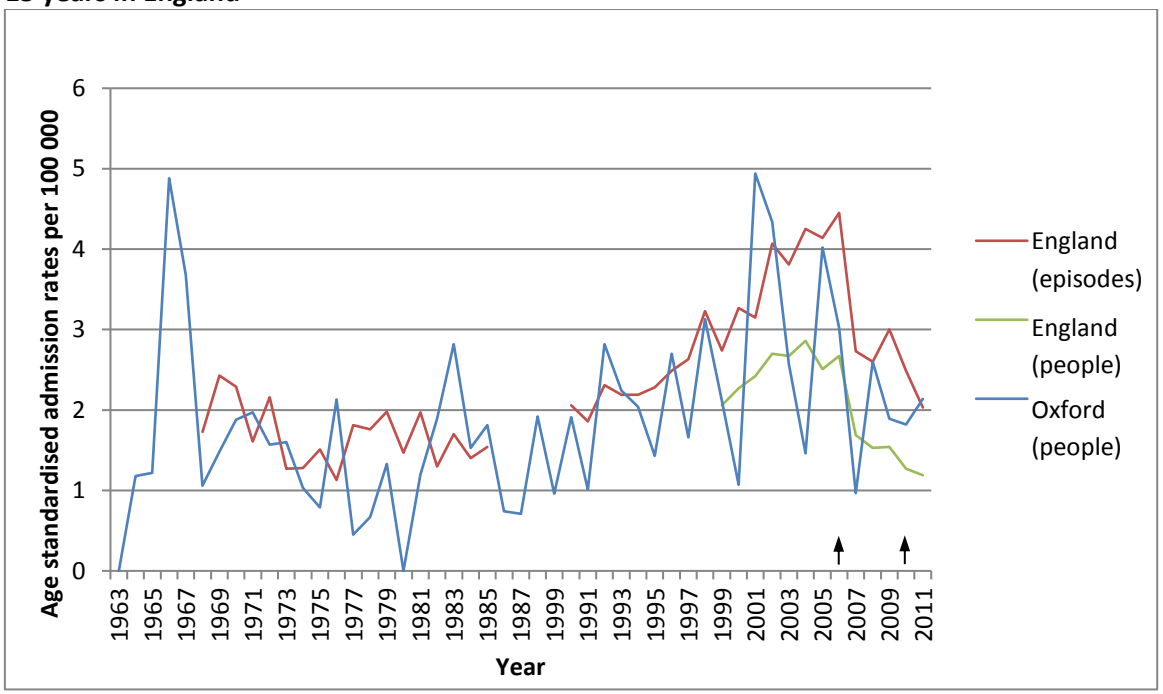
*3.3.3. Streptococcus pneumoniae*

From 1968-1985, the admission rate for pneumococcal meningitis in England ranged between 1·13 and 2·29/100 000 children per year (*figure 3.4*). Annual rates increased throughout the 1990s and early 2000s, reaching a peak in 2006 at 4·45 (95% CI 4-4.9) admission episodes per 100 000 children. A reduction in admissions occurred after 2006, coinciding with the introduction of the PCV7 vaccine, and the overall hospital admission rate in 2011 was similar to the rate in the early 1990s at 2.03 episodes (1.74-2.32) per 100 000 children. Using the linked data, the rate in 2006 was 2·67 (2·32-3·02) children per 100 000, and in 2011 was 1·19/ 100 000 (0·97-1·41), less than half that in 2006. A similar trend occurred for pneumococcal septicaemia (*figure 3.5*). A small increase in admissions occurred in 2009 for pneumococcal meningitis and 2010 for pneumococcal septicaemia, decreasing again following the replacement of PCV7 with PCV13 in the routine infant immunisation schedule. Person-based hospital admission rates for pneumococcal meningitis in children aged under 5 years from 2000-06 were 6·37 children per 100 000 per year compared to 3·18/100 000/year in laboratory surveillance data.[149] Similarly from 2008-10 person-based hospital admission rates in children under 5 years were 3·29 children per 100 000/year compared to 1·44/100 000 laboratory cases per year.[149] Hospital admissions and laboratory cases in this age group showed a similar reduction in disease between the two time periods.[149] Admission rates in all-England and Oxford are similar, although the Oxford rates show more fluctuation, which is likely contributed to by smaller sample size (*figures 3.4 and 3.5*).

There was no consistent change in hospital admission rates for pneumococcal meningitis or septicaemia combined in adults aged ≥65 years between 1999 and 2011. The rate in

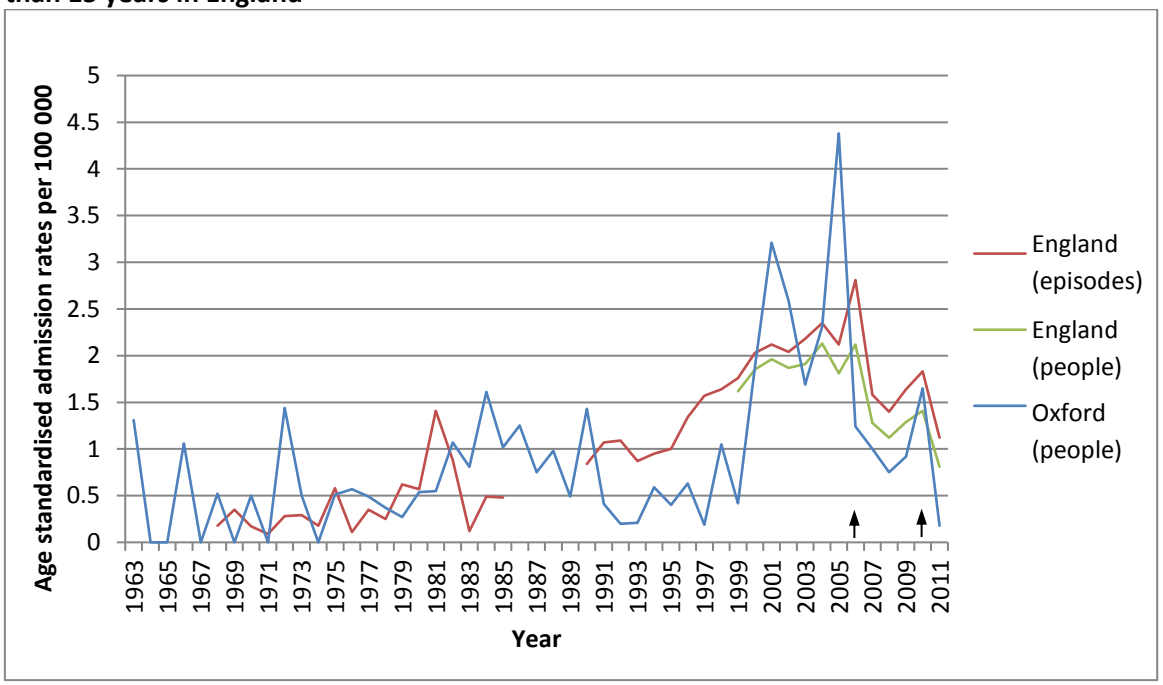
1999 was 4·79 people per 100 000, and in 2011 was 4·85/100 000. Annual rates fluctuated between 4·50 and 5·46 people per 100 000.

**Figure 3.4 Hospital admission rates for pneumococcal meningitis in children aged younger than 15 years in England**




Note: Arrows indicate (1) PCV7 vaccine introduced in September 2006; (2) PCV13 vaccine introduced in April 2010. *Routine collection of English national hospital statistics stopped in 1985, and usable data did not become available again until 1990. Linked person-based data for England became available from 1999.

**Figure 3.5 Hospital admission rates for pneumococcal septicaemia in children aged younger than 15 years in England**




Note: Arrows indicate (1) PCV7 vaccine introduced in September 2006; (2) PCV13 vaccine introduced in April 2010. *Routine collection of English national hospital statistics stopped in 1985, and usable data did not become available again until 1990. Linked person-based data for England became available from 1999.

*3.3.4. Burden of disease comparing sex and age*

For each disease, rates were higher in males than females: males comprised 56·3% of cases of meningococcal infection, 62·1% pneumococcal meningitis, 59·6% pneumococcal septicaemia, and 55·4% of *Haemophilus influenzae* meningitis. The greatest burden of disease occurs in infants (*table 3.2.*).

| Table 3.2 English hospital admission rates for bacterial meningitis and septicaemia per 100 000 | | | | |
|---|---|---|---|---|
| **Years** | **<1 year (episodes)** | **1-14 years (episodes)** | **<15 years age standardised (episodes) (95% CI)** | **<15 years age standardised (linked people) (95% CI)** |
| **Meningococcal Infection** | | | | |
| **1968-70** | 31·09 | 3· 91 | 5·89 (5·07-6·7) | |
| **1971-75** | 38·98 | 6·29 | 8·66 (7·87-9·46) | |
| **1976-80** | 27·22 | 3·91 | 5·61 (4·91-6·3) | |
| **1981-85** | 27·49 | 3·71 | 5·44 (4·73-6·14) | |
| **1986-90*** | | | | |
| **1991-96** | 68·87 | 11·51 | 15·68 (15·34-16·01) | |
| **1997-01** | 129·76 | 22·03 | 29·87 (29·19-30·54) | 23·35 (22·76-23·95) |
| **2002-06** | 89·15 | 12·83 | 18·38 (17·96-18·79) | 14·22 (13·85-14·58) |
| **2007-11** | 70·34 | 9·60 | 14·02 (13·67-14·36) | 10·37 (10·08-10·67) |
| **Pneumococcal Meningitis** | | | | |
| **1968-70** | 10·09 | 1·53 | 2·15 (1·66-2·64) | |
| **1971-75** | 8·81 | 1.00 | 1·57 (1·23-1·91) | |
| **1976-80** | 9·84 | 0·98 | 1·62 (1·25-2) | |
| **1981-85** | 10·06 | 0·92 | 1·59 (1·21-1·97) | |
| **1986-90*** | | | | |
| **1991-96** | 17·26 | 1·03 | 2·21 (2·09-2·34) | |
| **1997-01** | 27·02 | 1·17 | 3·05 (2·83-3·27) | 2·25 (2·06-2·44) |
| **2002-06** | 34·89 | 1·73 | 4·14 (3·94-4·34) | 2·68 (2·52-2·83) |
| **2007-11** | 19·66 | 1·23 | 2·57 (2·42-2·71) | 1·44 (1·33-1·55) |
| **Pneumococcal Septicaemia** | | | | |
| **1968-70** | 0·81 | 0·19 | 0·23 (0·07-0·39) | |
| **1971-75** | 1·07 | 0·22 | 0·28 (0·14-0·43) | |
| **1976-80** | 2·62 | 0·20 | 0·38 (0·19-0·56) | |
| **1981-85** | 2·68 | 0·52 | 0·68 (0·43-0·93) | |
| **1986-90*** | | | | |
| **1991-96** | 5·85 | 0·68 | 1·05 (0·96-1·14) | |
| **1997-01** | 12·06 | 1·18 | 1·97 (1·8-2·15) | 1·81 (1·64-1·97) |
| **2002-06** | 12·71 | 1·48 | 2·30 (2·15-2·45) | 1·97 (1·83-2·11) |
| **2007-11** | 7·94 | 1·01 | 1·51 (1·4-1·63) | 1·18 (1·08-1·28) |
| **Haemophilus Meningitis** | | | | |
| **1968-70** | 14·13 | 2·29 | 3·16 (2·56-3·75) | |
| **1971-75** | 16·02 | 2·40 | 3·39 (2·88-3·89) | |
| **1976-80** | 19·02 | 2·94 | 4·11 (3·5-4·72) | |
| **1981-85** | 26·49 | 3·35 | 5·04 (4·35-5·72) | |
| **1986-90*** | | | | |
| **1991-96** | 15·76 | 1·62 | 2·65 (2·51-2·79) | |
| **1997-01** | 2·67 | 0·30 | 0·47 (0·38-0·56) | 0·38 (0·3-0·45) |
| **2002-06** | 3·55 | 0·55 | 0·76 (0·68-0·85) | 0·52 (0·45-0·59) |
| **2007-11** | 2·19 | 0·11 | 0·26 (0·21-0·31) | 0·17 (0·13-0·21) |
| **Haemophilus Septicaemia** | | | | |
| **1968-90*** | | | | |
| **1991-96** | 0·32 | 0·03 | 0·05 (0·03-0·07) | |
| **1997-01** | 1·28 | 0·21 | 0·29 (0·22-0·36) | 0·26 (0·2-0·33) |
| **2002-06** | 1·18 | 0·29 | 0·35 (0·3-0·41) | 0·29 (0·24-0·34) |
| **2007-11** | 1·17 | 0·11 | 0·19 (0·15-0·23) | 0·16 (0·13-0·2) |

Note: *No data available

### 3.4. Discussion

These datasets, spanning five decades, show a striking reduction in childhood meningitis and septicaemia after the introduction of conjugate vaccines for *H. influenzae*, *N. meningitidis,* and *S. pneumoniae* in England. The long period of pre-immunisation surveillance provides a reliable baseline of admission rates against which to assess the recent post-immunisation decline. These are the most complete data available to report population-based incidence of these diseases over the five decades shown. Alternative sources are statutory infectious disease notifications,[354] which were very incomplete in the early years covered by this study,[355] laboratory confirmed cases reported to Public Health England,[356] and mortality statistics from death registrations.[357]

The decline in admission rates for these diseases, following the introduction of conjugate vaccines, has occurred at a time when emergency hospital admissions, generally, have increased in children in England[358] and when microbiological diagnostic methods have become more sensitive, most notably through the routine use of PCR.[20-28]

These data show that excellent disease control was achieved following the introduction of the Hib vaccine programme from 1992 in England. There was a 94% reduction in *H. influenzae* (any type) meningitis hospital admissions from 1992 to 1994. This has also been shown with laboratory surveillance data which indicated a 98% reduction in invasive Hib disease in children less than 5 years old by 1998.[12,107] However, from 1999 laboratory surveillance data showed an increase in Hib cases from an incidence of 0·26/100 000 children in 1998, to 1·8/100 000 in 2002, before falling to 0·27/100 000 in 2008.[159] The Hospital Episode Statistics (HES) data have shown a similar trend in hospital admission

rates for *H. influenzae* meningitis, suggesting that most of these hospitalised cases are likely to have been caused by Hib and that the trend for meningitis accurately reflects the overall burden of Hib disease. The resurgence in 2002 was thought to be caused by a greater than expected decline in protective Hib antibody concentrations following primary infant series, a decline in herd immunity after the cessation of the catch-up campaign, and a change in the combination Hib vaccine used.[159,359,360] A similar increase in invasive Hib disease cases was also seen in the Netherlands in 2002.[361] However, longitudinal hospital admissions data for Hib meningitis from Finland, reported a dramatic decline in admissions following the introduction of the Hib vaccine in 1986, but did not show disease resurgence in the early 2000s which may have been contributed to by differences in vaccine schedules.[362] Disease control in the UK was obtained with the use of a Hib catch-up booster vaccine in 2003 for children aged 6 months to 4 years and then with the introduction of a routine 12 month booster from 2006 in the second year of life.[107,360] Interestingly, the increase in total Hib disease reported by laboratory surveillance reached almost one-third of the peak number of cases in the early 1990s, whereas the increase in *H. influenzae* meningitis described in our hospital data were only around one-sixth of the previous peak.[159,235,363] This suggests that cases seen in the early 2000s may have been less severe than previous cases, which may reflect partial immunity from infant immunisation or a lower susceptibility of older children to meningitis during Hib infection compared to infants.

There was an increase in hospital admissions for meningococcal disease during the mid to late 1990s peaking in 1999. A similar trend was also reported by a longitudinal study in the Merseyside region.[364] This increase was seen in microbiological surveillance data and

was largely caused by the emergence of a hypervirulent capsular group C clone from the sequence type 11 clonal complex, which caused particularly high rates of disease in adolescents.[128,365] Although our database did not include national data between 1985 and 1991, data from microbiological surveillance demonstrated an increase in capsular group B meningococcal disease between 1984 and 1990.[366] Different capsular group B strains caused localised outbreaks during this time. Improved PCR-based diagnostic techniques may also have contributed to the increased number of laboratory confirmed cases identified in the late 1990s.[128,365] This is supported by a recent study which demonstrated that over 50% of confirmed invasive meningococcal disease cases in England in 2009-10 were diagnosed by positive PCR with a negative culture.[367]

The reduction in admissions from 1999 occurred following the introduction of routine immunisation in England with a MenC conjugate vaccine, with national surveillance of laboratory confirmed cases reporting a 97% reduction in MenC disease at all ages from 1998/99 to 2006/07.[128] During 1994-1998 when the hypervirulent strain was circulating, the proportion of disease caused by capsular group C increased from 26% to 34%.[365] The hospital admission data show an overall reduction in meningococcal infections (all groups) of 66% from 1999 to 2011. From the laboratory surveillance data it is clear that some of this decline is not due to immunisation but to stochastic changes in disease cause by capsular group B meningococci, for which no vaccine had yet been used.[110,128] Environmental factors, for example the introduction of legislation banning smoking in public places in England in July 2007, may also have contributed to the reduction in disease.[368]

These present data indicate higher rates of meningococcal disease from hospital discharge coding than microbiological surveillance data, which may be expected because of the stricter criteria required for laboratory confirmed cases (*table 3.1*.).[110] However, these linked person based rates are approximately 30-40% higher than laboratory data.[110] From 2006/07-2010/11, 87% of meningococcal disease (93·7% in infants) was caused by capsular group B in the UK, and capsular group C disease remained under tight control (accounting for 2·1% of disease at all ages).[110] The first broadly protective multicomponent MenB vaccine (4CMenB), based on non-serogroup specific subcapsular proteins, was introduced into the routine infant UK vaccine schedule in September 2015.[237,238]

These data demonstrate an increase in hospital admissions for pneumococcal meningitis and septicaemia during the 1990s and early 2000s before the introduction of routine pneumococcal vaccination. Microbiological surveillance data also showed an increase in invasive pneumococcal disease (IPD) between 1997/98-2005/06, from 8·8-11·9/100 000 at all ages, in addition to shifts in serotype distribution.[353] Another UK study reporting HES and Health Protection Agency (now part of Public Health England) data showed a similar trend in pneumococcal meningitis incidence from 1998-2005 at all ages, with the greatest disease burden occurring in infants aged 2-11 months.[115] A further study included reporting of HES data for pneumococcal meningitis in adults from 1996-1999.[369]

These hospital admission data demonstrate a reduction in pneumococcal meningitis and septicaemia in children following the introduction of PCV7 into the routine schedule in September 2006 at 2, 4 and 13 months along with a catch-up campaign. The impact of

vaccination on IPD hospital admissions statistics is less pronounced than for meningococcal and *H. influenzae* disease because these data are not restricted to vaccine type disease. Although the hospital admission rates are approximately double those reported in published microbiological surveillance data, there was a comparable reduction in pneumococcal meningitis reported in children less than 5 years old from 2000-06 to 2008-10.[149] The admission data also show a halving in pneumococcal meningitis rates in English children less than 15 years from 2006 to 2011 and a similar decline in pneumococcal septicaemia. Consistent with our data, laboratory surveillance data in England and Wales indicated an overall reduction in invasive pneumococcal disease (IPD) of 34% at all age groups, and 56% in children <2 years from 2000-06 to 2009-2010.[111,149] The overall reduction in pneumococcal meningitis reported in laboratory data from 2000-06 to 2008-10 was only significant in children less than 5 years of age (44% reduction) due to serotype replacement.[109,149]

No impact was observed in people aged ≥65 years on IPD hospital admissions from 1999 to 2011 which might have been expected as a result of increased herd immunity driven by the infant vaccine programme from 2006, or as a result of direct protection induced through the pneumococcal polysaccharide vaccine (PPV23) programme introduced in this age group from 2003.[370] In contrast to our study, laboratory surveillance data reported a 19% reduction in IPD from 2000-06 to 2009-10 in people ≥65 years following the introduction of the infant PCV7 vaccination programme in the UK.[149] This appears to be caused by a reduction in PCV7 serotypes through herd immunity rather than any impact of the PPV23 vaccine programme.[149,370]

Laboratory surveillance data indicated a greater reduction in IPD caused by PCV7 serotypes than the overall disease reduction because of serotype replacement.[16,18] There was a 98% reduction in IPD caused by PCV7 serotypes in children <2 years from 2000-06 to 2009-10 accompanied by a reduction in PCV7 serotypes of ≥75% in adults age ≥65 years through herd immunity.[111,149] Following the introduction of the PCV13 vaccine into the UK routine schedule from April 2010, microbiological surveillance data indicated that the number of IPD cases due to additional serotypes in PCV13 that are not in PCV7 halved in children under 2 years in the first epidemiological year following its introduction.[236]

Similar trends in hospital admissions from ICD coding for *H. influenzae* meningitis and IPD have been reported in the United States following the licensure of the Hib conjugate vaccine in December 1987 and PCV7 vaccine in 2000.[371,372] Following the licensure of the Hib conjugate vaccine there was an average decrease in admissions in children aged <5 years of 34% per year from 1988-91.[371] When comparing hospital admissions pre (1998-2000) and post (2001-2003) PCV7 licensure, pneumococcal meningitis reduced significantly by 67% at all ages. [372] The reduction in pneumococcal bacteraemia was only significant among adults aged ≥65 years.[372] Another USA study showed a reduction in pneumococcal meningitis when comparing hospital admissions from 1994-97 to 2001-04 of 66% among children aged <2 years, 51.5% in children aged 2-4 years, 26% in adults aged 18-39 years, and 33% in adults ≥65 years.[373]

There is a trend across the datasets to lower rates of hospital admissions in the 1960s-70s and a subsequent increase in incidence. There are various contributory factors that drive the observed fluctuations in incidence, though the relative contributions of each is not

well understood. Changes in diagnostics and coding are important causes of systematic changes in reporting, but various other factors including emergence and disappearance of new invasive bacterial clones, variation in population immunity, increases in transmission of predisposing viral infections, reduction in smoking rates and household crowding, and other social changes are all important.

Hospital admissions data show higher rates of meningitis and septicaemia than published microbiological surveillance data. This suggests that relying on microbiologically confirmed cases may underestimate disease incidence. Considering trends, however, similar profiles are seen in both hospital admission and microbiological surveillance data.

The study has several limitations. Data collection, including clinical coding, in the ORLS was managed by medically qualified epidemiologists from its establishment until 1985, and disease rates were broadly similar comparing ORLS and HIPE in the period covered independently by both suggesting both are likely to be reliable. Admission rates for meningococcal disease were a little lower in Oxford than all-England. Oxford is a relatively prosperous and healthy region and meningococcal disease in childhood tends to be associated with socio-economic disadvantage.[92,374] Rates in the Oxford data tended to be more fluctuant than the all-England rates, from instability in small numbers. There are no other regional datasets for comparison with Oxford in the years before HES. The quality and consistency of HES data are largely unknown. A recent systematic review in the UK suggests accuracy may be improving.[375] There is a potential that changes in hospital coding practise may have influenced the results. However, the trends shown in these data are consistent with published microbiological surveillance data.

Microbiological diagnostic techniques have improved during the study period which may account for some increase in reporting. Nevertheless, despite these limitations which would generally result in an increase in reported incidence, all the conditions studied have substantially reduced in recent years coincident with introduction of vaccine programmes targeted against them. Declining lumbar puncture rates are a potential confounding factor that may influence data precision. Although there has been discontinuity of ICD coding, because of revisions to the ICD itself, this does not appear to have impacted on trends. These data show no sudden changes in continuity at points of change of ICD.

In conclusion, vaccine preventable paediatric invasive bacterial disease has reduced dramatically in England with the advent of effective conjugate vaccines during the past 21 years. However, despite effective vaccine programmes, in England in recent years there were still appreciable numbers of children under 15 years admitted to hospital with meningococcal infection, pneumococcal septicaemia and meningitis, though relatively few with Hib meningitis or septicaemia, showing the importance of ongoing vaccine development and deployment to defend child health. Disease surveillance will continue to inform research into the priorities for development of effective vaccines and policy about immunisation schedules to further improve disease control.

## 4. Chapter 4: Hospital admission rates for viral meningitis in children in England over five decades: a population based observational study

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### 4.1. Introduction

A striking decline in bacterial meningitis caused by *Neisseria meningitidis, Haemophilus influenzae* type b, and *Streptococcus pneumoniae* has occurred in the UK following the implementation of highly effective conjugate vaccines (*chapter 3, section 3.3*).[169] Previous studies have reported that the majority of childhood meningitis in highly immunised populations is now aseptic, and most cases are caused by viruses when a cause is identified.[12,111,128,149,168,169,177] Before routine measles-mumps-rubella (MMR) vaccination, mumps was reported to be the commonest cause of viral meningitis in England and Wales, with epidemics occurring every 2-3 years.[182-184] Studies have suggested that enteroviruses are now responsible for most viral meningitis cases where a pathogen is identified.[172,178-180] However, for many cases of meningitis no cause is identified.[172,177-179]

Viral meningitis is usually a self-limiting disease and the majority of children require supportive management only. However, most children with cerebrospinal fluid (CSF) pleocytosis are admitted to hospital and receive intravenous antibiotics while a bacterial aetiology is excluded.[172,186] If a pathogen is not found, children often complete 10-14 days of treatment to cover the possibility of bacterial meningitis.[185,186]

During the past 40 years, introduction of vaccines, improvements in diagnostics, and the availability of antiviral therapy[36] for herpes simplex virus infection are likely to have impacted hospital admissions due to viral meningitis. MMR vaccination was introduced in the UK in 1988, and mumps disease almost disappeared.[182-184] The development of polymerase chain reaction (PCR) has significantly improved clinical diagnostics since the 1990s and is now widely applied to CSF samples.[20,22] Results from EV-PCR testing can be available rapidly where resources allow,[20,22] and it results in reduced length of hospital admission.[315,319,321]

Long-term trends in hospital admission rates for viral meningitis in children in England have not previously been reported. Improved knowledge of the epidemiology and true burden of hospital admissions is important to inform future research into prevention through vaccination, improved rapid diagnostic techniques, clinical management guidelines, and as the basis for healthcare cost-effectiveness analyses.

The aim of this study was to analyse trends in hospital admission rates for viral meningitis in children in England from 1968 to 2011.

### 4.2. Methods

The methods used in this chapter are 'Methods for analysis of retrospective epidemiology from hospital admission datasets' (*chapter 2, section 2.1*).

## 4.3. Results

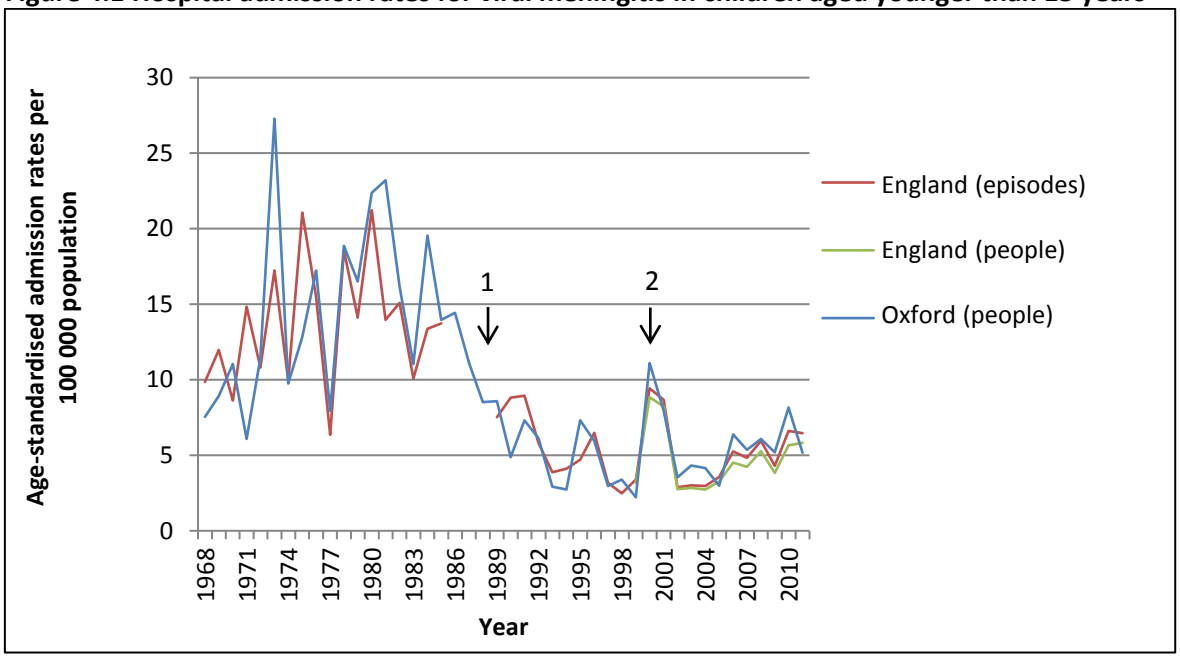
### *4.3.1. All cause viral meningitis*

There were 25 980 cases of childhood viral meningitis from 1968-1985 (mean 1443 per year) and 2584 in 2007-2011 (mean 517 per year). Overall admission rates from 1968-1985 varied annually, with peaks and troughs, but the mean was 13.5 episodes per 100 000 children per year (95% confidence interval 13.0-14.0) (*figure 4.1.*). Rates decreased in the mid to late 1980s, shown in the Oxford record linkage study (ORLS) for years in which national data were missing (*figure 4.1.*). Rates in England fell from 13.7 per 100 000 (11.3-16.2) in 1985 to 7.5 per 100 000 (7.0-8.1) in 1989. Subsequently rates remained relatively low, with a mean of 5.2 episodes per 100 000 children per year (5.1-5.3) in England from 1989-2011 (range 2·5 per 100 000 (2·2-2·8) to 9·4 per 100 000 (8·8-10·1)). There was a significant peak in 2000-01; the rate increased from 3.4 per 100 000 (3.0-3.8) in 1999 to 9.4 per 100 000 (8.8-10.0) in 2000, and fell to 2.9 per 100 000 (2.5-3.3) in 2002.

The decline only occurred in children aged 1-14 years (*figure 4.3.*). There was no decline in infants during the same period (*figure 4.2.*). The rates in children aged 1-4, 5-9 and 10-14 years were similar (*figure 4.3.*). In children aged 1-14 years, the mean rate from 1968-1985 was 12.8 per 100 000 children (12.3-13.3), and from 1989-2011 was 3.0 per 100 000 (2.9 to 3.1). There were small peaks in 2000 and 2001 (*table 4.1.*). The rates in infants fluctuated considerably from year to year. However, notably, the highest rates occurred in three of the last four years of the study: 59.9 per 100 000 infants (54.1-65.8) in 2008, 67 (60.9-73.3) in 2010, 70.0 (63.7-76.2) in 2011, caused by increased admission rates in infants aged ≤90 days (*figure 4.4.*).

The fall in rates in children aged 1-14 years has substantially changed the age profile of hospitalised children with viral meningitis. In 1968-1985, the vast majority of cases (89%, 23060/25980) were in children aged 1-14. The corresponding figures were 64% (3000/4669) in the 1990s, and 55% (1723/3144) in 2000-2006. By 2007-2011 the majority (73%, 1877/2584) were in infants. Male rates were higher than female rates, especially for children aged 1-14 years in the pre-1985 data (*figure 4.5*), and for infants after 1989 (*figure 4.6.*). The all-England data and the ORLS data, and the episode-based and person-based data, generally showed very similar rates (figures 4.1.-4.4.).

**Figure 4.1 Hospital admission rates for viral meningitis in children aged younger than 15 years**




Note: Arrow 1 shows the time of widespread introduction of MMR. Arrow 2 is the time of known outbreaks of Echovirus 13 and 30 in 2000 and 2001.

**Figure 4.2 Hospital admission rates for viral meningitis in infants aged younger than 1 year**

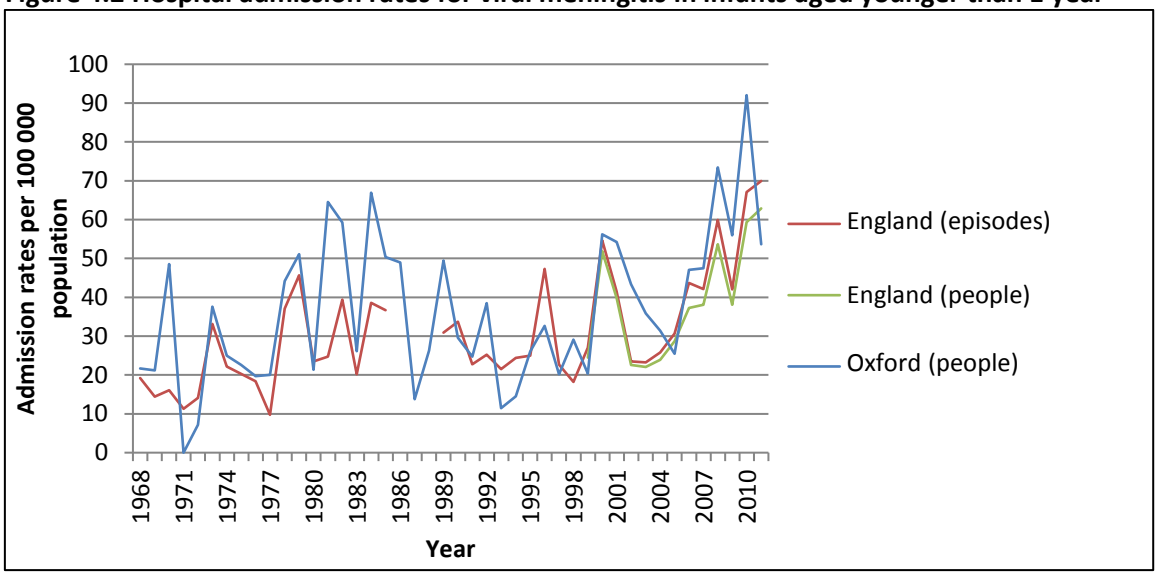



**Figure 4.3 Age-specific hospital admission rates for viral meningitis in age groups 1-4, 5-9 and 10-14 years, England 1968-2011**

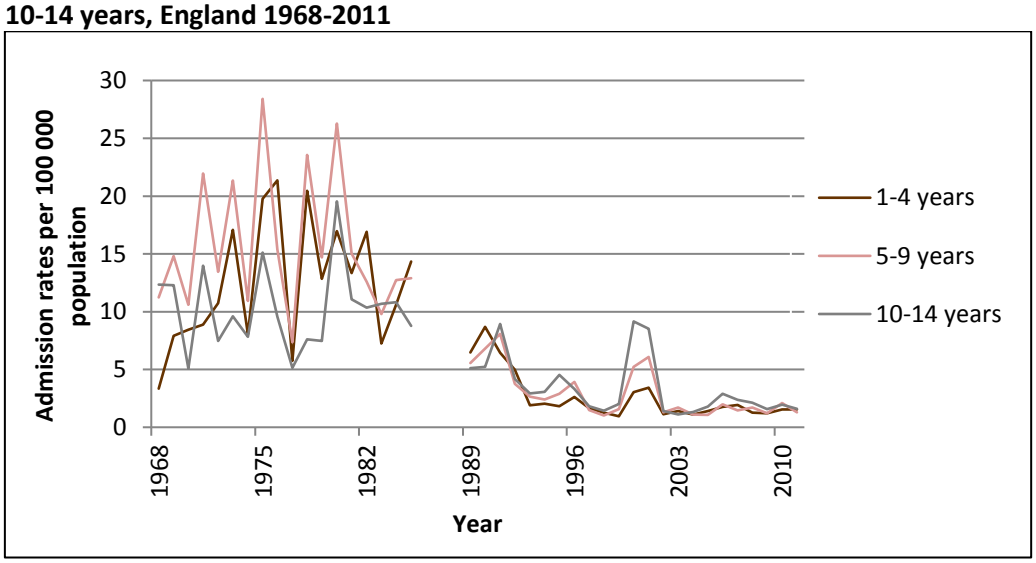
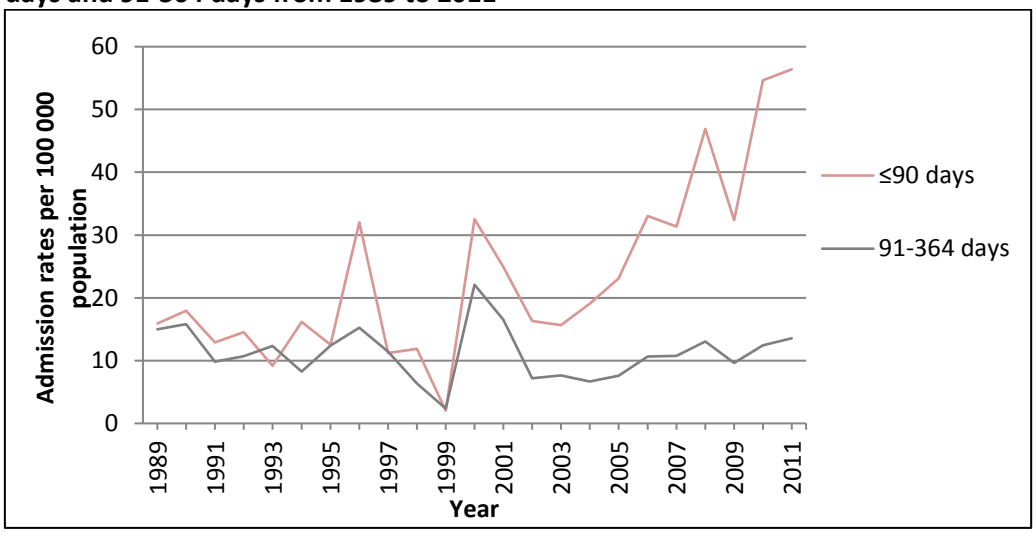



**Figure 4.4 Age-specific hospital admission rates for viral meningitis in infants in age groups ≤90 days and 91-364 days from 1989 to 2011**




Note: Figures 4.3 and 4.4 are episodes of care

**Figure 4.5 National hospital admission rates in children aged 1-14 years, comparing males and females**

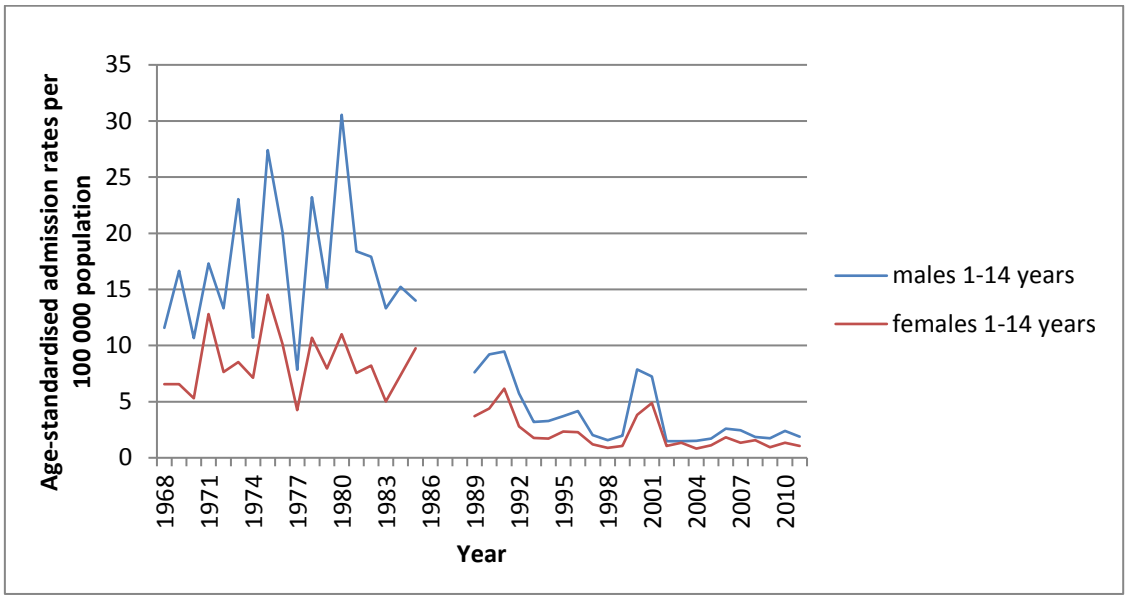
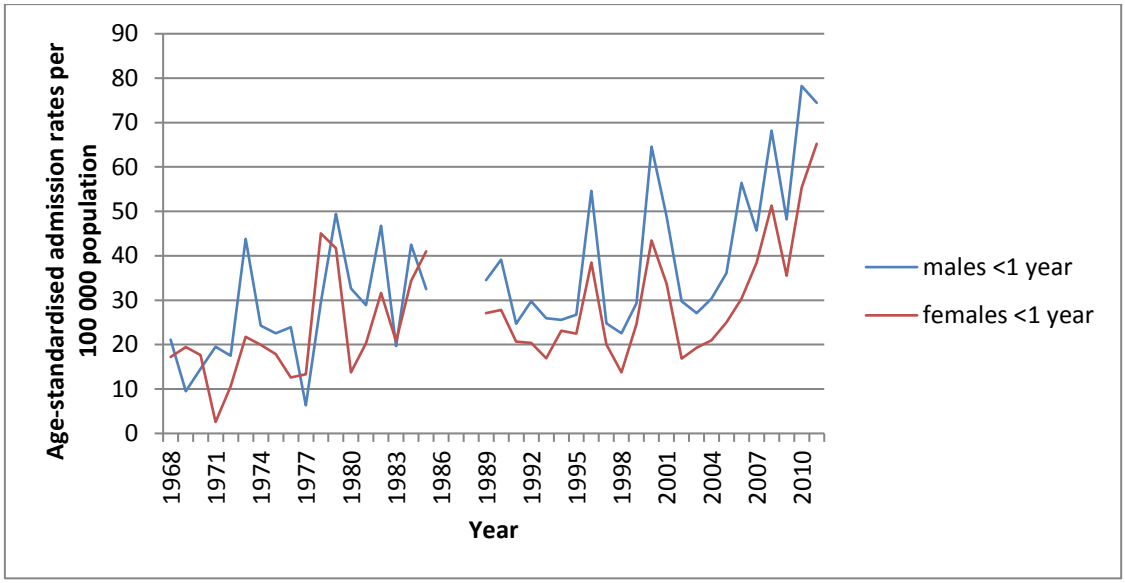



**Figure 4.6 National hospital admission rates in children aged younger than 1 year, comparing males and females**




The majority of viral meningitis cases were for aseptic meningitis without specification of virus type (*figures 4.7.-4.8.*). However, in recent years, an increasing percentage of cases had a specified virus, particularly in infants. From 1968-1985, 92% (2550/2770) of cases in infants and 89% (19820/22150) of cases in children aged 1-14 years were unspecified viral meningitis. By the last five years of the study, the corresponding figures were 50% (860/1716) in infants and 82% (544/666) in children aged 1-14 years.

The median length of hospital admission (LOS) for viral meningitis was longest from 1968-79. For infants, median LOS was five days from 1980-2004, and four days from 2005-2011. For children aged 1-14 years, median LOS was three days across the past three decades (*table 4.2.*).

**Figure 4.7 National hospital admission rates for all viral meningitis and for the subset of viral meningitis without specification of organism, children aged 1-14 years**

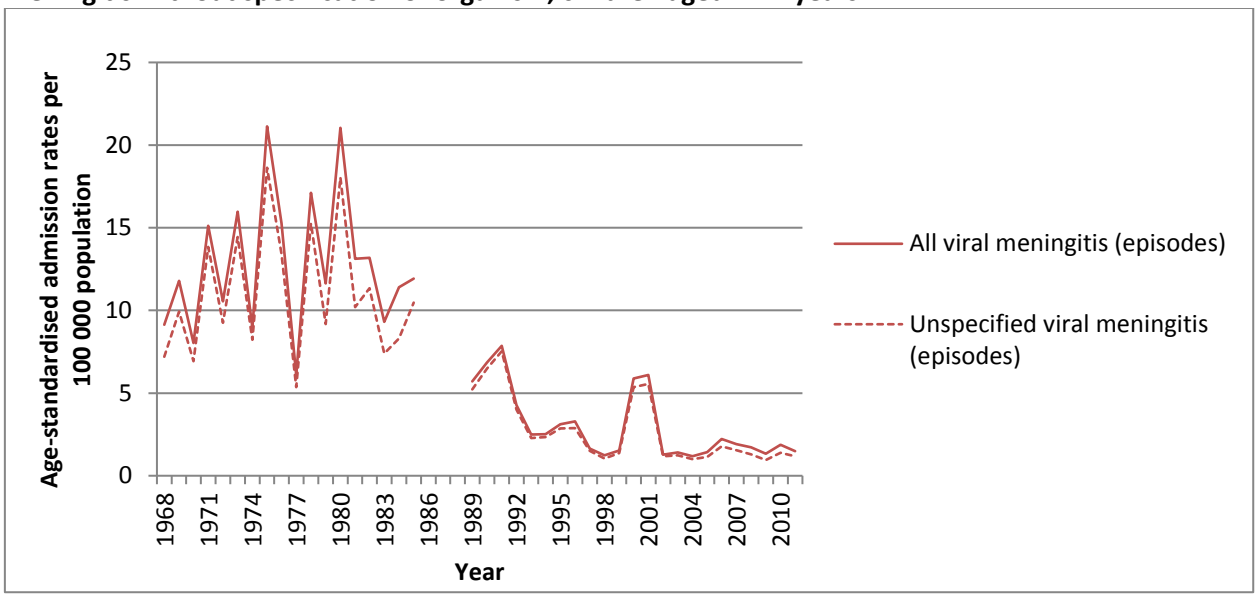



**Figure 4.8 National hospital admission rates for all viral meningitis and for the subset of viral meningitis without specification of organism, children aged <1 year**

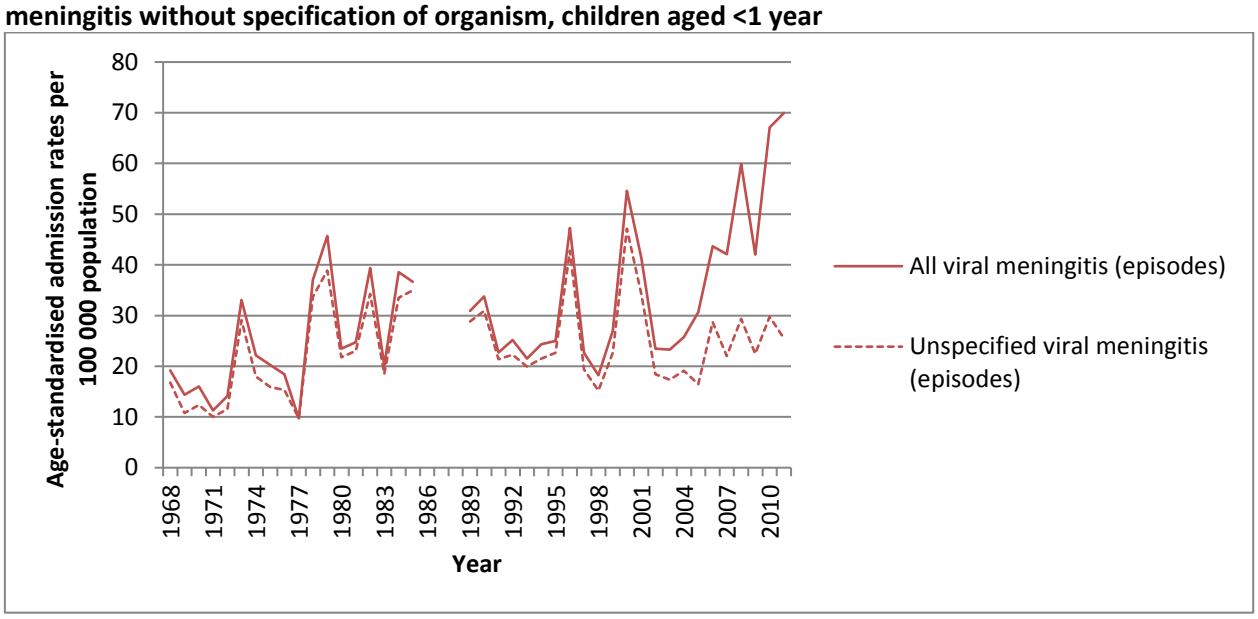


**Table 4.1 Hospital admission rates for viral meningitis in children younger than 15 years, England 1968-2011**

| Year | Age group (years) | | | | Age standardised rate | 95% CI |
|---|---|---|---|---|---|---|
| | < 1 | 1-4 | 5-9 | 10-14 | | |
| 1968 | 19·17 | 3·34 | 11·23 | 12·33 | 9·86 | 8.04 - 11.68 |
| 1969 | 14·41 | 7·90 | 14·81 | 12·30 | 11·97 | 9.97 - 13.98 |
| 1970 | 16·05 | 8·44 | 10·61 | 5·09 | 8·62 | 6.94 - 10.31 |
| 1971 | 11·28 | 8·88 | 21·94 | 13·97 | 14·83 | 12.64 - 17.02 |
| 1972 | 14·13 | 10·73 | 13·46 | 7·46 | 10·81 | 8.92 - 12.69 |
| 1973 | 33·1 | 17·09 | 21·33 | 9·60 | 17·22 | 14.82 - 19.62 |
| 1974 | 22·15 | 8·06 | 10·92 | 7·84 | 9·93 | 8.09 - 11.76 |
| 1975 | 20·25 | 19·77 | 28·41 | 15·1 | 21·07 | 18.39 - 23.75 |
| 1976 | 18·41 | 21·36 | 15·40 | 9·58 | 15·50 | 13.11 - 17.89 |
| 1977 | 9·74 | 5·75 | 7·37 | 5·13 | 6·36 | 4.83 - 7.89 |
| 1978 | 37·06 | 20·47 | 23·57 | 7·62 | 18·57 | 15.87 - 21.28 |
| 1979 | 45·68 | 12·85 | 14·71 | 7·46 | 14·11 | 11.72 - 16.50 |
| 1980 | 23·45 | 16·96 | 26·28 | 19·54 | 21·22 | 18.38 - 24.06 |
| 1981 | 24·71 | 13·34 | 15·02 | 11·06 | 13·97 | 11.62 - 16.33 |
| 1982 | 39·40 | 16·91 | 12·6 | 10·35 | 15·09 | 12.52 - 17.66 |
| 1983 | 20·19 | 7·24 | 9·8 | 10·67 | 10·09 | 8.02 - 12.16 |
| 1984 | 38·54 | 10·6 | 12·72 | 10·83 | 13·38 | 10.97 - 15.80 |
| 1985 | 36·64 | 14·33 | 12·89 | 8·77 | 13·73 | 11.26 - 16.19 |
| 1989 | 30·90 | 6·47 | 5·57 | 5·12 | 7·53 | 6.96 - 8.10 |
| 1990 | 33·74 | 8·70 | 6·81 | 5·23 | 8·81 | 8.20 - 9.43 |
| 1991 | 22·74 | 6·45 | 8·08 | 8·93 | 8·94 | 8.33 - 9.56 |
| 1992 | 25·19 | 4·98 | 3·77 | 4·17 | 5·80 | 5.32 - 6.31 |
| 1993 | 21·52 | 1·89 | 2·64 | 2·92 | 3·88 | 3.48 - 4.29 |
| 1994 | 24·38 | 2·05 | 2·41 | 3·07 | 4·11 | 3.70 - 4.53 |
| 1995 | 25·45 | 1·87 | 2·92 | 4·52 | 4·76 | 4.26 - 5.15 |
| 1996 | 47·29 | 2·62 | 3·91 | 3·30 | 6·49 | 5.96 - 7.03 |
| 1997 | 22·64 | 1·66 | 1·48 | 1·83 | 3·18 | 2.81 - 3.55 |
| 1998 | 18·24 | 1·26 | 1·02 | 1·44 | 2·48 | 2.15 - 2.81 |
| 1999 | 27·02 | 0·95 | 1·58 | 2·02 | 3·39 | 3.00 - 3.77 |
| 2000 | 54·61 | 3·03 | 5·23 | 9·16 | 9·43 | 8.79 - 10.07 |
| 2001 | 41·43 | 3·42 | 6·09 | 8·52 | 8·66 | 8.05 - 9.27 |
| 2002 | 23·46 | 1·12 | 1·30 | 1·41 | 2·89 | 2.53 - 3.26 |
| 2003 | 23·28 | 1·41 | 1·70 | 1·11 | 3·00 | 2.63 - 3.37 |
| 2004 | 25·78 | 1·11 | 1·09 | 1·33 | 2·97 | 2.60 - 3.33 |
| 2005 | 30·67 | 1·40 | 1·08 | 1·79 | 3·55 | 3.15 - 3.95 |
| 2006 | 43·70 | 1·76 | 1·99 | 2·91 | 5·25 | 4.77 - 5.73 |
| 2007 | 42·12 | 1·92 | 1·46 | 2·36 | 4·84 | 4.38 - 5.30 |
| 2008 | 59·93 | 1·26 | 1·72 | 2·13 | 5·95 | 5.45 - 6.45 |
| 2009 | 42·02 | 1·22 | 1·22 | 1·56 | 4·30 | 3.87 - 4.72 |
| 2010 | 67·07 | 1·54 | 2·10 | 1·95 | 6·61 | 6.09 - 7.14 |
| 2011 | 69·95 | 1·55 | 1·30 | 1·60 | 6·46 | 5.95 - 6.98 |

Note: no data 1986-88

| Table 4.2 Median length of hospital admission (LOS) in days for viral meningitis and enteroviral meningitis in children aged less than 14 years in England | | | | |
|---|---|---|---|---|
| **Viral meningitis** | | | | |
| **Year** | **0-1 year median LOS in days (interquartile range)** | **n** | **1-14 years median LOS in days (interquartile range)** | **n** |
| 1968-74 | 9 (5-14) | 141 | 6 (4-10) | 868 |
| 1975-79 | 7 (4-10) | 81 | 4 (2-7) | 722 |
| 1980-85 | 5 (3-7) | 109 | 3 (2-5) | 716 |
| 1989-94 | 5 (3-7) | 1021 | 3 (2-4) | 2511 |
| 1995-99 | 5 (3-7) | 849 | 3 (2-5) | 961 |
| 2000-04 | 5 (3-7) | 964 | 3 (2-4) | 1412 |
| 2005-09 | 4 (3-6) | 1406 | 3 (2-5) | 730 |
| 2010-11 | 4 (3-6) | 928 | 3 (2-5) | 288 |
| all years | 5 (3-7) | 5460 | 3 (2-5) | 8208 |
| **Enteroviral meningitis** | | | | |
| **Year** | **0-1 year median LOS in days (interquartile range)** | **n** | **1-14 years median LOS in days (interquartile range)** | **n** |
| 1968-74 | 15·5 (14-17) | 2 | 11 (8·5-15·5) | 12 |
| 1975-79 | 8 (5-27) | 3 | 3.5 (2-8) | 14 |
| 1980-85 | 10.5 (6·5-12·5) | 4 | 3.5 (2-4·5) | 8 |
| 1989-94 | 8 (5-10) | 29 | 2 (1-3·5) | 24 |
| 1995-99 | 7 (6-10) | 59 | 5 (2-6) | 21 |
| 2000-04 | 6 (4-8) | 132 | 3 (2-5) | 51 |
| 2005-09 | 5 (3-7) | 505 | 3 (2-5) | 57 |
| 2010-11 | 4 (3-6) | 452 | 3 (2-5) | 40 |
| all years | 5 (3-7) | 1186 | 3 (2-6) | 227 |

Note: no data 1986-88

*4.3.2. Enteroviral meningitis*

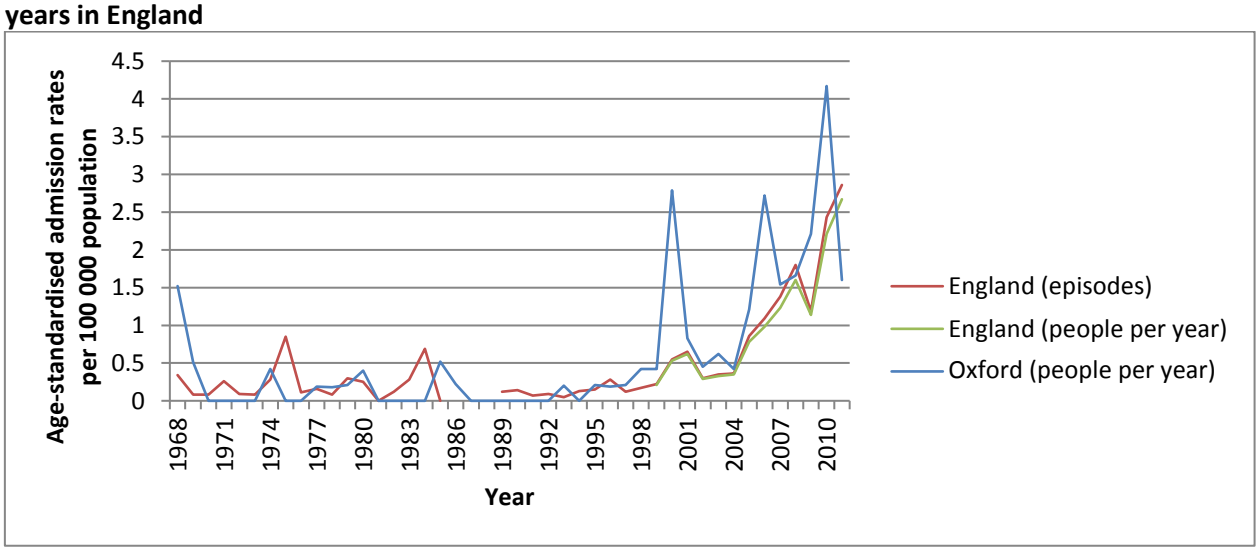
Hospital admission rates for enteroviral meningitis, including only the correct ICD codes (see below) for specific enteroviruses, remained low prior to 2000 at 0-0·9 episodes per 100 000 children (*figures 4.9.-4.10.*). There was an increase in admission rates from 2000, peaking at 2·9 episodes per 100 000 (2·5-3·2) for all children aged <15 years and 36·8 episodes per 100 000 (32.4-41.7) for infants in 2011 (*table 4.3.*). In 1968-1985 enteroviral meningitis was specified in 3% (90/2770) of viral meningitis cases in infants and 2% (340/22150) in children aged 1-14. By 2007-2011 enteroviral cases were specified in 47% (811/1716) of cases in infants and in 12% (77/666) of cases in children aged 1-14.

Consistent with viral meningitis trends overall, a peak in admission rates was seen in 2000-2001.

During the years covered by ICD versions 8 and 9, any unspecified viral or aseptic meningitis was classified within the three-digit code for 'meningitis due to enterovirus'. This is an error in the ICD. For completeness, we have included a separate analysis of these data including the codes wrongly classified as enteroviral meningitis, ICD-8 045·9 and ICD-9 047·9 (*figure 4.11.*), but the data for the early years are artefactual.

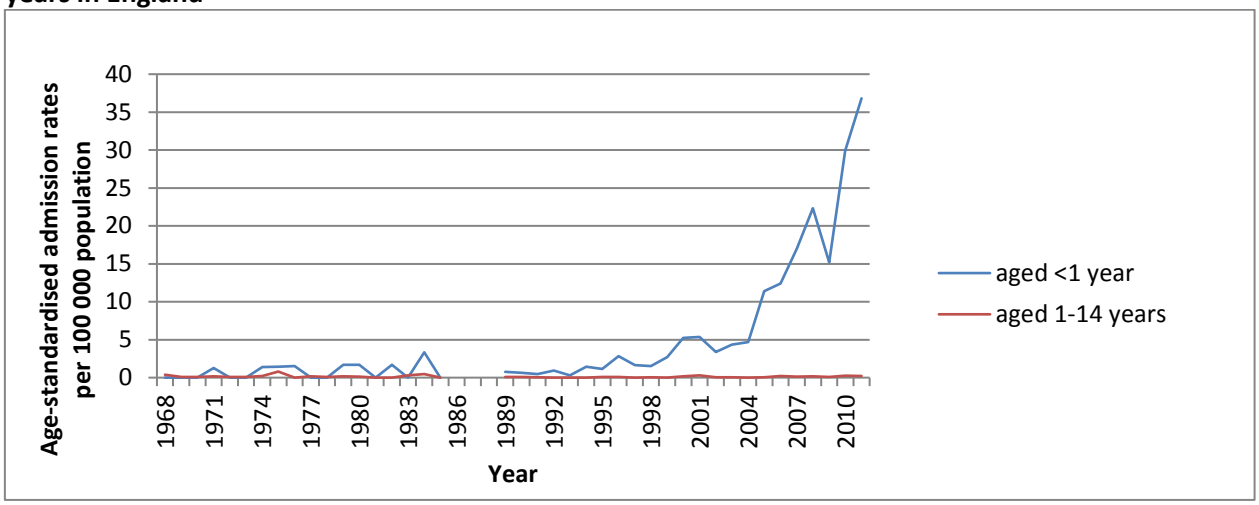
The median LOS for enteroviral meningitis (specific ICD codes) was 5 days for infants and 3 days for children 1-14 years (*table 4.2.*). In recent years, there was a reduction in LOS in infants from 6 days 2000-04 to 4 days 2010-11.

**Figure 4.9 Hospital admission rates for enteroviral meningitis in children aged younger than 15 years in England**




Note: These data exclude "unspecified" codes ICD9 047.9 and ICD8 045.9

**Figure 4.10 Hospital admission rates for enteroviral meningitis in children aged <1 and 1-14 years in England**




Note: These data exclude "unspecified" codes ICD9 047.9 and ICD8 045.9

**Figure 4.11 Hospital admission rates for "enteroviral" meningitis in children <15 years in England including "unspecified" codes ICD9 047.9 and ICD8 045.9 which the compilers of ICD9 erroneously classified as enteroviral**

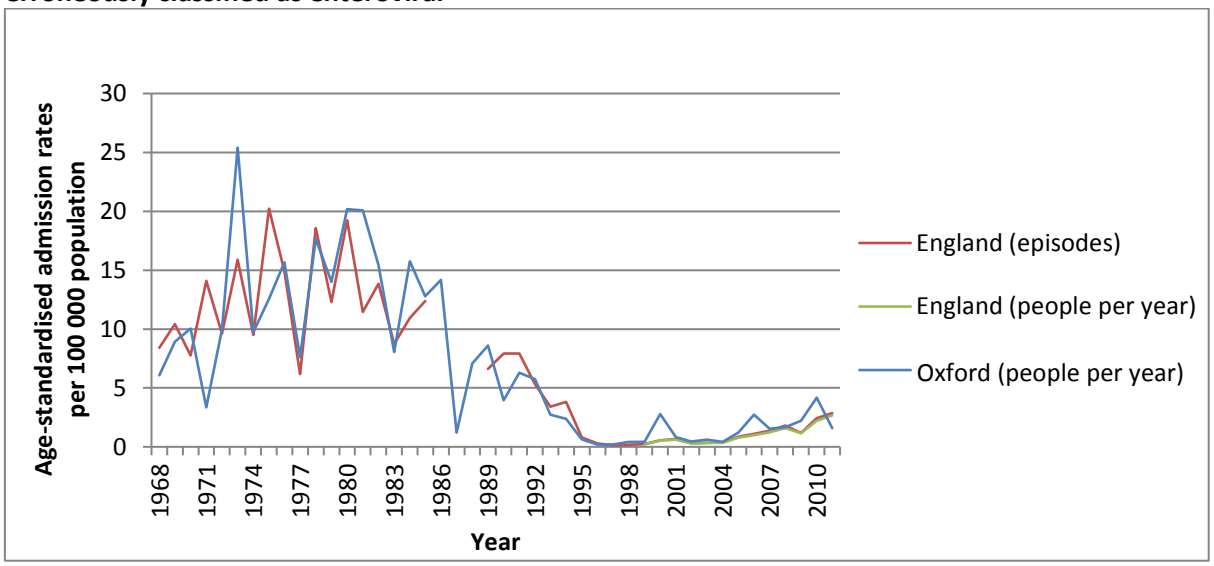


**Table 4.3 Hospital admission rates for EV meningitis in children younger than 15 years, England 1968-2011**

| Year | Age group (years) | | | | Age standardised rate | 95% CI |
|---|---|---|---|---|---|---|
| | < 1 | 1-4 | 5-9 | 10-14 | | |
| 1968 | 0 | 0·30 | 0·51 | 0·29 | 0·34 | 0.01 - 0.68 |
| 1969 | 0 | 0 | 0·26 | 0 | 0·08 | 0.00 - 0.24 |
| 1970 | 0 | 0 | 0·25 | 0 | 0·08 | 0.00 - 0.23 |
| 1971 | 1·25 | 0 | 0·25 | 0·27 | 0·26 | 0.00 - 0.55 |
| 1972 | 0 | 0·33 | 0 | 0 | 0·09 | 0.00 - 0.28 |
| 1973 | 0 | 0 | 0·25 | 0 | 0·08 | 0.00 - 0.23 |
| 1974 | 1·38 | 0·35 | 0 | 0·25 | 0·28 | 0.00 - 0.61 |
| 1975 | 1·45 | 0·37 | 1·27 | 0·74 | 0·85 | 0.32 - 1.38 |
| 1976 | 1·53 | 0 | 0 | 0 | 0·11 | 0.00 - 0.33 |
| 1977 | 0 | 0 | 0 | 0·49 | 0·16 | 0.00 - 0.37 |
| 1978 | 0 | 0 | 0 | 0·25 | 0·08 | 0.00 - 0.23 |
| 1979 | 1·69 | 0 | 0·57 | 0 | 0·30 | 0.00 - 0.65 |
| 1980 | 1·68 | 0·42 | 0 | 0 | 0·25 | 0.00 - 0.58 |
| 1981 | 0 | 0 | 0 | 0 | 0 | 0.00 - 0.00 |
| 1982 | 1·71 | 0 | 0 | 0 | 0·12 | 0.00 - 0.37 |
| 1983 | 0 | 0 | 0 | 0·87 | 0·28 | 0.00 - 0.59 |
| 1984 | 3·35 | 0 | 1·09 | 0·30 | 0·69 | 0.14 - 1.24 |
| 1985 | 0 | 0 | 0 | 0 | 0 | 0.00 - 0.00 |
| 1989 | 0·78 | 0 | 0·17 | 0·04 | 0·12 | 0.05 - 0.19 |
| 1990 | 0·62 | 0·08 | 0·13 | 0·11 | 0·14 | 0.07 - 0.22 |
| 1991 | 0·45 | 0·04 | 0·03 | 0·03 | 0·07 | 0.01 - 0.12 |
| 1992 | 0·92 | 0 | 0 | 0·07 | 0·09 | 0.03 - 0.15 |
| 1993 | 0·32 | 0·08 | 0 | 0 | 0·05 | 0.00 - 0.09 |
| 1994 | 1·42 | 0·08 | 0 | 0 | 0·13 | 0.05 - 0.20 |
| 1995 | 1·14 | 0·08 | 0·09 | 0·03 | 0·15 | 0.07 - 0.23 |
| 1996 | 2·82 | 0·08 | 0·03 | 0·13 | 0·28 | 0.17 - 0.39 |
| 1997 | 1·63 | 0 | 0 | 0 | 0·12 | 0.05 - 0.19 |
| 1998 | 1·51 | 0 | 0·09 | 0·10 | 0·17 | 0.08 - 0.26 |
| 1999 | 2·70 | 0·04 | 0 | 0·03 | 0·22 | 0.12 - 0.32 |
| 2000 | 5·22 | 0·04 | 0·13 | 0·38 | 0·55 | 0.39 - 0.71 |
| 2001 | 5·38 | 0·25 | 0·38 | 0·19 | 0·65 | 0.47 - 0.82 |
| 2002 | 3·40 | 0·04 | 0·10 | 0·03 | 0·30 | 0.18 - 0.42 |
| 2003 | 4·34 | 0 | 0·07 | 0·03 | 0·35 | 0.22 - 0.48 |
| 2004 | 4·69 | 0 | 0·03 | 0·03 | 0·36 | 0.23 - 0.49 |
| 2005 | 11·38 | 0 | 0·10 | 0 | 0·86 | 0.66 - 1.06 |
| 2006 | 12·42 | 0·21 | 0·14 | 0·26 | 1·09 | 0.87 - 1.31 |
| 2007 | 17·00 | 0·08 | 0·17 | 0·19 | 1·38 | 1.13 - 1.62 |
| 2008 | 22·33 | 0·08 | 0·28 | 0·20 | 1·80 | 1.52 - 2.07 |
| 2009 | 15·21 | 0·08 | 0·10 | 0·10 | 1·19 | 0.97 - 1.42 |
| 2010 | 29·91 | 0·31 | 0·34 | 0·17 | 2·43 | 2.11 - 2.75 |
| 2011 | 36·81 | 0·23 | 0·20 | 0·16 | 2·86 | 2.52 - 3.20 |

Note: no data 1986-88

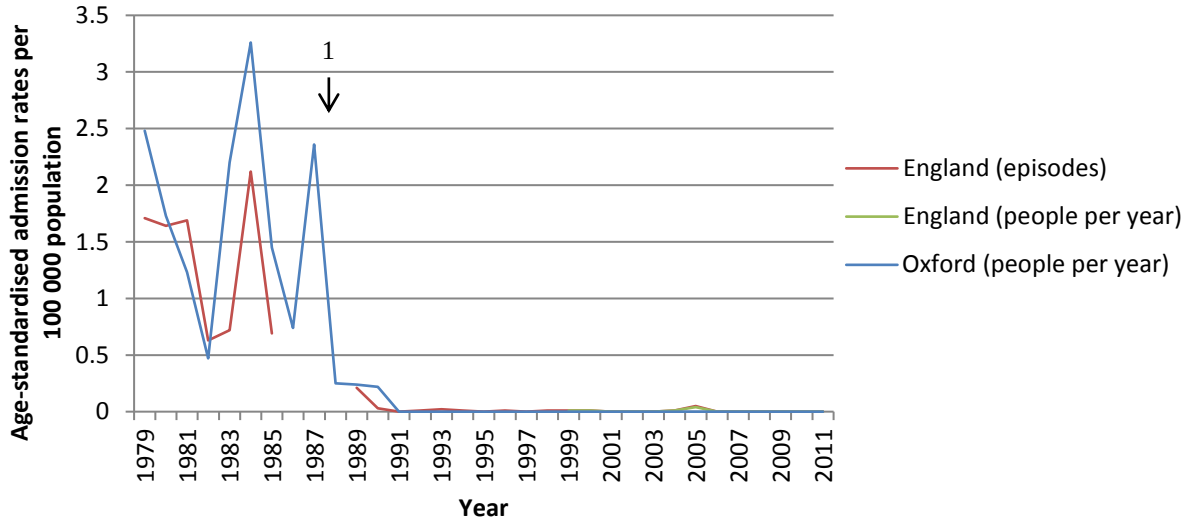
*4.3.3. Mumps and measles meningitis*

The hospital admission rate for mumps meningitis from 1979-1985 was between 0·6 (0·1-1·1) and 2·1 (1·2-3·1) episodes per 100 000 children (*figure 4.12*). Admissions for mumps meningitis almost disappeared from 1990 following the 1988 introduction of the MMR vaccination. A small peak in admissions occurred in 2005 at 0·05 episodes per 100 000 (0·01-0·10). Measles meningitis did not have a separate ICD code prior to 1995, and from 1995-2011, only one hospital admission was recorded.

*4.3.4. VZV and HSV meningitis*

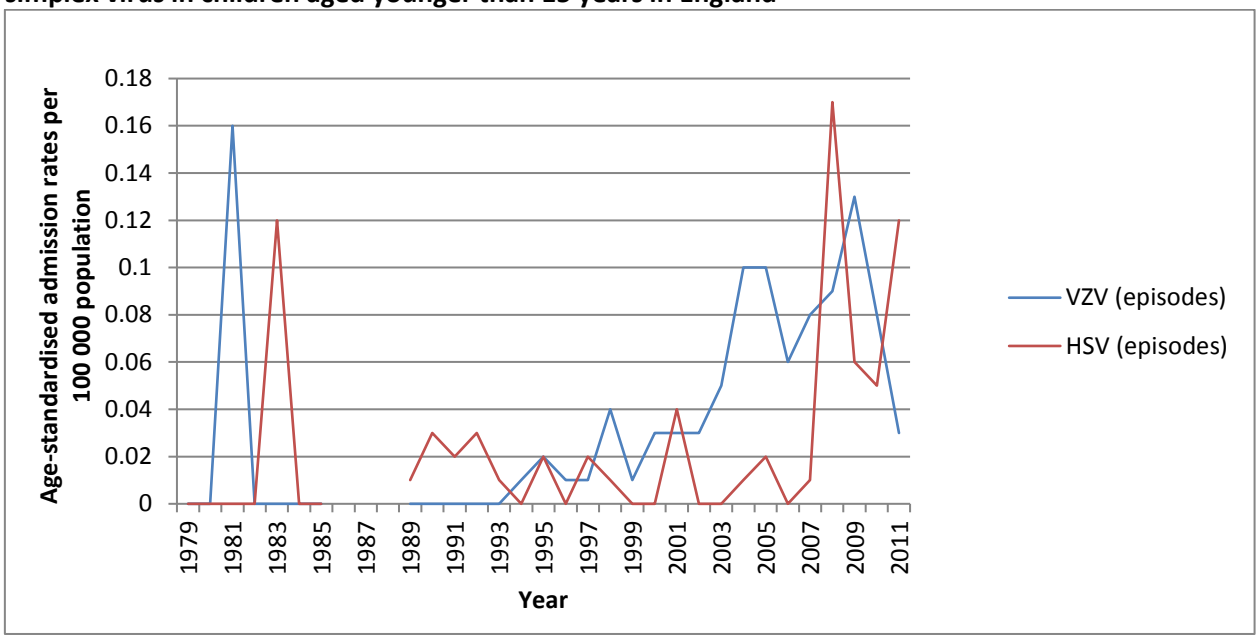
Hospital admissions coded as meningitis caused by VZV were low from 1979-2002 with the exception of a peak in 1981 (*figure 4.13.*). An increase in admission rates occurred from 2003-2011, coinciding with increased use of CSF PCR. There were similar low rates of hospital admissions coded as meningitis caused by HSV from 1979-2007 with a peak seen in 1983. HSV meningitis admission rates increased in the late 2000s, but remained low.

**Figure 4.12 Hospital admission rates for mumps meningitis in children aged younger than 15 years in England**




Arrow 1: the 1988 introduction of MMR vaccination.

**Figure 4.13 Hospital admission rates for meningitis caused by varicella zoster virus and herpes simplex virus in children aged younger than 15 years in England**




## 4.4. Discussion

Overall, admission rates for viral meningitis in children fell by almost two-thirds in the period covered by this study. The decline occurred only in the age group 1-14 years. Viral meningitis admission rates in infancy, conversely, increased in the last few years of the study. The contrasting changes over time in the two age groups have substantially altered the age-related epidemiological profile of viral meningitis in childhood. Mumps meningitis has almost disappeared.

The reduction in admissions due to childhood viral meningitis was abrupt, and occurred during the late-1980s. The great majority of cases of viral meningitis in hospital statistics, both historically and in recent years, are recorded without a specific virus type. It is therefore difficult to be precise about the aetiological components of the decline. One factor known to have changed is the 1988 widespread introduction of immunisation against mumps,[182,376] probably the commonest cause of childhood viral meningitis pre-

1988.[182-184] Other viruses, notably enteroviruses, also caused quite a high proportion of unspecified viral meningitis cases then.[172,178,319] However, it seems likely that much of the large decline in rates of 'unspecified' viral meningitis, between 1985 and 1989, was an unrecognised decline in mumps meningitis.

Age-related differences in clinical practice may also have contributed to differences in admission rates observed for children and infants. LPs have probably been performed less frequently recently.[256] Most LPs are performed in young infants as part of a sepsis screening protocol for febrile children, whereas older children will only have an LP if there is clinical suspicion of meningitis.[172,186] This may mean that diagnoses of viral meningitis are missed more often in children than infants. Furthermore, enteroviral meningitis in the absence of CSF pleocytosis has been increasingly described, specifically in young infants.[73,277] Although practices probably differ between hospitals for performing CSF PCR in acellular samples,[73] these cases are unlikely to have been detected prior to widespread use of PCR, and may have contributed to increased admission rates in young infants compared with older children.

Improved aetiological attribution has resulted from availability of PCR testing of CSF samples and this is reflected also in the arrival of specific coding for enteroviral meningitis. However, there does also seem to have been a recent absolute increase in total viral meningitis admission rates for young infants, indicating either a change in medical practice, or a true underlying increase in disease incidence. Stochastic changes in the biological properties of enteroviruses and changes in host immunity could contribute to an increase in incidence.[200] PCR testing is also likely to have significantly contributed to

the decrease in proportion of viral meningitis coded without specification of type. Improving microbiological diagnostics means that clinicians can diagnose viral meningitis more accurately. Prior to the widespread availability of viral PCR, CSF pleocytosis was usually treated as bacterial meningitis.[172,185,186,315,319]

Several studies have shown reduction in LOS and antibiotic use for children who are CSF EV PCR positive, compared with children who are tested and are EV PCR negative.[315,319,321] The median LOS of 3-5 days for viral and enteroviral meningitis observed in these present data is longer than has been previously reported in studies from other countries, which typically report LOS of 2-3 days,[22,172,185,186,313,315,317,319,321] suggesting room for improvement in availability of rapid PCR results in the UK.

These hospital datasets are the most complete data available in England to report historic population-based incidence of cases termed as viral meningitis. Statutory infectious disease notifications[354] are frequently incomplete,[377] and mortality statistics from death registrations[357] do not provide disease burden estimates. Microbiologically confirmed datasets are limited, because they do not include cases of with no pathogen identified, which are the majority in these data. The peak in admissions for viral meningitis noted in our data in 2000-2001 was also seen in laboratory notification data in the UK.[378,379] Although the peak in enteroviral meningitis hospital admissions during this period is smaller than the peak in overall viral meningitis, the increase in hospitalisations is likely to have been caused by reported outbreaks of laboratory confirmed cases of meningitis caused by echovirus 13 in 2000 and echovirus 30 in 2001 in England and Wales.[195,199] The increase in viral meningitis hospitalisations observed from 2002 to 2011 is consistent with

a recent study that reported an increase in meningoencephalitis laboratory confirmed cases from 0·6/100 000 in 2004 to 3·9/100 000 in 2013 at all ages in England and Wales.[180] One third of these cases occurred in children, with incidence peaking at 329/100 000 in infants <3 months in 2013.[180]

Although the proportion of viral meningitis admissions attributed to enteroviruses increased in 2007-11 to 47% in infants and 12% in children aged 1-14 years, as expected, these proportions are lower than were reported by a recent UK laboratory study,[180] which identified enteroviruses in 92% of cases with a pathogen found among infants <3 months, and 52% at all ages.[180] Microbiological surveillance from 1975 to 1994 identified CSF enteroviral isolates in 2/100 000 infants which is higher than our reported rates for specific enteroviral meningitis codes.[197]

Human parechovirus 3 causes central nervous system infection and sepsis-like illness in young infants.[201,217] Parechoviruses were only reclassified as a distinct genus from enteroviruses in 1999,[380] and therefore have not yet been coded separately within the ICD. Notably, peaks of viral meningitis hospital admissions in young infants in these present data appear to match reported peaks of parechoviral disease incidence occurring biennially in even years in studies from the UK and Europe since the mid-2000s.[196,201,217]

The small increase in mumps meningitis admissions seen in 2005 is consistent with a mumps outbreak in England which occurred in 2004-05.[376] This was partly caused by the presence of an unimmunised cohort in the population, and incomplete immunisation

coverage, demonstrating the importance of maintaining high two dose vaccine coverage even when rates of background disease are low.[376]

Although there are few comparable studies, hospital admission rates for viral meningitis in the UK are lower than are reported by studies from the USA, but appear consistent with data from other countries in Western Europe.[183,187-190] Hospitalization rates for viral meningitis in one USA study from 1988-99 were 213/100 000 for infants, and 14/100 000 for children aged 1-4 and 5-19 years.[187] Another USA study of emergency department admissions for all-cause meningitis from 1993-2008 identified 31 visits per 100 000 children <18 years, with 91% of visits for viral or unspecified meningitis in adults and children.[188] A birth cohort study of 12000 children in Finland from 1966 reported a viral meningitis incidence from hospital data of 28/100 000 per year in children <14 years and 219/100 000 in infants.[183] A Danish study based on ICD coding, from 1977-2001, observed 39/100 000 per year viral meningitis hospitalisations in the first 6 months of life, and between approximately 4-16/100 000 hospitalisations at ages 1-14.[189] Although the admission rates are higher in the Finnish study,[183] the rates reported in the Danish study[189] and a Greek study[190] are comparable with these present data, particularly before the reduction observed in the UK during the 1980s. The higher admission rates reported in the USA may be caused by differences in coding, healthcare behaviour or diagnostic practices.

The study has several limitations. As discussed in *chapter 3, section 3.4.*, the quality and consistency of HES data are largely unknown. Data precision is likely to have been affected by changes in clinical practice, for example changes in numbers of LPs performed

for febrile children, in addition to advances in microbiological diagnostic techniques. Changes in codes and terminology for viral meningitis across the relevant three editions of the ICD and changes in hospital coding practice could also have affected our findings. ICD coding for specific viral aetiologies are not always available, for example parechoviruses. Nonetheless, the trends shown are consistent with available microbiological surveillance data, rates reported by the ORLS and HIPE corroborate each other suggesting both datasets are probably reliable, and these are the only long term population datasets of their kind.[180,199,378]

In conclusion, viral meningitis hospital admission rates reduced significantly from 1968-85 to 1989-2011 in children aged 1-14 years. In contrast, viral meningitis admission rates in infants have increased in recent years. The impact of mumps vaccination, the widespread use of PCR allowing detection of specific viruses, and differences in clinical management of children related to their age likely contributed to these findings. Prospective cohort studies, like the UK-ChiMES study described in *chapters 5-8*, were needed to further assess the clinical disease burden of viral meningitis.

# 5. Chapter 5: The current aetiology of childhood meningitis in the UK: a prospective cohort study.

## 5.1. Introduction

Retrospective analyses of the epidemiology of childhood meningitis in the UK from the 1960s until 2011 using hospital coding data were reported in *chapters 3* and *4*. These studies demonstrated substantial changes over time in the causes of childhood meningitis, likely influenced by vaccine programmes, improvements in diagnostic capabilities and changes in circulating bacterial and viral strains. Prospective data were required to accurately assess the current causes of childhood meningitis in the UK.

Further changes to the UK vaccine programme relevant for bacterial meningitis have also occurred since 2011, with the 2015 introduction of both routine vaccination for infants to protect against MenB,[238,240] and MenACWY replacing MenC vaccination for adolescents.[238,381]

The aim of this study was to analyse the current aetiology of childhood meningitis, using data collected prospectively from children presenting with suspected meningitis to hospitals across the UK, from December 2012 to June 2016. The secondary aim was to assess antibiotic pre-treatment and the proportion of children receiving antibiotic management, length of stay, and the proportion of children investigated by PCR testing for sub-groups of children with meningitis of different aetiologies.

## 5.2. Methods

### *5.2.1. Participant inclusion and data collection*

General methods and inclusion criteria for the UK-ChiMES study were reported in *methods chapter 2, section 2.2*.

### *5.2.2. Definitions*

The CSF white blood cell (WBC) count was corrected for red blood cell count at a ratio of 500X$10^6$/L RBC:1 X$10^6$/L WBC.

A raised CSF WBC count (CSF pleocytosis) was defined as follows:[106]

Age 0-28 days: CSF WBC ≥20 X$10^6$/L

Age >28 days: CSF WBC >5 X$10^6$/L

Definite bacterial meningitis: Bacterial pathogen in CSF, or raised CSF WBC and relevant pathogen present in blood on culture or PCR, or CSF gram stain positive and corresponding pathogen present in blood. Relevant pathogens in blood included *N. meningitidis, S. pneumoniae, H. influenzae,* Group B Streptococcus, *E. coli*, *S. aureus* and Group A Streptococcus.

Definite viral meningitis: Raised CSF WBC and viral pathogen found in CSF or blood by PCR, culture or serology, OR no raised CSF WBC count and enterovirus, parechovirus or HSV present in CSF by PCR or culture.

Probable viral meningitis: Raised CSF WBC, and enterovirus or parechovirus present in stool by PCR or culture.

Probable bacterial meningitis: Raised CSF WBC and pure growth of bacterial pathogen in urine.

Aseptic meningitis: Raised CSF WBC and no bacterial pathogen identified. Aseptic meningitis includes atypical organisms, and any non-bacterial cause identified.

Aseptic meningitis with unknown aetiology: Raised CSF WBC and no causative pathogen identified.

Possible meningitis: Pathogen known to cause bacterial meningitis in blood and no evaluable LP result, or pathogen of uncertain significance in CSF and either no available or no raised CSF WBC, or discharge diagnosis of meningitis but with no other features of confirmed or probable meningitis.

Meningoencephalitis: Meningitis as previously defined and discharge diagnosis of encephalitis or meningoencephalitis.

Possible encephalitis: No raised or no available CSF WBC, and discharge diagnosis of encephalitis.

Definite encephalitis cases were not defined for this analysis because of incomplete neuroimaging and neurophysiology data, which are required for the encephalitis international consensus case definition.[382]

The following organisms were defined as contaminants in CSF or blood cultures unless not treated as a contaminant by the hospital site: coagulase-negative staphylococci (because the cohort only included neonates admitted from home, not neonates in the

neonatal intensive care unit), diptheroids, micrococci, bacillus species, corynebacterium, alpha haemolytic Streptococci, *Pseudomonas oryzihchitens*, *Pantoea dispersa*, Kocuria species, *Moraxella osloensis*, *Granulicatella elegans*, *Neisseria elongata*, and *Proprionibacterium acnes*.

*5.2.3. Aetiology data analysis*

Meningitis aetiology was analysed in age groups 0-28 days, 29 days-<3 months, 3-11 months, 1-4 years, and 5-15 years. Cause of meningitis was defined by analysis of results from routine laboratory tests performed at hospital sites including CSF, blood or other site culture and PCR results, CSF WBC, CSF Gram stain, serum serology, and by discharge diagnosis recorded in the CRF. Tests performed at hospital sites were determined by the clinical team managing the patient. The diagnosis for children who did not have meningitis was also analysed by results of laboratory tests performed at hospital sites and discharge diagnosis recorded in the CRF. The number of cases recruited by month and year was analysed separately for EV, parechoviral, pneumococcal, meningococcal, and all meningitis, all participants. Children with inadequate data to assess diagnosis were excluded from analysis. Participants who withdrew were included if adequate data were obtained prior to withdrawal.

*5.2.4. Other clinical data analysis*

Demographic data including age and sex, and mortality, were analysed. The number of participants who had CSF or blood PCR tests performed by meningitis aetiology, and length of hospital admission and proportion of children receiving antibiotics including

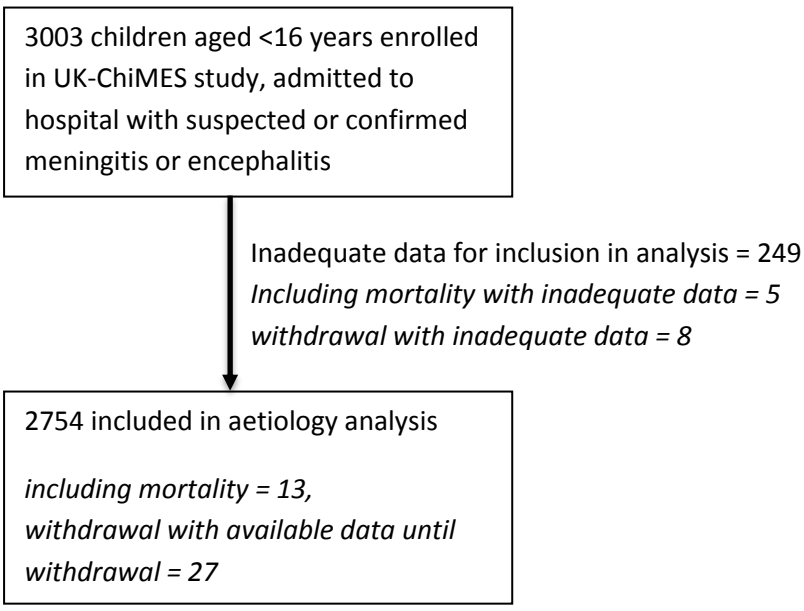
pre-treatment prior to LP for specified meningitis aetiologies, was analysed. The Pearson Chi-Squared tests was used to analyse categorical variables and the Wilcoxon rank-sum test was used to compare non-parametric continuous data. A P-value of <0.05 was defined as significant. Participants were excluded from specific analyses if relevant data were missing. All analyses were performed using SPSS.

## 5.3. Results

### *5.3.1. Participant inclusion, demographics, mortality and overall diagnoses*

There were 2754 out of 3003 children aged <16 years recruited to the UK-ChiMES study from December 2012 to June 2016 included in this analysis of aetiology. 249 children were excluded from the aetiology analysis due to inadequate data *(figure 5.1)*. Of 27/35 participants who withdrew, adequate data for inclusion in analysis were obtained prior to withdrawal. Reasons for withdrawal were patient or relative request (80%, 28/35), clinician request (11%, 4/35), social issues or change in social circumstances (6%, 2/35), and intercurrent illness (3%, 1/35). Mortality for any reason including during the 18 months follow-up period was 18/3003 enrolled children and infants *(table 3.1)*.

**Figure 5.1 Participant enrolment in UK-ChiMES study, and inclusion in aetiology analysis**



| **Table 5.1 Mortality for enrolled children and infants who died at any time during the UK-ChiMES study** | | | |
|---|---|---|---|
| Age at admission | Presenting diagnosis | Timing of death | Reason for death |
| Presented with meningitis or meningoencephalitis | | | |
| <1 month | Enteroviral meningitis | 2 weeks following discharge | Respiratory failure, spinal muscular atrophy type 2 |
| 2 months | Aseptic meningitis unknown aetiology | 14 months following discharge | Uncertain |
| 10 years | Aseptic meningitis unknown aetiology | 3 days following two-month admission | Uncertain, discharge diagnosis suggests encephalitis with mild raised CSF WBC, results do not indicate aetiology |
| 15 years | Pneumococcal meningitis | During admission | Pneumococcal meningitis |
| Presented with a non-meningitis illness | | | |
| 1 month | Mitochondrial encephalopathy | During admission | Related to chronic conditions |
| 1 month | Possible encephalitis | 21 months following discharge | Uncertain |
| 2 months | Lower respiratory tract infection, epilepsy | 3 months following discharge | Related to chronic conditions |
| 9 months | Neurodegenerative condition | During admission | Neurodegenerative condition |
| 1 year | Status epilepticus, regression motor skills | 3 months following discharge | Related to chronic conditions |
| 1 year | Possible encephalitis, previous seizures | 11 months following discharge | Uncertain |
| 3 years | Chronic metabolic disease | During admission | Related to chronic conditions |
| 7 years | Upper airway obstruction, global developmental delay, seizures, genetic syndrome | 1 day following discharge | Related to chronic conditions |
| 14 years | UTI | 3 months following discharge | Uncertain |
| Participants excluded from analyses due to inadequate data | | | |
| <1 month | Disseminated HSV2 infection | During admission | Presumed relating to presenting illness |
| 2 months | Uncertain, chronic medical conditions | 9 months following admission | Uncertain |
| 1 year | Neurodegenerative condition, presumed | During admission | Neurodegenerative condition |
| 2 years | Status epilepticus | During admission | Presumed relating to presenting illness |
| 8 years | Pneumonia, cerebral palsy, chronic renal impairment | 1 month following admission | Uncertain |

Demographics of included children and infants are shown in *table 5.2.* The median age was two months, and 57.7% (1588/2754) were male. Overall, 32.4% (892/2754) had meningitis (from here onwards 'meningitis' refers to meningitis or meningoencephalitis) *(table 5.3).*

| **Table 5.2 Demographics of children and infants included in aetiology analysis** | |
|---|---|
| Age at admission, *median (IQR), n* | 2 months (0-11 months), n=2754 |
| Male, *% n/N* | 57.7%, 1588/2754 |
| Ethnicity, *% n/N* | |
| *white* | 76.1%, 2096/2754 |
| *mixed* | 4.3%, 118/2754 |
| *Asian or Asian British (included Chinese)* | 9.8%, 270/2754 |
| *Black* | 4.5%, 123/2754 |
| *Other* | 0.3%, 8/2754 |
| *Unknown* | 5.0%, 139/2754 |
| Immunized*, *% n/N* | 82.2%, 1765/2147 |
| Development reported normal, *% n/N* | 96.4%, 2572/2667 |

Note: *Fully immunized by age defined as all primary immunisations by age 6 months, boosters by age 15 months, preschool boosters by age 4 years.

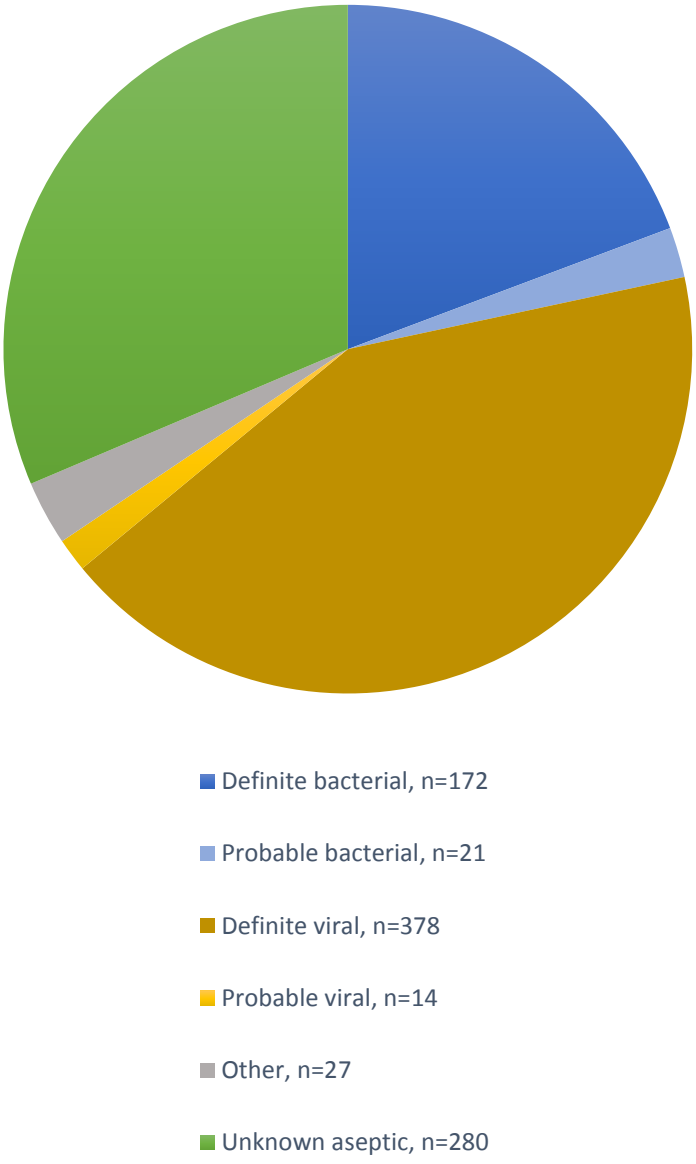
| Table 5.3 Diagnosis of all participants in UK-ChiMES study | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0-28 days, n | 29 days - <3months, n | 3-23 months, n | 2-4 years, n | 5-15 years, n | All ages, percentage of meningitis, possible or non-meningitis, % (n/N) | All ages, total % of all cases, % (n/N) |
| Meningitis cases, n=892 | | | | | | | |
| Bacterial meningitis | 40 | 29 | 64 | 20 | 16 | 19.3% (172/892) | Meningitis 32.4% (892/2754) |
| Bacterial meningoencephalitis | | | 3 | | | | |
| Probable bacterial meningitis | 3 | 9 | 9 | | | 2.4% (21/892) | |
| Viral meningitis | 136 | 158 | 41 | 1 | 11 | 42.4% (378/892) | |
| Viral meningoencephalitis | 5 | 6 | 8 | 2 | 10 | | |
| Viral and antibody*, or viral and atypical meningoencephalitis | | | | 1 | 2 | 0.3% (3/892) | |
| Probable viral meningitis | 2 | 4 | 2 | 2 | | 1.6% (14/892) | |
| Probable viral Meningoencephalitis | | | 2 | | 2 | | |
| Atypical bacteria/ TB meningitis | | | | | 2 | 0.6% (5/892) | |
| Atypical bacteria meningoencephalitis | | | 1 | | 2 | | |
| Antibody mediated meningoencephalitis | | | | | 2 | 0.2% (2/892) | |
| Other aseptic meningitis | | | 3 | 1 | 1 | 0.6% (5/892) | |
| ADEM with meningitis | | 1 | 1 | 3 | 7 | 1.3% (12/892) | |
| Meningitis unknown cause | 44 | 94 | 51 | 21 | 34 | 31.4% (280/892) | |
| Meningoencephalitis unknown cause | 1 | 1 | 4 | 13 | 17 | | |
| Possible meningitis cases, n=110 | | | | | | | |
| Possible meningitis | 27 | 15 | 39 | 9 | 20 | 100% (110/110) | Possible meningitis 4.0% (110/2754) |
| Non-meningitis cases, n=1752 | | | | | | | |
| Possible encephalitis | 4 | 2 | 7 | 14 | 37 | 3.7% (64/1752) | Not meningitis 63.6% (1752/ 2754) |
| ADEM without meningitis | | | 3 | 4 | 1 | 0.5% (8/1752) | |
| Other not meningitis | 449 | 531 | 471 | 87 | 142 | 95.9% (1680/1752) | |
| Total meningitis and non-meningitis cases, n=2754 | | | | | | | |
| TOTAL, % (n/N) | 25.8% (711/2754) | 30.9% (850/2754) | 25.7% (709/2754) | 64.6% (178/2754) | 11.1% (306/2754) | 100% (2754/2754) | |

* Positive for antibody associated with antibody mediated encephalitis and a viral pathogen

*5.3.2. Meningitis aetiology*

Definite bacterial meningitis comprised 19.3% (172/892) of all meningitis *(table 5.3, figure 5.2)*. Overall 40% (69/172) of all bacterial meningitis cases were young infants aged <3 months, and 79% (136/172) were aged <2 years *(table 5.4)*. Group B Streptococcus (53%, 21/40) and *E. coli* (33%, 13/40) caused most neonatal meningitis. Overall *S. pneumoniae* caused slightly more cases (31%, 53/172), compared with *N. meningitidis* (29%, 51/172). Both *S. pneumoniae* and *N. meningitidis* caused a greater proportion of bacterial meningitis after the neonatal period; 62% (33/53) of pneumococcal and 71% (36/51) of meningococcal meningitis occurred in children aged 29 days -23 months *(table 5.4)*. For bacterial meningitis, 49.4% (85/172) of cases were diagnosed by culture only, 42.4% (73/172) by PCR and culture/Gram stain, and 8.1% (14/172) by PCR only. Considering only meningococcal meningitis, 37.3% (19/51) were diagnosed by culture only, 45.0% (23/51) by PCR and culture and 17.6% (9/51) by PCR only. Of the two children with *S. aureus* meningitis diagnosed by positive blood culture only, one had orbital cellulitis and an anterior cranial fossa collection, and the other child was also diagnosed with *S. aureus* endocarditis. The neonate with *Enterobacter cloacae* meningitis with a positive CSF culture, had a congenital intestinal enteropathy and recurrent central venous line infections.

**Figure 5.2 Aetiology in children and infants with meningitis or meningoencephalitis**
**n=892**



| **Table 5.4 Aetiology in children and infants with definite bacterial meningitis or meningoencephalitis** | | | | | | |
|---|---|---|---|---|---|---|
| | 0-28 days, n | 29 days - < 3 months, n | 3-23 months, n | 2-4 years, n | 5-15 years, n | All ages, % (n/N) |
| *S.pneumoniae* meningitis | 2 | 8 | 23 | 11 | 7 | 30.8% (53/172) |
| *S.pneumoniae* meningoencephalitis | | | 2 | | | |
| *N.meningitidis* meningitis | 1 | 5 | 30 | 6 | 8 | 28.7% (51/172) |
| *N.meningitidis* meningoencephalitis | | | 1 | | | |
| *N.meningitidis* and *S.pneumoniae* PCRs both reported positive | | | 1* | 1† | | 1.2% (2/172) |
| GBS | 21 | 8 | 2 | | | 18.0% (31/172) |
| *E.coli* | 13 | 8‡ | 1 | | | 12.8% (22/172) |
| *H.influenzae* | | | 4 | 1 | | 2.9% (5/172) |
| *S.aureus* | | | 2§ # | 1 # | | 1.7% (3/172) |
| *Listeria monocytogenes* | 1 | | | | 1 | 1.2% (2/172) |
| GAS | | | 1 ^ | | | 0.6% (1/172) |
| *Klebsiella oxytoca* | 1 | | | | | 0.6% (1/172) |
| *Enterobacter cloacae* | 1 | | | | | 0.6% (1/172) |
| **TOTAL definite bacterial,** % (n/N) | **23.3% (40/172)** | **16.9% (29/172)** | **39.0% (67/172)** | **11.6% (20/172)** | **9.3% (16/172)** | **172** |

Notes *Positive blood PCR for *N.meningitidis* and *S.pneumoniae* and CSF diplococci (Gram stain unspecified) seen, †Positive CSF and blood PCRs for *N.meningitidis* and *S.pneumoniae* reported, ‡One infant CSF E.coli culture positive and CSF EV-PCR+, § One *S.aureus* CSF culture positive, # two *S.aureus* blood culture positive, ^Group A Streptococcus blood culture positive. GAS = Group A Streptococcus, GBS = Group B Streptococcus

Most definite viral meningitis was caused by enteroviruses (81%, 305/378), and the majority of enteroviral meningitis occurred in young infants aged <3 months (83%, 253/305) *(table 5.5)*. Parechoviral meningitis comprised 13% (49/378) of definite viral meningitis, including six cases that were CSF-PCR positive for both parechovirus and enterovirus. 94% (46/49) of parechovirus meningitis occurred in infants aged <3 months. Other viral aetiologies were HSV, HHV6, adenovirus, measles, rhinovirus, VZV and CMV *(table 5.5).* All enteroviral and parechoviral meningitis cases were diagnosed by PCR. All

parechoviral meningitis cases (49/49) were diagnosed by a CSF-PCR+ result, including the six participants who were EV and parechovirus CSF-PCR+. 97% (296/305) of enteroviral meningitis cases were diagnosed by a CSF-PCR+ result, including two who were also CSF HHV6-PCR+. A further 8/305 enteroviral meningitis cases were diagnosed by both positive blood and CSF PCR results, and 1/305 was diagnosed by blood PCR only. Thirteen HSV meningitis were diagnosed by CSF-PCR+, and one was diagnosed by a raised CSF WBC count and blood HSV1-PCR+. There were several children with more than one positive PCR result.

Overall, for 31% (280/892) of all meningitis, no aetiology was identified from tests performed at hospital sites *(table 5.3, figure 5.2).* The percentage of meningitis with no cause identified from tests performed at hospital sites increased with age *(table 5.5)*. In neonates aged <29 days, 19.5% (45/231) with meningitis had no aetiology identified. The proportion of meningitis with no aetiology increased after the neonatal period to 31.5% (95/302) in infants aged 29 days to <3 months and 29.1% (55/189) in infants and young children aged 3-23 months. A further increase occurred in children aged ≥2 years, to 53.1% (34/64) in children aged 2-4 years and 48% (51/106) in aged 5-15 years. The probable bacterial meningitis cases were all young children and infants aged <2 years with *E. coli* cultured in urine, and a raised CSF WBC count *(table 5.5)*. All meningitis identified as caused by *Mycoplasma pneumoniae* was diagnosed by serum mycoplasma IgM.

The possible meningitis category included a wide variety of different aetiologies *(table 5.6)*. Notably, 21% (23/110) of children with possible meningitis had meningococcal

bacteraemia, none of whom had a LP performed. Only 1/5 children with pneumococcal bacteraemia had a LP performed which showed no raised CSF WBC. Therefore, the cases with no LP performed may have represented bacterial meningitis.

| **Table 5.5 Aetiology in children and infants with aseptic meningitis and meningoencephalitis, including atypical organisms and probable bacterial meningitis** | | | | | | |
|---|---|---|---|---|---|---|
| | 0-28 days, n | 29 days - <3months, n | 3-23 months, n | 2-4 years, n | 5-15 years, n | All ages, % (n/N) |
| **Viral meningitis** | | | | | | |
| enterovirus | 111 | 135 | 39 | 1 | 10 | 41.1% (296/720) |
| enterovirus and HHV6 | 1* | 1* | | | | 0.3% (2/720) |
| parechovirus and enterovirus | 2* | 3* | | | 1* | 0.8% (6/720) |
| parechovirus | 22 | 18 | 2 | | | 58.3% (42/720) |
| rhinovirus | | 1* | | | | 0.1% (1/720) |
| **Viral meningoencephalitis** | | | | | | |
| enterovirus | 3 | 2 | 2 | | | 1.0% (7/720) |
| HSV1 | | 3 | 4 | 1 | 4 | 1.7% (12/720) |
| HSV1 and EV | | 1* | | | | 0.1% (1/720) |
| VZV | | | | | 2* | 0.3% (2/720) |
| HSV2 | | | 1 | | | 0.1% (1/720) |
| parechovirus | 1 | | | | | 0.1% (1/720) |
| HHV6 | 1* | | | | 1* | 0.3% (2/720) |
| CMV | | | | | 1† | 0.1% (1/720) |
| adenovirus | | | 1‡ | | | 0.1% (1/720) |
| measles | | | | 1* | | 0.1% (1/720) |
| EBV and HHV6 | | | | | 1§ | 0.1% (1/720) |
| EBV and CMV | | | | | 1# | 0.1% (1/720) |
| **Probable viral meningitis or meningoencephalitis** | | | | | | |
| enteroviruses | 2 | 4 | 3 | 2 | 2 | 1.8% (13/720) |
| parechovirus | | | 1 | | | 0.1% (1/720) |
| **Other aseptic meningitis** | | | | | | |
| Kawasaki | | | 3 | | | 0.4% (3/720) |
| Guillain Barre | | | | 1 | | 0.1% (1/720) |
| Optic neuritis | | | | | 1 | 0.1% (1/720) |
| **ADEM with meningitis** | | | | | | |
| *M. pneumoniae* | | | | | 2** | 0.3% (2/720) |
| adenovirus | | | | | 1* | 0.1% (1/720) |
| No pathogen | | 1 | 1 | 3 | 4 | 1.3% (9/720) |
| **Other aseptic meningoencephalitis** | | | | | | |
| Antibody mediated | | | | | 2^ | 0.3% (2/720) |
| Viral and antibody | | | | | 2¶ | 0.3% (2/720) |
| **Atypical meningitis, n=2** | | | | | | |
| *M. pneumoniae* | | | | | 1** | 0.1% (1/720) |
| TB Optic Neuritis | | | | | 1 | 0.1% (1/720) |
| **Atypical meningoencephalitis, n=4** | | | | | | |
| *M. pneumoniae* | | | 1** | | 2** | 0.4% (3/720) |
| *M. pneumoniae* + EBV | | | | 1†† | | 0.1% (1/720) |
| **Probable bacterial meningitis, n=21** | | | | | | |
| *E. coli* | 3 | 9 | 9 | | | 2.9% (21/720) |
| **Unknown cause‡‡** | 45 | 95 | 55 | 34 | 51 | 38.9% (280/720) |
| TOTAL ASEPTIC, % (n/N) | 26.5% (191/720) | 37.9% (273/720) | 16.9% (122/720) | 6.1% (44/720) | 12.5% (90/720) | 720 |

*CSF PCRs, †blood CMV PCR+, ‡blood adenovirus PCR+, §CSF EBV PCR+ and blood HHV6 PCR+, #CSF CMV-PCR+ and EBV-PCR+, ^NMDA Receptor Ab positive (blood), ¶ both CSF EBV+ and vgkc antibody low positive[383],**Blood *Mycoplasma pneumoniae* IgM+, ††Blood *Mycoplasma pneumoniae* IgM+ and blood EBV DNA+, ‡‡ Meningitis or meningoencephalitis of unknown cause.

| Table 5.6 Aetiology in children and infants with possible meningitis | | | | | | |
|---|---|---|---|---|---|---|
| | 0-28 days, n | 29 days - < 3 months, n | 3-23 months, n | 2-4 years, n | 5-15 years, n | All ages, % (n/N) |
| **CSF relevant bacterial pathogen in blood** | | | | | | |
| *N. meningitidis* | | 1 | 16 | 1 | 5 | 20.9% (23/110) |
| *S. pneumoniae* | | 1 | 1 | 1 | 2 | 4.5% (5/110) |
| GBS | 4 | | | | | 3.6% (4/110) |
| *E. coli* | 1 | | | | | 0.9% (1/110) |
| **CSF relevant bacterial pathogen other site** | | | | | | |
| urine *E. coli* and discharge diagnosis states meningitis | 1 | | | | | 0.9% (1/110) |
| **CSF viral pathogen PCR+ or CSF pathogen uncertain significance, and no raised CSF WBC or no evaluable CSF WBC result** | | | | | | |
| HHV6 | | 1 | 5 | | | 5.5% (6/110) |
| VZV | | | | 2 | | 1.8% (2/110) |
| EBV and CMV | 1 | | | | 1 | 1.8% (2/110) |
| adenovirus | | 1 | | | | 0.9% (1/110) |
| adenovirus and rhinovirus | | | 1 | | | 0.9% (1/110) |
| rhinovirus | | 1 | | | | 0.9% (1/110) |
| rhinovirus and parainfluenzae virus | | | 1 | | | 0.9% (1/110) |
| coronavirus | | 1 | | | | 0.9% (1/110) |
| EBV | | | | | 1 | 0.9% (1/110) |
| Parvovirus B19 | | | | | 1 | 0.9% (1/110) |
| Multiple positive CSF viral PCRs reported* | | | | 1 | | 0.9% (1/110) |
| *H. parainfluenzae* 1 colony forming unit | | 1 | | | | 0.9% (1/110) |
| *Toxoplasma gondii* | 1 | | | | | 0.9% (1/110) |
| **CSF relevant viral pathogen positive PCR in non-CSF site** | | | | | | |
| enterovirus | | | 1 | 2 | 1 | 3.6% (4/110) |
| **Discharge diagnosis states meningitis** | 19 | 8 | 14 | 2 | 9 | 47.2% (52/110) |
| **Total,** % (n/N) | 24.5% (27/110) | 13.6% (15/110) | 35.5% (39/110) | 8.2% (9/110) | 18.2% (20/110) | 110 |

*Reported CSF PCRs positive for HSV2, VZV, EV and *parechovirus*

### *5.3.3. Participant recruitment by month and year*

Recruitment to the study in children with EV meningitis peaked in summer months (June-September) each year (*figure 5.3a*). Of children with parechoviral meningitis, recruitment also peaked in summer months during 2014 and 2016 only, with few cases occurring in 2015 (*figure 5.3b)*. Peaks in pneumococcal and meningococcal meningitis occurred in winter (*figures 5.3c-d*), including a peak in the number of pneumococcal meningitis cases in winter 2016, when overall recruitment to the study was reducing (*figures 5.3c, e-f)*.

**Figure 5.3 Number of children recruited to UK-ChiMES study by month and year by aetiology**

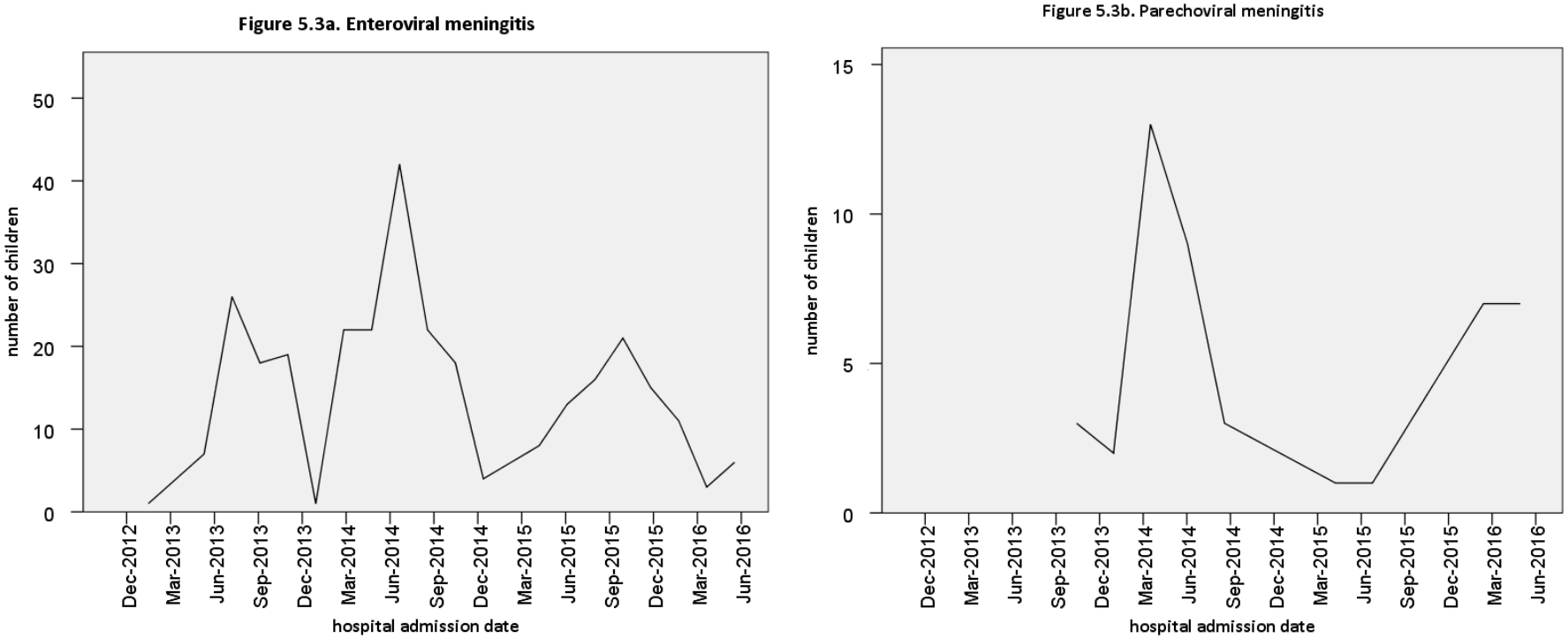


Figure 5.3c Pneumococcal meningitis

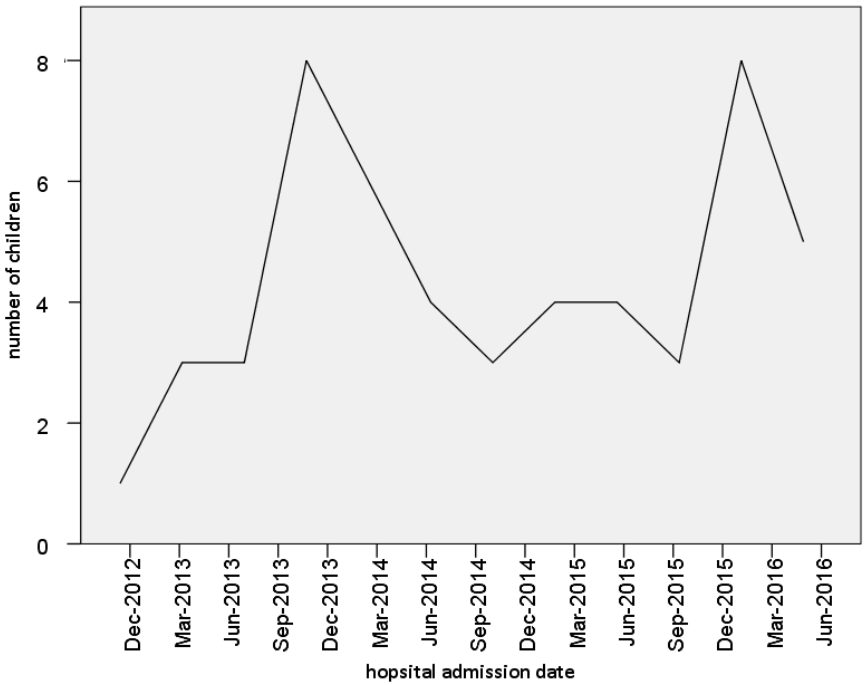



Figure 5.3d Meningococcal meningitis

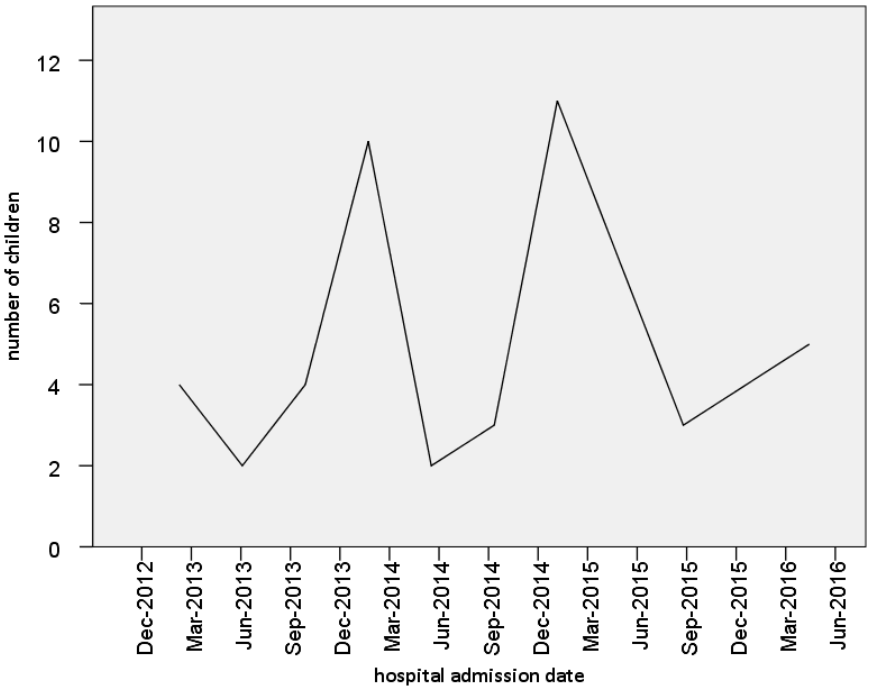


Figure 5.3e Total recruitment to UK-ChiMES study

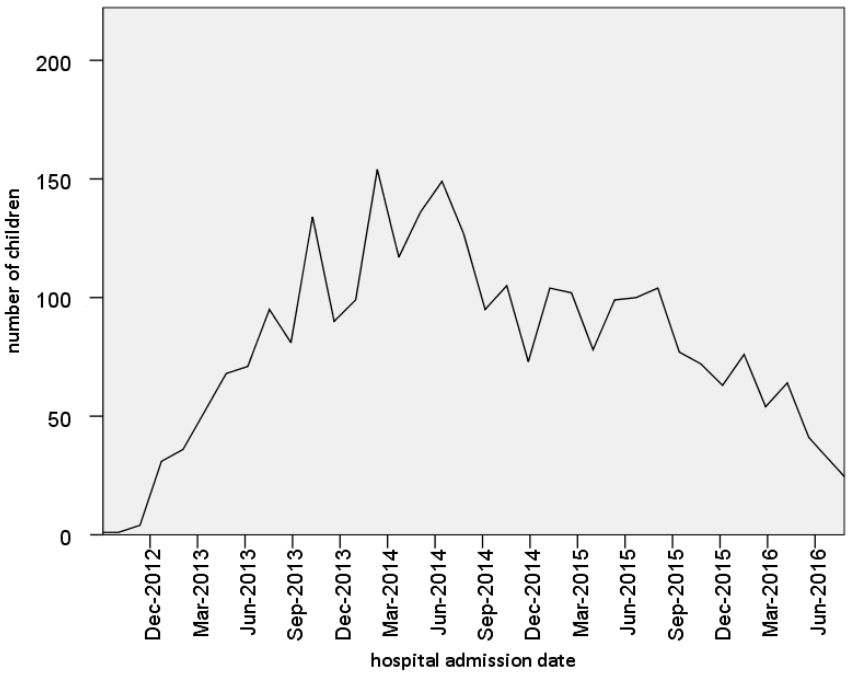
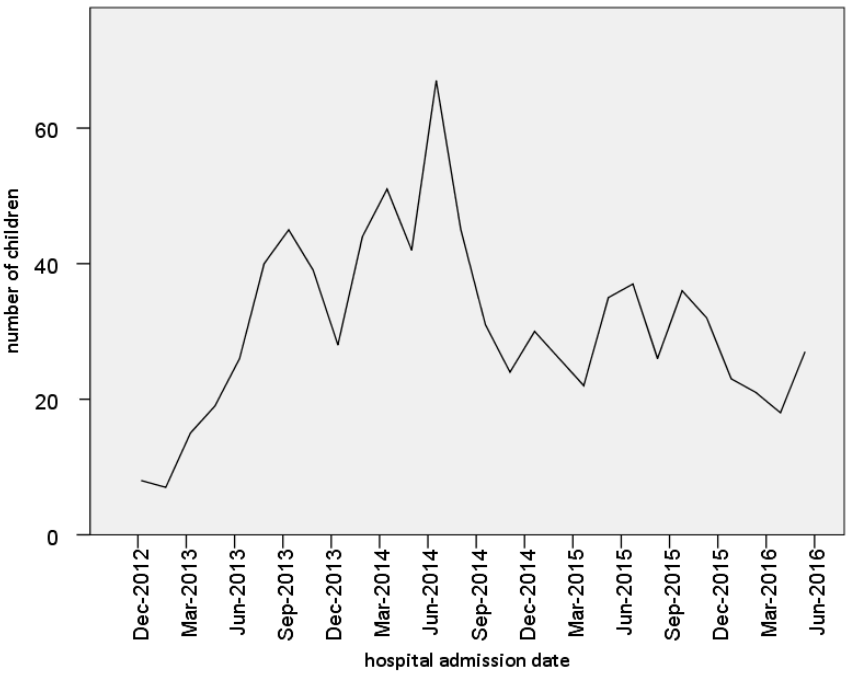



Figure 5.3f All meningitis



*5.3.4. Aetiology in participants with a non-meningitis diagnosis*

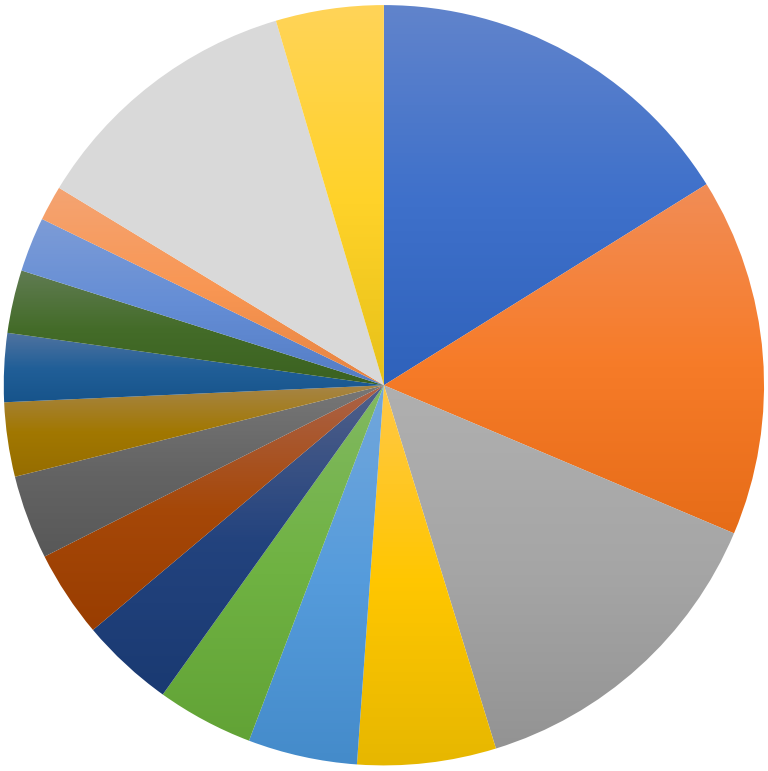
Possible aetiologies in children with a discharge diagnosis of encephalitis without meningitis are shown in *table 5.7*.

Final diagnoses in children who did not have meningitis are shown in *figure 5.4* and *table 5.8,* with urinary tract infections, suspected sepsis, probable viral illnesses or upper respiratory tract infections accounting for more than half of cases. Infants aged <3 months comprised 60% (533/892) of meningitis and 58% (980/1680) of participants who did not have meningitis. In children aged 3-23 months these proportions were 21% (302/892) of meningitis and 28% (471/1680) of non-meningitis cases, children aged 2-4 years comprised 7% of meningitis (64/892) and 5% (87/1680) of non-meningitis cases, and children aged 5-15 years comprised 12% (106/892) of meningitis and 8% (142/1680) of non-meningitis cases.

| Table 5.7 Aetiology in children and infants with possible encephalitis and ADEM without meningitis | | | | | | |
|---|---|---|---|---|---|---|
| | 0-28 days, n | 29 days - < 3 months, n | 3-23 months, n | 2-4 years, n | 5-15 years, n | All ages, % (n/N) |
| **Encephalitis without meningitis** | | | | | | |
| *Mycoplasma pneumoniae* ^ | | | | | 1 | 1.4% (1/72) |
| *Mycoplasma pneumoniae* ^ and adenovirus** | | | | 1 | | 1.4% (1/72) |
| Antibody mediated | | | | 1* | 1* | 2.8% (2/72) |
| Antibody and HSV1 | | | | 1† | | 1.4% (1/72) |
| Antibody low titre | | | | | 1‡ | 1.4% (1/72) |
| *Campylobacter jejuni* | | | | | 1§ | 1.4% (1/72) |
| *Borrelia burgdorferi* | | | | 1 | | 1.4% (1/72) |
| enterovirus (non-CSF) | 2# | | | | 1# | 4.2% (3/72) |
| HHV6 ** | | | 1 | | | 1.4% (1/72) |
| VZV ** | | | | 1 | | 1.4% (1/72) |
| Autoimmune encephalitis discharge diagnosis | | | | 1 | 2 | 4.2% (3/72) |
| Unknown aetiology | 2 | 2 | 6 | 8 | 30 | 66.7% (48/72) |
| **ADEM** | | | | | | |
| ADEM without meningitis | | | 3 | 4 | 1 | 11.1% (8/72) |
| **Total,** % (n/N) | **5.6% (4/72)** | **2.8% (2/72)** | **13.9% (10/72)** | **25.0% (18/72)** | **52.8% (38/72)** | **72** |

*NMBA titire positive, †NMDA positive, HSV-1 PCR+ on brain biopsy, ‡vkgc low positive[383], §blood culture, #from non-CSF site, ^*Mycoplasma pneumoniae* blood IgM+, ¶ Blood IgM+, ** CSF PCR+

**Figure 5.4 Aetiology in children and infants with a non-meningitis illness n=1680**



- Urinary tract infection, n=271
- Suspected sepsis, n=256
- Non-specific viral illness, n=233
- Febrile seizure, n=99
- Febrile illness or fever unknown origin, n=78
- Seizure other, n=69
- URTI, n=67
- Bacteraemia, n=62
- Bronchiolitis, n=60
- Pneumonia, n=53
- Chronic condition presenting with acute illness, n=49
- Gastroenteritis, including viral or bacterial, n=45
- Viral illnes with positive PCR, n=39
- enteroviral infection, n=25
- Other, n=197
- Unknown, n=77

| Table 5.8 Aetiology in children and infants with a non-meningitis illness | | | | | | |
|---|---|---|---|---|---|---|
| | 0-28 days, n | 29 days - < 3 months, n | 3-23 months, n | 2-4 years, n | 5-15 years, n | All ages, % (n/N) |
| enteroviral infection* | 3 | 8 | 10 | 4 | | 1.5% (25/1680) |
| Non-specific viral illness, including PCR positive | 60 | 108 | 74 | 11 | 19 | 16.2% (272/1680) |
| Urinary tract infection† | 89 | 113 | 64 | 2 | 3 | 16.1% (271/1680) |
| Suspected sepsis | 107 | 93 | 48 | 3 | 5 | 15.2% (256/1680) |
| Febrile seizure, includes respiratory viral PCR+ ^§ | 2‡ | | 80 | 9 | 8 | 5.9% (99/1680) |
| Febrile illness or fever unknown origin | 15 | 36 | 23 | 2 | 2 | 4.6% (78/1680) |
| Seizure (afebrile or not specified) | 2 | 2 | 33 | 11 | 21 | 4.1% (69/1680) |
| URTI** | 7 | 25 | 29 | 2 | 4 | 4.0% (67/1680) |
| Bacteraemia | 29 | 20 | 12 | | 1 | 3.7% (62/1680) |
| Bronchiolitis | 29 | 25 | 5 | 1 | | 3.6% (60/1680) |
| Pneumonia/ LRTI | 13 | 21 | 15 | 3 | 1 | 3.2% (53/1680) |
| Chronic condition†† | 5 | 10 | 15 | 7 | 12 | 2.9% (49/1680) |
| Gastroenteritis | 4 | 17 | 18 | 5 | 1 | 2.7% (45/1680) |
| Brief resolving episode | 9 | 5 | 2 | 1 | 1 | 1.1% (18/1680) |
| Kawasaki or atypical Kawasaki | | | 6 | 2 | 3 | 0.6% (11/1680) |
| Headache/ migraine | | | | 1 | 6 | 2.2% (38/1680) |
| Surgical‡‡ | 4 | 3 | | | | |
| Guillain Barre syndrome | | | | 2 | 2 | |
| HHV6 infection | | | 2 | 1 | 1 | |
| HSV infection | 4 | | | | | |
| Idiopathic ICH | | | | | 3 | |
| Chickenpox | | 1 | 1 | 2 | | |
| Optic neuritis | | | | | 3 | |
| Transverse myelitis | | | 1 | | 1 | |
| Other acute illness§§ | 42 | 25 | 16 | 13 | 34 | 7.7% (130/1680) |
| Unknown | 25 | 19 | 17 | 5 | 11 | 4.5% (77/1680) |
| **TOTAL** | **26.7% (449/1680)** | **31.6% (531/1680)** | **28.0% (471/1680)** | **5.2% (87/1680)** | **8.4% (142/1680)** | **1680** |

*****non-CSF EV-PCR+ included hand foot mouth and including with seizure**,** †included pyelonephritis, but not urosepsis (urosepsis classified as bacteraemia), ^included status epilepticus but not known epilepsy, § including associated with URTI/pneumonia/ gastro ‡outside usual age - one associated with pneumonia, one presumed associated with viral illness, ¶adenovirus or rhinovirus, ** included tonsillitis and otitis media, uncomplicated sinusitis, croup, pharyngitis, ††Chronic illness presenting with acute illness, chronic illness included seizure disorder included seizure disorder or epilepsy, congenital brain or other abnormalities, developmental delays, immunological conditions, neuro-regression, ‡‡included pyloric stenosis, gastroschisis, intussusception, small bowel obstruction, §§ Other acute illness included cellulitis, osteomyelitis, septic arthritis, poor feeding, failure to thrive, other rashes and skin infection, symptoms post vaccination, acute resolving confusion or lethargy or ataxia or transient neurological symptoms which not described as encephalitis, tics, lymphadenitis, medication overdose, musculoskeletal neck pain, adrenal crisis, any electrolyte abnormalities, mastoiditis, conjunctivitis, upper respiratory tract obstruction or abscess, skull fracture, head injury. LRTI=lower respiratory tract infection. RSV = respiratory syncytial virus

For the purpose of further analyses in this thesis, a non-meningitis control group was established for comparison with meningitis groups, including children and infants who had an acute resolving illness and no reported history of chronic illness or neurological disease. The following non-meningitis diagnoses were included in the control group (n=1199): non-specific viral illness (n=233), viral illness with respiratory PCR+ (not including enterovirus or parechovirus) (n=37), febrile seizure (specified as febrile) including with viral PCR+ (n=99), febrile illness (n=78), suspected sepsis (n=256), bronchiolitis (n=60), gastroenteritis (n=45), URTI (n=67), UTI (not including urosepsis) (n=271), and pneumonia (n=53).

*5.3.5. Analysis of PCR tests performed, hospital length of stay and proportion of antibiotic management and pre-treatment by aetiology*

The number of children with CSF or blood PCR performed by meningitis aetiology is shown in *table 5.9*. Of children who had aseptic meningitis with no defined aetiology, CSF EV-PCR was performed for 74% (208/280), and CSF parechovirus-PCR was performed for 50% (139/280) (*table 5.9.*). Greater proportions of children with confirmed viral meningitis had CSF-PCR performed for EV (98%, 370/378) and parechovirus (67%, 252/378). However, there was a trend to more blood bacterial PCR testing in the children with aseptic meningitis of unknown aetiology than children with viral meningitis (*N. meningitidis* 24% versus 14%, *S. pneumoniae* 20% versus 11%).

The median length of hospital admission for children <16 years was 9 days (IQR 6-16 days, n=166/172) for bacterial meningitis, 3 days (IQR 3-5 days, n=299/305) for EV meningitis, 3

days (IQR 2-5 days, n=49/49) for parechoviral meningitis, and 6 days (4-10 days, n=276/280) for aseptic meningitis of unknown aetiology. Hospital LOS in days was significantly longer for children with aseptic meningitis of unknown aetiology compared with definite EV or parechoviral meningitis (6 days (IQR 4-10 days) versus 3 days (IQR 3-5 days), p=<0.001).

The proportion of children who were pre-treated with antibiotics prior to LP was 38% (66/172) for bacterial, 21% (64/305) for enteroviral and 16% (8/49) for parechoviral meningitis, and 36% (102/280) for aseptic meningitis of unknown aetiology. No aetiology was defined for a greater proportion of pre-treated children with meningitis compared with children who were not pre-treated (37.1% (102/275) versus 28.8% (178/617), p=0.016). 96% (292/305) of all children <16 years with enteroviral meningitis, 100% (49/49) with parechoviral meningitis, and 92% (258/280) with aseptic meningitis of unknown aetiology received at least one dose of either intravenous or intramuscular antibiotics.

**Table 5.9 Blood and CSF PCRs performed in children and infants with meningitis of different aetiologies or a non-meningitis diagnosis**

| Blood PCR | | | | | | |
|---|---|---|---|---|---|---|
| | Bacterial meningitis<br><br>n=172<br>% (n) | Viral meningitis<br><br>n=378<br>% (n) | Other aseptic meningitis<br><br>n=62<br>% (n) | Aseptic meningitis unknown aetiology<br>n=280<br>% (n) | Possible meningitis<br><br>n=110<br>% (n) | Not meningitis<br><br>n=1752<br>% (n) |
| *N. meningitidis* | 34% (58) | 14% (52) | 19% (12) | 24% (66) | 35% (39) | 12% (204) |
| *S. pneumoniae* | 31% (53) | 11% (41) | 11% (7) | 20% (55) | 30% (33) | 10% (169) |
| Enterovirus | 1% (2) | 3% (11) | 8% (5) | 3% (7) | 3% (3) | 2% (42) |
| Parechovirus | 1% (1) | 1% (2) | 5% (3) | 1% (4) | 1% (1) | 1% (23) |
| CSF PCR* | | | | | | |
| | Bacterial meningitis<br><br>n=172<br>% (n) | Viral meningitis<br><br>n=378<br>% (n) | Other aseptic meningitis<br><br>n=62<br>% (n) | Aseptic meningitis unknown aetiology<br>n=280<br>% (n) | Possible meningitis<br><br>n=80<br>% (n) | Not meningitis<br><br>n=1736<br>% (n) |
| *N. meningitidis* | 37% (63) | 22% (83) | 32% (20) | 25% (70) | 19% (15) | 10% (175) |
| *S. pneumoniae* | 32% (55) | 22% (83) | 23% (14) | 21% (58) | 15% (12) | 9% (152) |
| *H. influenzae* | 3% (3) | 1% (3) | 25 (1) | 2% (5) | 0% | <1% (5) |
| Other bacterial PCR | 4% (7) | 3% (12) | 2% (1) | 1% (3) | 1% (1) | <1% (5) |
| Enterovirus | 48% (82) | 98% (370) | 85% (53) | 74% (208) | 64% (51) | 58% (1000) |
| Parechovirus | 29% (50) | 67% (252) | 55% (34) | 50% (139) | 46% (37) | 38% (657) |
| Herpes simplex virus | 49% (84) | 90% (339) | 81% (50) | 73% (203) | 69% (55) | 56% (967) |
| Varicella zoster virus | 41% (70) | 78% (296) | 79% (49) | 64% (179) | 58% (46) | 48% (831) |
| Epstein-Barr Virus | 2% (4) | 5% (19) | 18% (11) | 6% (17) | 8% (6) | 4% (67) |
| Cytomegalovirus | 3% (5) | 7% (28) | 13% (8) | 7% (19) | 6% (5) | 5% (94) |
| Human herpesvirus-6 | 7% (12) | 8% (31) | 8% (5) | 8% (22) | 11% (9) | 7% (124) |
| Human herpesvirus-7 | 0% | <1% (1) | 2% (1) | 1% (2) | 0% | 1% (10) |
| Influenza A virus | 4% (7) | 2% (8) | 5% (3) | 1% (3) | 6% (5) | 1% (18) |
| Influenza B virus | 3% (6) | 2% (8) | 5% (3) | 1% (4) | 8% (6) | 1% (17) |
| Adenovirus | 10% (17) | 20% (76) | 23% (14) | 16% (44) | 21% (17) | 14% (248) |
| Parvovirus B19 | 0% | 1% (4) | 2% (1) | 1% (2) | 3% (2) | 1% (13) |
| Other viral PCR | 2% (4) | 1% (5) | 5% (3) | 2% (6) | 6% (5) | 1% (23) |

*Note: No LP result for 30 participants with possible meningitis, and 16 participants with non-meningitis

## 5.4. Discussion

This is the first UK-wide study to prospectively define the current causes of childhood meningitis. These data, collected across 31 UK hospital sites from December 2012 to June 2016, demonstrated that the vast majority of childhood meningitis in the UK is currently aseptic. Enteroviruses caused most aseptic meningitis when a cause was identified, with most enteroviral infections occurring in young infants. However, no aetiology was defined from routine tests performed at hospital sites in many meningitis cases, particularly in older children. In children aged <16 years, *S. pneumoniae* was a slightly more common cause of bacterial meningitis than *N. meningitidis*. Almost all children with viral meningitis were treated with antibiotics and children with aseptic meningitis of unknown aetiology had longer hospital LOS than children with confirmed viral meningitis, suggesting that prompt identification of non-bacterial cases could reduce unnecessary antibiotic management and hospital LOS.

Overall 19.3% (172/892) of meningitis was bacterial, similar to the prospective pilot study performed from June 2011 to June 2012 at three UK-ChiMES sites which included 59 definite cases childhood meningitis, of which 22% were bacterial.[177] Young infants aged <3 months comprised 60% of all meningitis cases. Retrospective studies from the USA and other European countries performed between 1996-2008 also reported bacterial aetiologies for between 4-15% of childhood meningitis.[168,174-176] These retrospective studies all identified children by searching patient records, laboratory records or hospital discharge coding, and all studies excluded neonates aged <1 month.[168,174-176] Apart from the pilot work to this present study,[177] there had been no such study directly defining the current causes of all childhood meningitis in the UK. There have also been further

changes to meningitis epidemiology in countries like the UK since these studies were performed,[168,174-176] influenced by changes in vaccine schedules (*chapters 3 and 4*). The difference in reported proportion of children with bacterial meningitis in this present study compared to a USA study,[168] which included 3295 children with meningitis and reported a bacterial cause for only 4% is likely contributed to by different inclusion criteria. The USA study identified children retrospectively by ICD discharge coding, and in contrast to the present study, children who presented to the emergency department but were not admitted to hospital were included, and neonates were excluded.

No cause was identified for 38.9% of aseptic meningitis, compared with 30-76% reported by other studies from Europe and Canada, although there was variation in study design,[172,178,179] and 39.1% (18/46) in the UK-ChiMES pilot study.[177] Notably, there was an increase in the proportion of meningitis with no identified aetiology with increasing age, from 20% in infants <29 days, to approximately 30% from age 29 days to <2 years, and a further increase to about half of all children aged 2-15 years, which may suggest that clinicians are more rigorous about investigating a cause in younger infants and children. No aetiology was identified for meningitis in a greater proportion of children who received IV or IM antibiotics before LP compared to children who were not pre-treated (pre-treated 37.1% versus not pre-treated 28.8%, p=0.016). Although CSF-PCR has been reported to remain sensitive with antibiotic pre-treatment,[384] of children with no aetiology identified 26% did not have CSF EV-PCR performed and the majority did not have bacterial PCRs performed. This suggests that there is still room for improvement with performing PCR testing to define meningitis aetiology in these children.

The median LOS of three days for EV and parechoviral meningitis was shorter than the median LOS of five days for infants and three days in children >1 year with EV meningitis reported in the retrospective analysis of viral meningitis from 1968-2011 (*chapter 4*), which suggests improved diagnostic capabilities in recent years may have influenced earlier discharge for children with a definite viral cause. The longer LOS of six days for aseptic meningitis of unknown aetiology (aseptic unknown meningitis median LOS =6 days versus EV and parechoviral meningitis median LOS =3 days, p=<0.001) also suggests that diagnostic uncertainty may contribute to longer hospitalisations. The vast majority (92-100%) of children with EV and parechoviral meningitis, or meningitis of unknown aetiology received intravenous or intramuscular antibiotics, similar to previous studies.[172,186,312] Previous studies have shown that EV-PCR testing and reduced EV-PCR turnaround time reduce LOS and unnecessary antibiotic management in EV meningitis.[257,315,316,318,319] The high proportion of antibiotic treatment and substantial hospital LOS further emphasises the importance of obtaining timely PCR testing. Ongoing improvements in diagnostic capabilities, for example with recent advances in multiplexed PCRs[322,323] may further reduce unnecessary hospitalisations and antibiotics for children with viral meningitis.

The reduction in bacterial meningitis which occurred in the UK until 2011, most notably starting with the 1993 introduction of a *H. influenzae* vaccine, and subsequent introduction of different vaccines for bacterial meningitis, is discussed in *results chapter one*.[12,110,128,149,159,169,235,236] During the years included in the present study, further changes relevant to the UK vaccine schedule were implemented, and some laboratory confirmed data have been reported for invasive meningococcal and pneumococcal

disease in England and Wales.[129-132,150,152] However, since 2011,[104] no further published studies have directly compared incidence of different bacterial causes of childhood meningitis in the UK.

In contrast to these present data, which showed that *S. pneumoniae* (31% of cases) was a slightly more frequent cause of bacterial meningitis than *N. meningitidis* (29% of cases), the study reporting laboratory confirmed data during an earlier period from 2004-11 in England and Wales indicated that *N. meningitidis* (34%) was most common cause of bacterial meningitis in children <15 years, followed by *S. pneumoniae* (16%).[104] These differences are likely to be contributed to by natural fluctuations in disease. The *Okike et al* paper reported that the all-age incidence for meningococcal meningitis was 0.31/100 000 and for pneumococcal meningitis was 0.26/100 000 from 2004-11.[104] Consistent with the current study, the UK-ChiMES pilot study from June 2011-June 2012, also reported that more bacterial meningitis cases in children were caused by *S. pneumoniae* (4/13) than *N. meningitidis* (1/13).[177] In the USA, the incidence of pneumococcal meningitis is higher than meningococcal meningitis.[153]

There were five cases of *H. influenzae* (type not reported) meningitis. A few cases of *H. influenzae* (type not reported) meningitis were also reported at all ages in the *Okike et al* study from 2004-11.[104] Although PHE only reported three cases of invasive Hib disease in children in 2014,[163] an earlier PHE study reported 15 cases of Hib meningitis in children between 2009-2012.[385] A previous UK study found oropharyngeal Hib carriage in >4% of school aged children, which may be the reservoir for transmission to younger children without protection.[386]

Group B Streptococcus was the most common cause of neonatal bacterial meningitis in this current study from 2012-2016, causing 53% of cases in infants aged <29 days and 42% in infants <3 months. A similar proportion was reported by a surveillance study in the UK and Ireland with GBS causing 50% of bacterial meningitis in infants <90 days in 2010-11.[103] Other frequent causes of bacterial meningitis in infants aged <3 months were *E. coli* 21%, *S. pneumoniae* 15% and *N. meningitidis* 9%. The 2010-11 surveillance study reported that 11% of bacterial meningitis was caused each by *E. coli* and *N. meningitidis*, and only 7% was caused by *S. pneumoniae* although this study would have included infants in the neonatal intensive care unit (NICU) which is likely to contribute to differences in causative pathogens.[103] Previous studies have reported a rise in GBS disease in recent years, with a study from England and Wales reporting an increase in all neonatal GBS disease from 1991 to 2010,[167] and another study reporting an increase in bacterial meningitis in infants aged <3 months (7.4% per year) from 2004 to 2011 caused mostly by increased GBS disease.[104]

Enteroviral meningitis comprised 81% of all definite viral meningitis (305/378), and a further 13 cases of probable EV meningitis were identified. Parechoviral meningitis was the second most common cause accounting for 13% (49/378) of definite viral meningitis. Previous studies from Europe and Canada have reported that EV causes between 54-88% when a pathogen is isolated.[172,178-180] A UK study found that in England and Wales, EV caused 87.8% of laboratory confirmed viral meningoencephalitis reported to PHE in children aged <15 years in 2013, and HSV was second most common cause (6%).[180] Considering only definite viral meningitis, in these present data 81% of cases were caused EV, 13% by parechoviruses and 4% (14 cases) by HSV, which is similar to the *Kadambari et*

*al* study although they did not specifically report parechoviral infection.[180] An apparent recent increase in reported viral meningitis was also been observed by the *Kadambari et al* study,[180] and is discussed in *chapter 4*.

The vast majority (83%) of EV meningitis occurred in infants aged <3 months, and 47% (n= 253 cases) of all meningitis cases in infants <3 months were caused by definite EV. Unpublished data from a surveillance study in the UK and Ireland in infants <90 days from June 2014-June 2015, identified 710 cases of EV or parechoviral meningitis, 95% of which were enteroviral (including infants in the NICU).[202] Consistent with previous studies which have reported that the vast majority of parechoviral meningitis occurs in young infants,[200,217] but a wider age distribution for EV meningitis,[200] 96% (47/49) of parechoviral meningitis occurred in infants aged <90 days.

Infants and children with aseptic meningitis and a bacterial UTI were classified as 'probable bacterial meningitis'. Some infants with urinary tract infections are bacteraemic which is a potential mechanism for comorbid meningitis, although recent studies report reasonably low bacteraemia rates, around 4-8% in young infants with UTIs.[387-389] A study from the USA reported that 18% (214/1190) of infants aged 29-60 days with a UTI had aseptic meningitis.[388] This study excluded infants with bacterial meningitis from their secondary analysis, however prior to exclusions only 5/1895 infants with a UTI had bacterial meningitis.[388] An Australian study reported that 2/163 neonates with a UTI had meningitis, but no infants or children aged >1 month with a UTI had meningitis.[389]

The less common causes identified for bacterial meningitis in these data have been previously reported as rare causes of meningitis.[104,389-396] *Enterobacter cloacae* meningitis has been previously reported,[394] and *Klebsiella oxytoca* has been described including in newborns.[389,391,395] A recent study reported that most *Staphlococcus aureus* meningitis occurred in people who had previous neurosurgery although other cases were also reported,[396] and the *Okike et al* study from 2004-11 in England and Wales reported *Staphlococcus aureus* as cause for 5% of childhood meningitis.[104] Group A Streptococcus has been previously reported as a rare cause of childhood.[390,393] A 30-year surveillance study from the Netherlands reported that *Listeria monocytogenes* meningitis incidence peaks in neonates and older people.[392] In these present data, *M. pneumoniae* was defined as the cause of aseptic meningitis if there was a positive serum *M. pneumoniae* IgM serology. Serum *M. pneumoniae* results have also been used in previous studies to assign aetiology although specificity can be poor.[227,382,397]

Interestingly, six children had positive CSF PCRs for both parechovirus and enterovirus, which may depend on whether EV primers used in PCR assays at hospital sites also detect parechovirus. These cases were classified as parechoviral meningitis. There were several other participants with more than one positive PCR result, which may be caused by coincidental detection, contamination, or by poor specificity of PCR assays at hospital sites as has been demonstrated for example by studies of blood pneumococcal PCR,[398,399] and infrequent false positive results in a CSF multiplex assay.[323] Dual infections, although apparently rare, have been previously reported, including a case report of a child with culture positive bacterial meningitis for both *S. pneumoniae* and *N. meningitidis*.[400] Notably, CSF HHV-6+ results have been previously reported in children with past HHV-6

infection.[223] Dual CSF infection with HHV-6 and EBV[401] and HHV-6 and EV[402] have been reported, with a previous study proposing mechanisms including reactivation of HHV-6 or EBV which both may latently infect lymphoid cells.[401] Dual CSF infection with EBV and CMV could represent latency or reactivation of either virus, and has also been previously reported by a study which found that in a quarter of CSF EBV-PCR+ participants, another pathogen was also detected.[403]

Of all bacterial meningitis, 49% of cases were diagnosed by culture only with the remaining cases diagnosed by PCR, or PCR and culture. Considering only meningococcal meningitis, 37% were diagnosed by culture only, which is similar to data from PHE which reported that 38% of all IMD at all ages in 2015/16 was diagnosed by culture only, although fewer (18%) meningococcal meningitis cases in this present study were diagnosed by PCR only compared with all IMD (40%) in the PHE data.[114,310]

Consistent with these previous studies, EV meningitis peaks occurred annually in summer and autumn months,[171,172,180,191] and as previously reported in the UK and other European countries, biennial peaks in parechoviral infections occurred in summer months in even-numbered years.[200,201,216,217] Also accordant with previous data, recruitment of children with meningococcal and pneumococcal peaked in winter months.[113,126]

The inclusion of children with suspected meningitis, who did not have a confirmed meningitis diagnosis, allowed the development of a non-meningitis control group for the purpose of further analyses in this thesis.

Limitations of this study include variation in laboratory investigations performed at different hospital sites, and exclusion of participants with incomplete data for analysis. The requirements for recruitment to a prospective study also restrict all meningitis cases at hospital sites from being included in the study.

In summary, this is the largest study of childhood meningitis in the UK to report prospectively collected data, and provides detailed contemporary knowledge about the current causes of meningitis, which is important to inform priorities for disease prevention and management, including implementation of vaccine programmes, further research into improved diagnostic tests and vaccines, and improved clinical guidelines. There are still substantial numbers of bacterial meningitis cases, and early identification and management of these cases, and prevention strategies remain important. These data support the need for ongoing development of vaccinations for Group B Streptococcus.[250] Continued cost-effective strategies are needed to implement new effective vaccines to further prevent pneumococcal and meningococcal disease. However, this study confirmed that most childhood meningitis in the UK is now aseptic, with the majority of cases caused by enteroviruses. Ongoing research into multivalent EV vaccines is required.[214,246-248] Almost all children with viral meningitis receive antibiotic management, and hospital LOS is longer if an aetiology is not identified. In addition to adequate investigation to promptly identify aetiology, robust methods are also needed to help clinicians assess the probability of whether a child has viral or bacterial meningitis on presentation to hospital, to ensure prompt treatment of bacterial cases and reduce unnecessary antibiotics and hospital LOS in viral meningitis. This is explored in *chapter 6*.

# 6. Chapter 6: Clinical and laboratory features of childhood meningitis of different aetiologies and development of a new clinical decision rule to distinguish bacterial and aseptic meningitis.

## 6.1. Introduction

Contemporary, prospective data collected across the UK confirm that the great majority of childhood meningitis cases is aseptic nowadays (*chapter 5, figure 5.2*). Therefore, reliable approaches are needed to identify, at presentation to hospital, children who do not have bacterial meningitis to avoid unnecessary antibiotic management and days in hospital. Previous studies from different countries, mostly completed prior to the current vaccine schedule, have described some clinical and laboratory features of meningitis of different aetiologies including well established CSF parameters (*chapter 1, section 1.9*).[44,76,174,177,252,260,261,263,268,270-272,283,285-287,294] However, no previous UK study has described comprehensive clinical and laboratory features of meningitis of different aetiologies from current prospective data, or investigated a clinical decision model to distinguish bacterial and aseptic meningitis in UK children.

The Bacterial Meningitis Score (BMS) predicts children aged at least 1 month with CSF pleocytosis to be at very low risk of bacterial meningitis if they have none of the following five features: positive Gram stain, CSF protein ≥80mg/dL, peripheral neutrophil count ≥10 000 cells/μL, seizure at or before presentation, CSF neutrophil count ≥1000 cells/ μL.[168,295] The BMS has been previously developed in the USA, and validated in other populations with a combined sensitivity of 99.3%, specificity of 62.1% and negative predictive value of

99.7% in a meta-analysis including 5312 participants,[168,173,295] but has not been validated in the UK.

Methods for developing clinical prognostic rules have been widely described.[404-410] A recent paper described external validation of clinical prediction rules using big datasets.[411] Previously published guidance suggests that the development and validation of multivariable clinical decision rules requires steps including a development study, in which predictors are identified and assigned relative weights, and the model's predictive performance is assessed.[405] Model performance can be determined by calibration, which assesses how well observed risk matches predicted risk,[410] and by discrimination which assesses how well the model distinguishes individuals with and without the outcome.[410] Validation studies are then required to assess the rule's predictive performance in a new population.[405] Impact studies, usually requiring a control group, may also be performed to evaluate whether the rule improves day to day clinical decision making and patient outcome.[405]

The aims of this study were to describe clinical and laboratory features of meningitis of different aetiologies or suspected meningitis in infants and children, validate the BMS in the UK population, and develop a new clinical decision rule to distinguish bacterial and aseptic meningitis, for participants recruited prospectively to the UK-ChiMES study.

## 6.2. Methods

Methods for the UK-ChiMES study are reported in the general methods *chapter 2, section 2.2*. All statistical analyses were performed with SPSS software.

### *6.2.1 Methods for descriptive analysis of clinical features*

Demographics, clinical and laboratory features were described in infants and children aged <16 years recruited to the UK-ChiMES study in the following groups defined in *chapter 5, section 5.2.2*:

1. Definite bacterial meningitis or meningoencephalitis
2. Probable bacterial meningitis
3. Definite enteroviral meningitis or meningoencephalitis
4. Definite parechoviral meningitis or meningoencephalitis
5. Other definite viral meningitis or meningoencephalitis
6. Probable enteroviral meningitis
7. Aseptic meningitis or meningoencephalitis of unknown cause
8. Possible meningitis
9. A non-meningitis control group, including children with an acute resolving illness and no reported history of chronic illness or neurological disease.

Participants were excluded from the clinical features descriptive analysis with meningitis caused by atypical organisms, antibody mediated meningoencephalitis, 'other' meningitis associated with Guillain Barre syndrome, optic neuritis, Kawasaki disease or acute disseminated encephalomyelitis (ADEM), possible encephalitis or ADEM without

meningitis, and non-meningitis illnesses not included in the control group (described in *chapter 5, section 5.3*). Because there was only one child with probable parechoviral meningitis, they were not included in this analysis.

Data that were either not reported or not applicable, were excluded from analysis of each clinical or laboratory feature separately. For infants, headache, photophobia, Kernig's and Brudzunski's signs were not analysed, because these features cannot be reliably determined, and were frequently not reported. The proportion of infants with these features unreported were: Kernig's sign 81% (1431/1772), Brudzunski's sign 82% (1445/1772), photophobia 61% (1078/1772), and headache 49% (870/1772). The proportion of children ≥12 months with these features unreported were: Kernig's sign 71% (283/401), Brudzunski's sign 80% (321/401), 43% photophobia (171/401) and headache 24% (97/401). For children, categorical clinical features were not reported for probable bacterial (n=1) or parechoviral meningitis (n=2) because of limited sample size.

Considering participants with more than one lumbar puncture performed, results were analysed from the first successful lumbar puncture. Of infants, 98.8% of peripheral WBC counts and 98.9% of CRP tests were performed on day 0 or day 1 following admission, and of children 98.2% peripheral WBC counts and 97.5% of CRP tests were performed on day 0 or day 1 following admission. Blood results obtained >2 days following admission were excluded.

*6.2.2. Methods for comparison of enteroviral meningitis with and without a raised CSF WBC count*

A univariable analysis was performed to compare clinical and laboratory features, time to lumbar puncture and length of hospital admission in 293 children and infants with enteroviral meningitis, with (n=175) and without (n=118) a raised CSF WBC (for definition see *chapter 5, section 5.2.2*). Participants (n=12) without an evaluable CSF WBC count were not included in this analysis. T-tests were used to compare continuous variables with a normal distribution. The Wilcoxon rank-sum test was used to compare non-parametric continuous data. A P-value of <0.05 was defined as significant, and adjustments were not made for multiple comparisons.

*6.2.3. Methods for validation of the 'Bacterial Meningitis Score' in the UK-ChiMES study population*

The BMS was validated in the prospective UK-CHiMES study. Participants were included in the BMS validation study if they were aged 29 days to <16 years, and had CSF pleocytosis, defined as CSF WBC >5 $X10^6$/L on first lumbar puncture only. For this analysis, the definition of bacterial meningitis required either a bacterial pathogen in CSF, or CSF pleocytosis and a relevant bacterial pathogen present in blood, or CSF Gram stain positive for a likely pathogen and corresponding pathogen present in blood.[168] The definition of aseptic or viral meningitis required CSF pleocytosis, in addition to either no relevant pathogen identified or a relevant viral pathogen in CSF or a relevant viral pathogen found in blood.[168] Participants with a positive CSF viral PCR without CSF pleocytosis were not included. Participants with meningitis caused by atypical organisms, antibody mediated meningoencephalitis, and other meningitis associated with Guillain

Barre syndrome, optic neuritis, Kawasaki disease or ADEM were included. Children were classified as very low risk of bacterial meningitis if all BMS criteria were not present (positive Gram stain, CSF protein ≥80mg/dL, peripheral neutrophil count ≥10 000 cells/μL, seizure at or before presentation, CSF neutrophil count ≥1000 cells/μL). Children with adequate data for analysis who subsequently withdrew from the study or subsequently died at any time during the study were included. Participants were defined as having missing data if data were unreported for any BMS predictor unless there was a positive BMS finding from another predictor.[168] Blood neutrophil counts obtained >2 days following admission were classified as missing. The sensitivity, specificity, positive and negative predictive values for the BMS were calculated for children aged >28 days without intravenous or intramuscular antibiotic pre-treatment, which were inclusion criteria for the original BMS development study.[173] Separate analyses were also performed including neonates (age 0-28 days, raised CSF WBC defined as ≥20 $X10^6$/L), including participants who were pre-treated with antibiotics, and including participants with missing data.

### *6.2.4. Methods for development of a new multivariable rule to predict probability of bacterial meningitis*

A binary multivariable logistic regression model was developed using clinical features and results of clinical investigations available within a day of admission to hospital to assess the predicted probability of bacterial meningitis in children presenting with bacterial or aseptic meningitis, who were recruited to the UK-ChiMES study. Methods for building and assessing the predictive model were developed following recently published recommendations.[407-410]

*Participant inclusion*

Participants were included who were recruited prospectively to the UK-ChiMES study (*see methods chapter 2, section 2.2.2.* for study inclusion criteria), and were aged >28 days to <16 years and had a CSF WBC>5 $X10^6$/L, or definite bacterial meningitis. Therefore, participants with meningitis caused by atypical organisms, antibody mediated meningoencephalitis, and other meningitis associated with Guillain Barre syndrome, optic neuritis, Kawasaki disease or ADEM were included. Neonates aged <29 days were not included, because of different accepted definitions for CSF parameters in this age group and different bacterial organisms causing disease.[104-106] Participants were excluded who had probable bacterial meningitis defined as a raised CSF WBC and pure growth of relevant bacterial pathogen in urine, because these children were unable to be accurately assigned to either group. Participants were also excluded who were pre-treated with IV or IM antibiotics, because pre-treatment may have affected presenting clinical and laboratory features and participants with aseptic meningitis who were pre-treated may have represented treated bacterial meningitis.

Aseptic meningitis was defined by a CSF WBC>5 $X10^6$/L, and not bacterial meningitis, regardless of discharge diagnosis or results of further investigations. Bacterial meningitis was defined by pathogen in CSF, or CSF WBC>5 $X10^6$/L and relevant pathogen present in blood on culture or PCR, or CSF Gram stain positive for a likely pathogen and corresponding pathogen present in blood.

*Univariable analysis*

A univariable analysis was performed for clinical and laboratory features available at presentation to hospital, comparing participants who had bacterial meningitis with all aseptic meningitis. Clinical and laboratory features included symptoms recorded from patient history, clinical examination findings, and results of initial blood and CSF tests. Blood WBC and CRP were excluded if they were collected >2days from admission. In total eight WBC results and seven CRP results were excluded.

T-tests were used to compare continuous variables with a normal distribution. The Wilcoxon rank-sum test was used to compare non-parametric continuous data. Categorical variables were analysed with Pearson Chi-Squared tests or the Fisher's Exact test if any expected values were <5. Confidence intervals and p-values were calculated.

*Variable selection for the multivariable model*

Variables were considered for inclusion in the model that were clinically relevant, with <10% data missing, and $p<0.25$ at univariable analysis to ensure that variables are not overlooked that may be significant in multivariable analysis.[408] It is widely recommended to include not more than one variable per ten participants with the outcome of interest.[406,408]

*Building a multivariable model*

Three binary multivariable logistic regression analyses were performed including selected variables. For each model, participants were included if they had complete data for all

considered variables. Because data were collected prospectively it is likely that missing data are representative of available data for children presenting in the UK population with suspected bacterial meningitis. All continuous variables were kept continuous and not categorised to avoid loss of predictive information.[407,409]

The first full model included all nine selected variables. A second model was developed by stepwise backward elimination by likelihood ratio starting with the nine selected variables.[407,408] A third model was included based on the model developed by backward elimination with inclusion of the CSF WBC variable.

Bootstrapping by simple, percentile method based on 1000 bootstrap samples was performed to calculate confidence intervals and P-values for the odds ratio for included variables. For each variable included in each model the beta coefficient and the odds ratio, with 95% confidence interval and P-value calculated by bootstrapping, were reported.

*Assessing model performance*

For each model, calibration was assessed by 'calibration-at-large' which was performed by comparing observed and predicted means, although notably these are expected to be equal during model development;[407,409] and by plotting the regression slope for the observed proportion with bacterial meningitis, versus the predicted probability in ten percent groups.[409] Model discrimination was assessed by calculating the c-statistic, which is the area under the receiver operating curve (ROC).[409] The ROC curve is a graphical plot of sensitivity (true positive) versus 1-specificity (false positive).[409]

*Analysis for participants who were pre-treated, participants with viral meningitis without a raised CSF WBC count, and neonates*

In separate analyses, predicted probabilities were calculated in the third model for pre-treated participants aged >28 days, and participants with viral meningitis and no raised CSF WBC count aged >28 days. The performance of the third model was also assessed in neonates.

## 6.3. Results

### *6.3.1. Demographics for descriptive analysis of clinical features*

Demographics, clinical and laboratory features were analysed for 2173 out of 2754 infants and children aged <16 years recruited to the UK-ChiMES study included in the aetiology analysis (*chapter 5*), with definite bacterial meningitis or meningoencephalitis (n= 172), probable bacterial meningitis (n= 21), definite enteroviral meningitis or meningoencephalitis (n= 305), definite parechoviral meningitis or meningoencephalitis (n= 49), other definite viral meningitis or meningoencephalitis (n= 24), probable enteroviral meningitis (n= 13), aseptic meningitis or meningoencephalitis of unknown cause (n= 280), possible meningitis (n= 110), and the non-meningitis control group (n= 1199). There were 580/2754 participants excluded from the clinical features analysis with meningitis caused by atypical organisms or antibody mediated meningoencephalitis (n=10), 'other' meningitis associated with Guillain Barre syndrome, optic neuritis, Kawasaki disease or ADEM (n=17), possible encephalitis or ADEM without meningitis (n=72), and non-meningitis illnesses not included in the control group (n=480). A child

with probable parechoviral meningitis was also excluded because there was only one participant in the group (n=1).

Males comprised 58% (1259/2173) of all participants, including 61% (105/172) of definite bacterial meningitis cases, and 57% (174/305) of definite enteroviral meningitis cases. Lower reported rates for 'fully immunized' in infants compared with children may reflect the definition used for infants, which required all primary immunizations by age 6 months. ICU admission was reported for 36% (60/169) of bacterial meningitis, 4% (13/301) of EV meningitis and 10% (5/49) of parechovirus meningitis. High rates of ICU admission for possible meningitis (32%, 34/107) may be contributed to by participants with bacteraemia for a CSF relevant pathogen, for example *N. meningitidis (tables 6.1 and 6.2)*.

| Table 6.1 Demographic features of infants <1 year with meningitis of different aetiologies or a non-meningitis illness | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ALL | Bacterial meningitis | Probable bacterial meningitis | Enterovirus meningitis | Parechovirus meningitis | Other viral meningitis | Probable enteroviral meningitis | Possible meningitis | Aseptic meningitis unknown aetiology | Not meningitis (control) |
| n | 1772 | 119 | 20 | 288 | 47 | 10 | 7 | 73 | 184 | 1024 |
| Age in months at admission median (IQR) | 1 (0-2) | 2 (0-5) | 1 (1-4) | 1 (0-1) | 0 (0-1) | 2 (1-5) | 2 (0-2) | 1 (0-4) | 1 (1-2) | 1 (0-3) |
| Sex | | | | | | | | | | |
| male, % (n/N) | 58.7% (1040/1772) | 57.1% (68/119) | 45.0% (9/20) | 56.9% (164/288) | 51.1% (24/47) | 70.0% (7/10) | 71.4% (5/7) | 49.3% (36/73) | 62.5% (115/184) | 59.8% (612/1024) |
| Fully immunized % (n/N) | 77%, (1008/1308) | 73.2% (74/101) | 78.6% (11/14) | 77.9% (152/195) | 75.9% (22/29) | 87.6% (7/8) | 60% (3/5) | 81.4% (44/54) | 77.9% (109/140) | 76.9% (586/762) |
| Development normal% (n/N) | 98.3% (1681/1710) | 98.2% (112/114) | 100% (19/19) | 98.9% (273/276) | 100% (46/46) | 90% (9/10) | 100% (7/7) | 95.8% (68/71) | 97.7% (172/176) | 98.4% (975/991) |
| Ethnicity | | | | | | | | | | |
| *white* | 1386 | 94 | 16 | 235 | 40 | 10 | 6 | 56 | 141 | 788 |
| *mixed* | 77 | 6 | 0 | 16 | 0 | 0 | 0 | 6 | 9 | 40 |
| *Asian or Asian British*[*] | 147 | 4 | 3 | 18 | 5 | 0 | 0 | 2 | 17 | 85 |
| *Black* | 74 | 5 | 0 | 7 | 0 | 0 | 1 | 5 | 8 | 48 |
| *Other* | 7 | 0 | 1 | 2 | 0 | 0 | 0 | 2 | 0 | 2 |
| *Unknown* | 81 | 10 | 0 | 10 | 2 | 0 | 0 | 2 | 9 | 48 |
| ICU % (n/N) | 9.2% (162/1755) | 32.5% (38/117) | 0% (0/19) | 3.8% (11/284) | 10.6% (5/47) | 20% (2/10) | 0% (0/7) | 28.2% (20/71) | 8.2% (15/182) | 7.0% (71/1018) |
| Mortality # | | | | 1 | | | | | 1 | |

Note: *Asian British including Chinese, #Mortality at any time during study including unrelated to presenting illness

| Table 6.2 Demographic features of children ≥1 year with meningitis of different aetiologies or a non-meningitis illness | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ALL | Bacterial meningitis | Probable bacterial meningitis | Enterovirus meningitis | Parechovirus meningitis | Other viral meningitis | Probable enteroviral meningitis | Possible meningitis | Aseptic meningitis unknown aetiology | Not meningitis (control) |
| n | 401 | 53 | 1 | 17 | 2 | 14 | 6 | 37 | 96 | 175 |
| Age in months at admission median (IQR) | 36 (18-88) | 33 (22-66) | 13 | 68 (16-122) | 96 (12-179) | 116 (45-155) | 40 (18-95) | 67 (30-112) | 62 (36-115) | 23 (15-59) |
| Sex | | | | | | | | | | |
| male, % (n/N) | 54.6% (219/401) | 67.9% (36/53) | 100% (1/1) | 58.8% (10/17) | 100% (2/2) | 64.3% (9/14) | 83.3% (5/6) | 48.6% (18/37) | 58.3% (56/96) | 46.9% (82/175) |
| Fully immunized % (n/N) | 98.1% (361/378) | 96.2% (50/52) | 100% (1/1) | 93.8% (15/16) | 100% (2/2) | 83% (10/12) | 100% (6/6) | 100% (35/35) | 96.7% (86/89) | 94.5% (156/165) |
| Development normal % (n/N) | 93.7% (371/396) | 96.2% (51/53) | 100% (1/1) | 100% (17/17) | 100% (2/2) | 78.6% (11/14) | 100% (5/5) | 89.1% (33/37) | 93.6% (88/94) | 94.2% (163/173) |
| Ethnicity | | | | | | | | | | |
| *white* | 296 | 39 | 1 | 16 | 2 | 9 | 6 | 28 | 68 | 127 |
| *mixed* | 15 | 5 | | 0 | | 0 | | 2 | 3 | 5 |
| *Asian or Asian British*[*] | 54 | 7 | | 1 | | 4 | | 3 | 17 | 22 |
| *Black* | 15 | 0 | | 0 | | 0 | | 2 | 4 | 9 |
| *Other* | 1 | 0 | | 0 | | 0 | | 0 | 0 | 1 |
| *Unknown* | 20 | 2 | | 0 | | 1 | | 2 | 4 | 11 |
| ICU % (n/N) | 25.9% (103/398) | 42.3% (22/52) | 0% (0/1) | 11.8% (2/17) | 0% (0/2) | 28.6% (4/14) | 20% (1/5) | 38% (14/36) | 16.7% (16/96) | 25.1% (44/175) |
| Mortality# | | 1 | | | | | | | 1 | 1 |

Note: *Asian British including Chinese, #Mortality at any time during study including unrelated to presenting illness

*6.3.2. Descriptive analysis of clinical features*

In the descriptive analysis of clinical features, infants with meningitis of different aetiologies, or a non-meningitis illness investigated for suspected meningitis, presented with similar constellations of symptoms and signs *(figures 6.1-6.2)*. Children ≥12 months with confirmed meningitis or suspected meningitis also had similar presenting clinical features *(figures 6.3-6.4).* In infants and children with bacterial meningitis, a history of altered consciousness, vomiting, respiratory signs and non-blanching rash on examination were frequently reported. In infants with possible meningitis, non-blanching rash was frequently reported which likely represents infants with meningococcal bacteraemia. In infants with 'other viral' meningitis, seizures and vesicular rash were often reported, probably due to HSV infection, which comprised 7/10 of this group. In children ≥1 year, headache, neck stiffness and photophobia were often reported in the meningitis groups (*figures 6.1-6.4)*.

**Figure 6.1 Symptoms from clinical history in infants aged <12 months with meningitis of different aetiologies or a non-meningitis diagnosis**

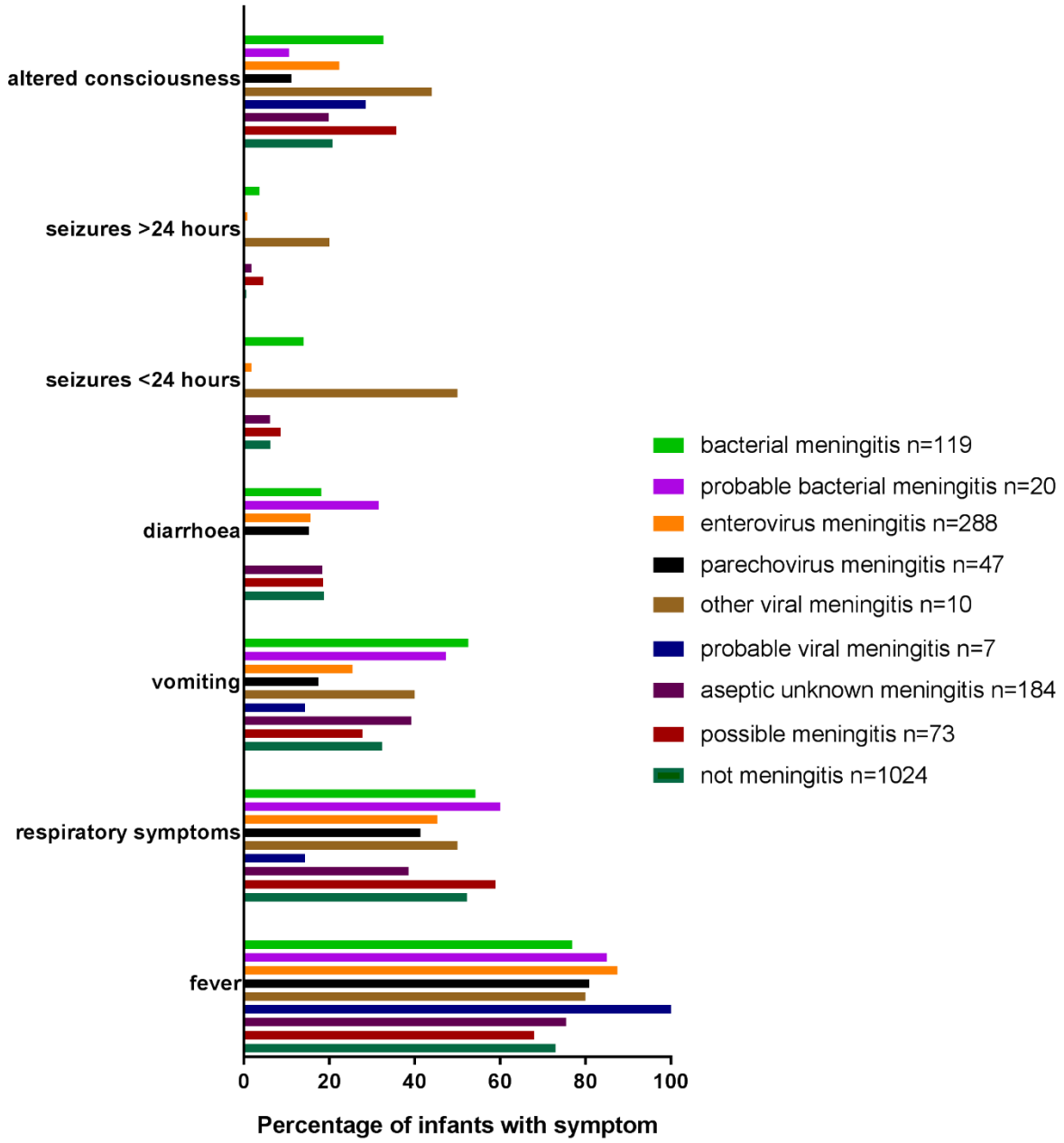
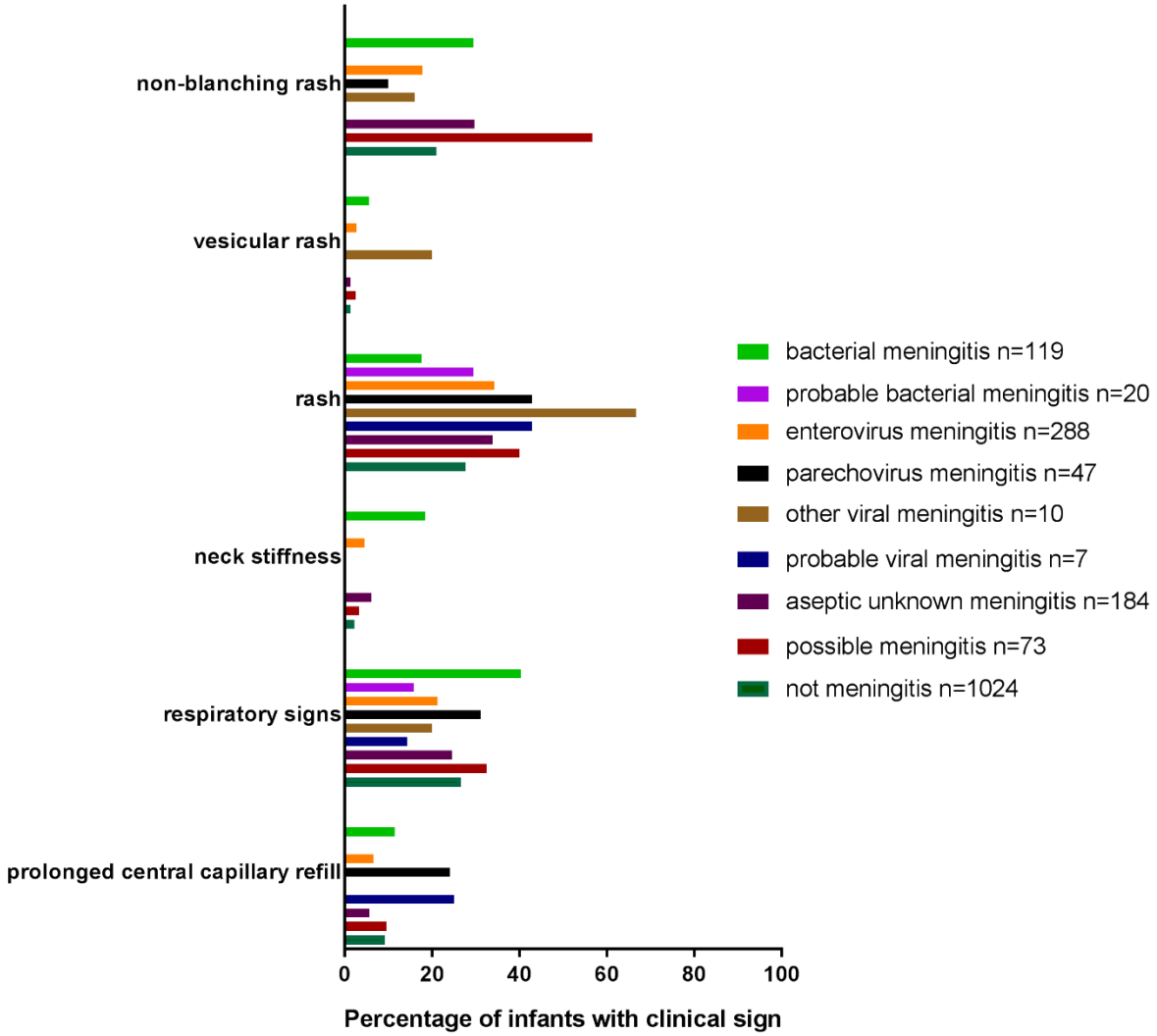


**Figure 6.2 Clinical signs in infants aged <12 months with meningitis of different aetiologies or a non-meningitis diagnosis**




Note: Prolonged capillary refill was defined as ≥3 seconds

**Figure 6.3 Symptoms from clinical history in children aged ≥12 months with meningitis of different aetiologies or a non-meningitis diagnosis**

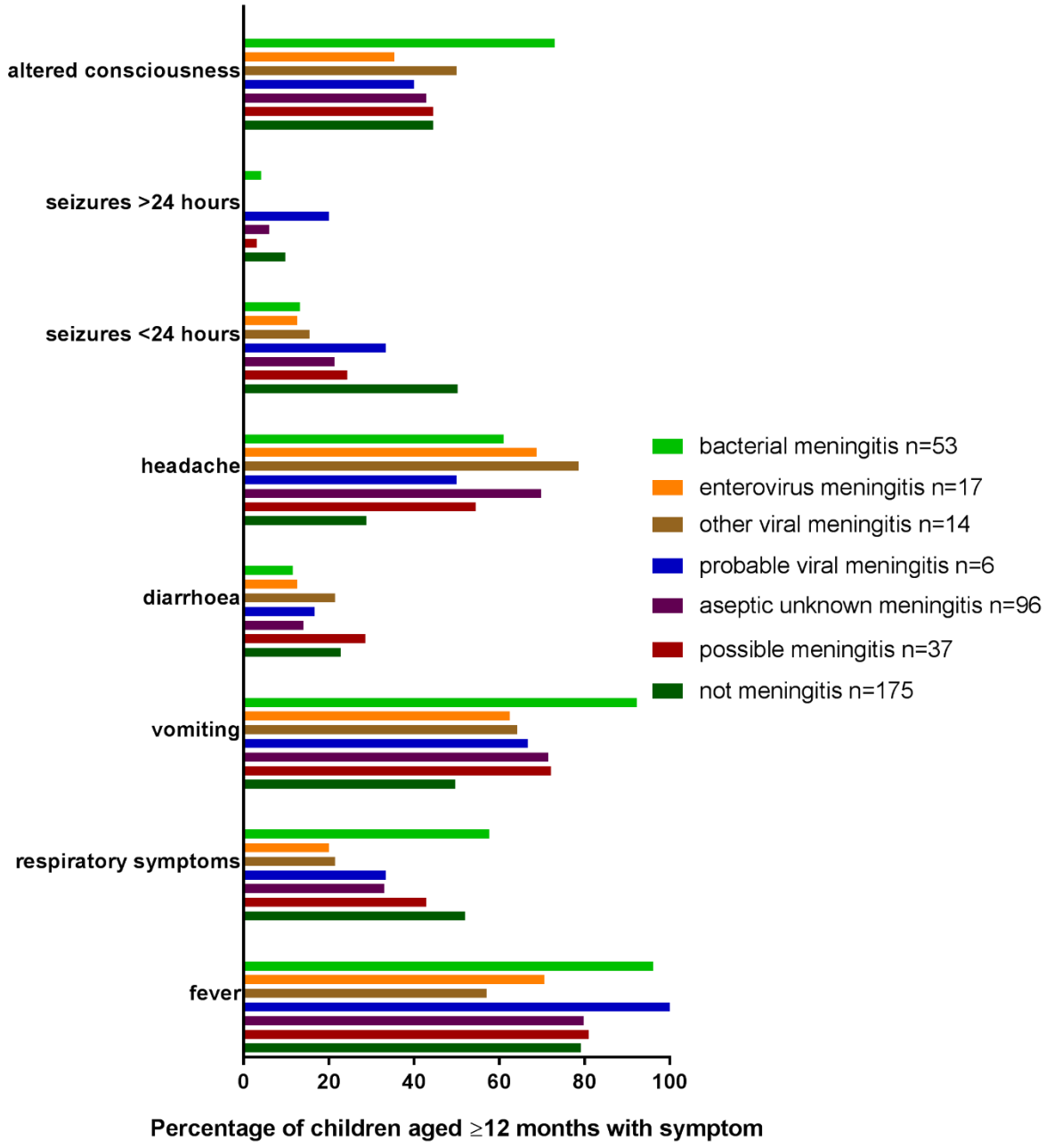
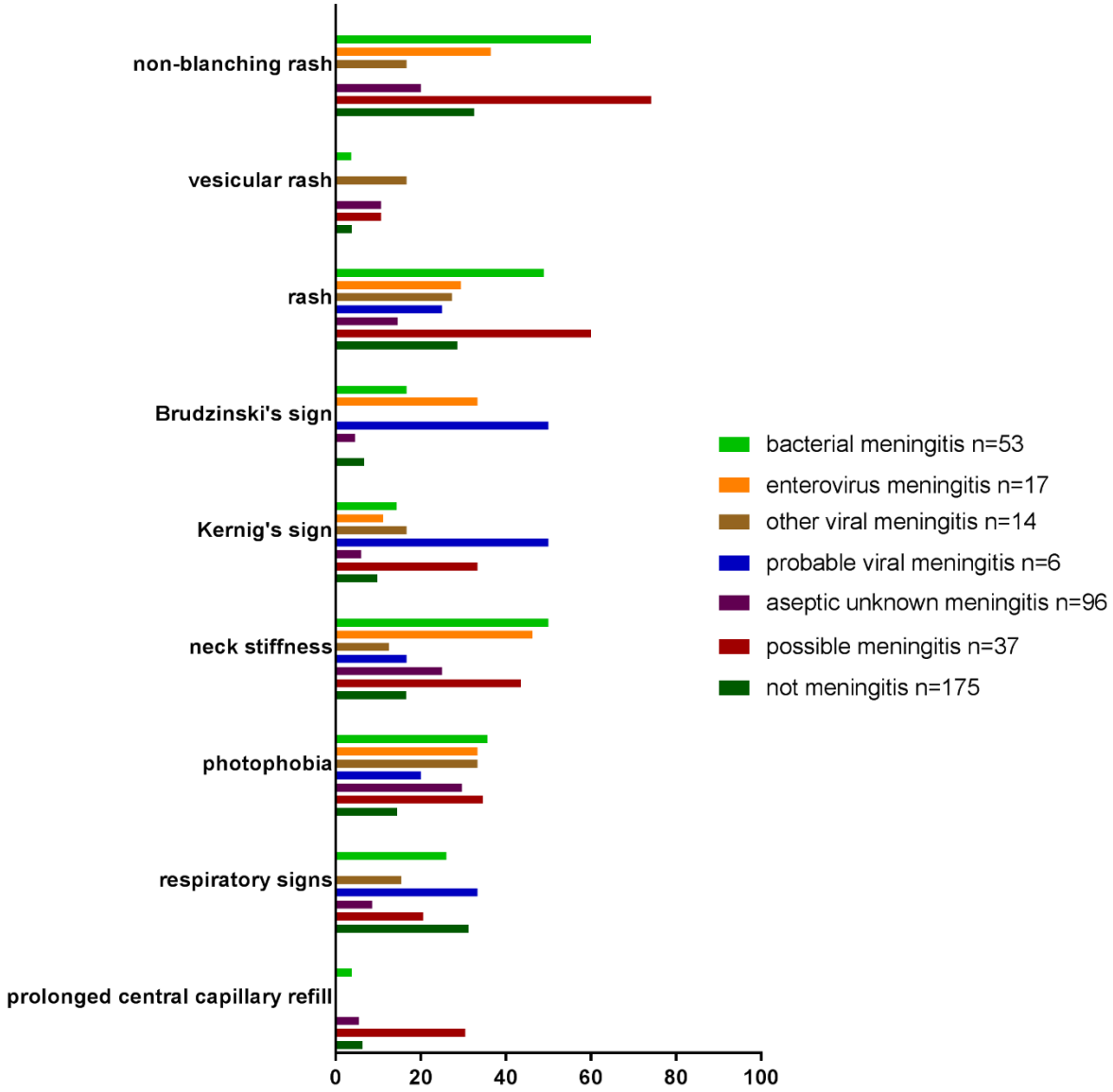


**Figure 6.4 Clinical signs in children aged ≥12 months with meningitis of different aetiologies or a non-meningitis diagnosis**




Note: Prolonged capillary refill was defined as ≥3 seconds

In this descriptive analysis, respiratory rate and temperature on arrival at hospital appeared similar across all groups including non-meningitis, in infants (*tables 6.3-6.4*), and in children (*tables 6.5-6.6*). Infants with aseptic meningitis of unknown aetiology had generally similar CSF parameters to the viral meningitis groups. In infants, median CSF WBC was 483 $X10^6$/L (IQR 100-1484) for bacterial meningitis and 47 $X10^6$/L (1-322) for EV meningitis, median CSF protein was 1.6 g/L (IQR 0.7-2.7) for bacterial meningitis and 0.6 g/L (0.4-0.9) for EV meningitis, median CSF:plasma glucose ratio was 0.3 (0.1-0.5) for bacterial meningitis and 0.5 (0.5-0.6) for EV meningitis, and median blood CRP was 116 mg/L (38-204) for bacterial meningitis and 6 mg/L (1-15) for EV meningitis (*tables 6.3-6.4*). For probable bacterial meningitis (all *E. coli* UTI) in infants, there was a trend to a higher median blood WBC count (18 $X10^9$/L, 11-25) than for other diagnoses, however the median CSF WBC count (33 $X10^6$/L, 11-110) was similar to viral meningitis, which is less suggestive of bacterial meningitis (*tables 6.4-6.5*). 80.5% (33/41) of infants with parechoviral meningitis and an evaluable CSF WBC result did not have a raised CSF WBC count (median 1 $X10^6$/L, IQR 0-6) (*table 6.4*). In children ≥1 year, CRP and CSF parameters also appeared to most reliably distinguish between bacterial and non-bacterial meningitis, and children with aseptic meningitis of unknown aetiology also had similar CSF parameters to the viral meningitis groups (*tables 6.5-6.6*).

A positive CSF Gram stain was a frequent finding in bacterial meningitis (*tables 6.7-6.8*). A GCS<15 was a presenting feature for bacterial meningitis in 70% of infants and 32% of children, for EV meningitis in 0% of infants and 11% of children, but also occurred in 50% of infants with a non-meningitis illness (*tables 6.7-6.8*).

| | **Table 6.3 Clinical signs and laboratory parameters in infants with meningitis or different aetiologies or a non-meningitis diagnosis** | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bacterial meningitis | | | Probable bacterial meningitis | | | Aseptic meningitis unknown aetiology | | | Possible meningitis | | | Not meningitis (control) | | |
| | **median** | IQR | n | **median** | IQR | n | **median** | IQR | n | **median** | IQR | n | **median** | IQR | n |
| Temperature (◦C) | **38.6** | 37.9-39.1 | 115 | **38.4** | 37.6-39.0 | 20 | **38.2** | 37.7-38.7 | 177 | **38.1** | 37.4-39.0 | 71 | **38.2** | 37.5-38.9 | 1015 |
| Heart rate (/min) | **165** | 146-186 | 116 | **168** | 160-180 | 20 | **163** | 145-180 | 178 | **176** | 147-190 | 70 | **166** | 149-180 | 1017 |
| Respiratory rate (/min) | **44** | 36-56 | 115 | **43** | 40-48 | 20 | **45** | 38-50 | 177 | **47** | 38-56 | 66 | **44** | 38-52 | 999 |
| Blood WBC count ($X10^9$/L) | **11** | 6-18 | 116 | **18** | 11-25 | 20 | **12** | 9-17 | 179 | **11** | 7-14 | 69 | **11** | 8-16 | 1003 |
| Blood neutrophil count ($X10^9$/L) | **6** | 3-13 | 116 | **9** | 5-14 | 20 | **5** | 3-9 | 177 | **4** | 3-8 | 69 | **5** | 3-8 | 995 |
| Blood lymphocyte count ($X10^9$/L) | **2** | 2-4 | 113 | **7** | 5-8 | 20 | **5** | 4-7 | 178 | **4** | 2-5 | 68 | **4** | 3-6 | 994 |
| Blood CRP (mg/L) | **116** | 38-204 | 116 | **59** | 29-118 | 20 | **9** | 1-50 | 177 | **24** | 1-55 | 68 | **13** | 1-44 | 1004 |
| Corrected CSF WBC count# ($X10^6$/L) | **483** | 100-1485 | 110 | **33** | 11-110 | 20 | **37** | 14-141 | 184 | **1** | 0-4 | 47 | **0** | 0-2 | 1021 |
| CSF neutrophil number ($X10^6$/L) | **371** | 74-2345 | 52 | **12** | 7-50 | 9 | **18** | 8-50 | 70 | **4** | 1-9 | 16 | **1** | 0-2 | 87 |
| CSF lymphocyte number ($X10^6$/L) | **126** | 31-382 | 67 | **15** | 2-90 | 10 | **26** | 10-100 | 106 | **3** | 0-12 | 18 | **1** | 0-3 | 144 |
| CSF glucose (mmol/L) | **2.0** | 0.6-2.9 | 105 | **3.1** | 2.7-3.2 | 18 | **2.8** | 2.5-3.1 | 168 | **2.8** | 2.5-3.4 | 47 | **3.1** | 2.8-3.5 | 973 |
| CSF:plasma glucose ratio | **0.3** | 0.1-0.5 | 56 | **0.5** | 0.5-0.7 | 12 | **0.6** | 0.5-0.6 | 71 | **0.6** | 0.5-0.8 | 23 | **0.6** | 0.5-0.7 | 469 |
| CSF protein (g/L) | **1.6** | 0.7-2.7 | 99 | **0.4** | 0.3-0.6 | 18 | **0.6** | 0.4-0.8 | 162 | **0.6** | 0.3-1.0 | 44 | **0.4** | 0.2-0.6 | 952 |

Note: #corrected by $500X10^6$/L RBC:1 $X10^6$/L WBC

| | **Table 6.4 Clinical signs and laboratory parameters in infants with viral or probable viral meningitis** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Enterovirus meningitis | | | Parechovirus meningitis | | | Other viral meningitis | | | Probable enteroviral meningitis | | |
| | **median** | IQR | n | **median** | IQR | n | **median** | IQR | n | **median** | IQR | n |
| Temperature (◦C) | **38.6** | 38.2-39.1 | 284 | **38.7** | 38.4-39.2 | 47 | **37.8** | 37.1-38.5 | 10 | **38.1** | 37.5-38.2 | 7 |
| Heart rate (/min) | **169** | 154-183 | 282 | **186** | 171-195 | 46 | **163** | 144-180 | 8 | **169** | 164-172 | 7 |
| Respiratory rate (/min) | **45** | 40-52 | 278 | **48** | 40-56 | 43 | **47** | 35-52 | 10 | **54** | 46-64 | 6 |
| Blood WBC count ($X10^9$/L) | **10** | 8-13 | 286 | **6** | 5-8 | 46 | **12** | 8-17 | 10 | **12** | 10-15 | 7 |
| Blood neutrophil count ($X10^9$/L) | **4** | 3-6 | 285 | **3** | 2-4 | 45 | **6** | 2-7 | 10 | **5** | 3-9 | 7 |
| Blood lymphocyte count | **4** | 3-6 | 284 | **2** | 1-3 | 46 | **5** | 4-6 | 9 | **5** | 3-6 | 7 |
| Blood CRP (mg/L) | **6** | 1-15 | 280 | **6** | 1-15 | 46 | **3** | 1-9 | 10 | **8** | 5-11 | 7 |
| Corrected CSF WBC count[#] ($X10^6$/L) | **47** | 1-322 | 274 | **1** | 0-6 | 41 | **56** | 10-151 | 10 | **90** | 10-514 | 6 |
| CSF neutrophil number ($X10^6$/L) | **71** | 10-225 | 65 | **1** | 0-4 | 5 | | | 0 | **8** | 2-108 | 3 |
| CSF lymphocyte number ($X10^6$/L) | **84** | 15-251 | 140 | **7** | 0-17 | 12 | **32** | 24-40 | 5 | **35** | 14-314 | 4 |
| CSF glucose (mmol/L) | **2.7** | 2.4-3.1 | 272 | **3.2** | 2.9-3.4 | 42 | **2.9** | 2.3-3.2 | 7 | **2.8** | 2.4-3.2 | 7 |
| CSF:plasma glucose ratio | **0.5** | 0.5-0.6 | 152 | **0.6** | 0.5-0.7 | 17 | **0.4** | 0.4-0.4 | 2 | **0.6** | 0.5-0.6 | 4 |
| CSF protein (g/L) | **0.6** | 0.4-0.9 | 258 | **0.4** | 0.3-0.6 | 36 | **0.5** | 0.3-0.8 | 7 | **0.6** | 0.5-0.9 | 7 |

*Note: If CRP was recorded as <5 then a value of 1 was used.* [#]corrected by $500X10^6$/L RBC:1 $X10^6$/L WBC

| | **Table 6.5 Clinical signs and laboratory parameters in children ≥12 months with meningitis or different aetiologies or a non-meningitis diagnosis** | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bacterial meningitis | | | Probable bacterial meningitis | | | Aseptic meningitis unknown aetiology | | | Possible meningitis | | | Not meningitis (control) | | |
| | **median** | IQR | n | **median** | IQR | n | **median** | IQR | n | **median** | IQR | n | **median** | IQR | n |
| Temperature (◦C) | **38.7** | 38.2-39.1 | 51 | **40.0** | | 1 | **38.4** | 37.5-39.2 | 85 | **38.4** | 37.7-39.2 | 36 | **38.7** | 37.9-39.5 | 169 |
| Heart rate (/min) | **129** | 110-150 | 50 | **150** | | 1 | **110** | 90-139 | 91 | **128** | 106-160 | 37 | **148** | 122-170 | 171 |
| Respiratory rate (/min) | **32** | 25-38 | 49 | **36** | | 1 | **26** | 22-32 | 89 | **28** | 22-40 | 35 | **30** | 25-36 | 166 |
| Blood WBC count ($X10^9$/L) | **16** | 10-25 | 51 | **22** | | 1 | **14** | 10-20 | 94 | **11** | 8-17 | 35 | **12** | 8-17 | 169 |
| Blood neutrophil count ($X10^9$/L) | **12** | 7-20 | 51 | **15** | | 1 | **9** | 6-16 | 93 | **9** | 5-15 | 35 | **8** | 5-13 | 170 |
| Blood lymphocyte count | **1** | 1-2 | 48 | **5** | | 1 | **2** | 1-4 | 92 | **2** | 1-3 | 35 | **3** | 1-4 | 169 |
| Blood CRP (mg/L) | **184** | 82-225 | 51 | **260** | | 1 | **18** | 1-75 | 92 | **41** | 6-90 | 34 | **16** | 1-60 | 166 |
| Corrected CSF WBC count[#] ($X10^6$/L) | **506** | 129-1480 | 51 | **39** | | 1 | **48** | 18-154 | 95 | **0** | | 14 | **0** | 0-2 | 175 |
| CSF neutrophil number ($X10^6$/L) | **99** | 26-680 | 23 | | | 0 | **9** | 2-68 | 41 | | | 0 | **0** | 0-1 | 6 |
| CSF lymphocyte number ($X10^6$/L) | **162** | 45-317 | 32 | **39** | | 1 | **43** | 19-126 | 60 | | | 0 | **1** | 0-2 | 11 |
| CSF glucose (mmol/L) | **2.4** | 1.1-3.4 | 45 | **2.8** | | 1 | **3.2** | 2.8-3.8 | 87 | **3.3** | 3.1-3.8 | 15 | **3.5** | 3.1-3.9 | 165 |
| CSF:plasma glucose ratio | **0.5** | 0.2-0.6 | 16 | **0.6** | | 1 | **0.6** | 0.6-0.7 | 41 | **0.6** | 0.5-0.7 | 8 | **0.7** | 0.6-0.7 | 72 |
| CSF protein (g/L) | **1.0** | 0.6-1.3 | 42 | **0.1** | | 1 | **0.3** | 0.2-0.5 | 85 | **0.2** | 0.2-0.2 | 13 | **0.2** | 0.1-0.2 | 162 |

*Note: If CRP was recorded as <5 then a value of 1 was used.* [#]corrected by 500X$10^6$/L RBC:1 X$10^6$/L WBC

| | **Table 6.6 Clinical signs and laboratory parameters in children ≥12 months with viral or probable viral meningitis** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Enterovirus meningitis | | | Parechovirus meningitis | | | Other viral meningitis | | | Probable enteroviral meningitis | | |
| | **median** | IQR | n | **median** | IQR | n | **median** | IQR | n | **median** | IQR | n |
| Temperature (◦C) | **38.2** | 37.9-38.9 | 17 | **39.2** | 39.1-39.2 | 2 | **38.0** | 37.2-39.2 | 11 | **37.3** | 36.9-39.2 | 6 |
| Heart rate (/min) | **122** | 94-143 | 17 | **133** | 75-190 | 2 | **120** | 94-135 | 13 | **125** | 93-134 | 6 |
| Respiratory rate (/min) | **24** | 20-30 | 17 | **35** | 20-50 | 2 | **27** | 20-29 | 12 | **33** | 32-39 | 5 |
| Blood WBC count ($X10^9$/L) | **12** | 9-19 | 16 | **6** | 4-8 | 2 | **10** | 8-11 | 13 | **13** | 11-14 | 6 |
| Blood neutrophil count ($X10^9$/L) | **10** | 7-12 | 16 | **4** | 2-5 | 2 | **6** | 5-9 | 13 | **9** | 5-11 | 6 |
| Blood lymphocyte count | **1** | 1-2 | 15 | **1** | 1-2 | 2 | **2** | 1-2 | 13 | **5** | 1-6 | 5 |
| Blood CRP (mg/L) | **1** | 1-18 | 16 | **43** | 20-66 | 2 | **1** | 1-6 | 12 | **1** | 1-1 | 6 |
| Corrected CSF WBC count# ($X10^6$/L) | **19** | 12-37 | 17 | **126** | 126-126 | 1 | **9** | 0-63 | 14 | **110** | 49-162 | 5 |
| CSF neutrophil number ($X10^6$/L) | **2** | 0-6 | 5 | **0** | 0-0 | 1 | **78** | 4-110 | 3 | **13** | 1-91 | 4 |
| CSF lymphocyte number ($X10^6$/L) | **36** | 15-57 | 11 | **126** | 126-126 | 1 | **39** | 13-187 | 4 | **89** | 57-124 | 4 |
| CSF glucose (mmol/L) | **3.3** | 2.9-3.4 | 16 | **2.9** | 2.3-3.5 | 2 | **3.2** | 3.0-4.1 | 13 | **3.2** | 2.7-4.1 | 6 |
| CSF:plasma glucose ratio | **0.6** | 0.5-0.6 | 6 | **1.1** | 1.1-1.1 | 1 | **0.8** | 0.4-1.0 | 3 | **0.5** | 0.4-0.7 | 3 |
| CSF protein (g/L) | **0.3** | 0.2-0.5 | 17 | **0.6** | 0.4-0.8 | 2 | **0.4** | 0.2-0.8 | 13 | **0.5** | 0.3-0.9 | 6 |

*Note: If CRP was recorded as <5 then a value of 1 was used.* #corrected by $500X10^6$/L RBC:1 $X10^6$/L WBC

| Table 6.7 Percentage of infants with decreased GCS scores and positive CSF Gram stain, with meningitis of different aetiologies or a non-meningitis diagnosis | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bacterial meningitis | | Probable bacterial meningitis | | Enterovirus meningitis | | Parechovirus meningitis | | Other viral meningitis | | Probable viral meningitis | | Possible meningitis | | Aseptic meningitis unknown aetiology | | Not meningitis (control) | |
| | % | n/N | % | n/N | % | n/N | % | n/N | % | n/N | % | n/N | % | n/N | % | n/N | % | n/N |
| GCS less than 15 | 32.4 | 12/37 | 0 | 0/6 | 11.3 | 7/62 | 20 | 2/10 | 25 | 1/4 | | | 25 | 6/24 | 18.8 | 9/48 | 9.6 | 24/249 |
| Positive CSF Gram stain | 41.3 | 45/109 | 5.9 | 1/17 | 2.7 | 7/264 | 2.4 | 1/42 | 11 | 1/9 | 0 | 0/7 | 7.1 | 4/56 | 3.1 | 5/164 | 2.6 | 23/874 |

| Table 6.8 Percentage of children ≥12 months with decreased GCS scores and positive CSF Gram stain, with meningitis of different aetiologies or a non-meningitis diagnosis | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bacterial meningitis | | Probable bacterial meningitis | | Enterovirus meningitis | | Parechovirus meningitis | | Other viral meningitis | | Probable viral meningitis | | Possible meningitis | | Aseptic meningitis unknown aetiology | | Not meningitis (control) | |
| | % | n/N | % | n/N | % | n/N | % | n/N | % | n/N | % | n/N | % | n/N | % | n/N | % | n/N |
| GCS less than 15 | 70.3 | 26/37 | | | 0 | 0/11 | | | 66.7 | 4/6 | 50 | 2/4 | 50 | 10/20 | 32 | 18/56 | 50 | 50/100 |
| Positive CSF Gram stain | 34.7 | 17/49 | 0 | 0/1 | 0 | 0/15 | 0 | 0/2 | 0 | 0/14 | 0 | 0/6 | 6.7 | 1/15 | 3.5 | 3/85 | 2.0 | 3/148 |

**6.4. Comparison of enteroviral meningitis with and without a raised CSF WBC count**

Of participants with EV meningitis and an evaluable CSF WBC result (293/305), 40% (118/293) did not have a raised CSF WBC count *(table 6.9)*. Infants and children without a raised CSF WBC count compared with those who had a raised CSF WBC were younger (median age 31 days versus 44 days, p<0.008), had lower peripheral WBC counts (median 8 $X10^9$/L versus 12 $X10^9$/L, p<0.001), and shorter length of hospital stay (3.4 versus 4.2 days, p=0.001). Participants without a raised CSF WBC also had significant differences in CSF parameters (lower CSF protein and higher CSF glucose), higher heart rate on arrival at hospital, and slightly higher blood CRP, compared with the raised CSF WBC group. There was no significant difference in time of LP after onset of symptoms, but there was a trend to earlier LP in participants without raised CSF WBC *(table 6.9)*.

Of infants and children with EV meningitis without a raised CSF WBC count compared with those who had a raised CSF WBC, there was no difference at presentation to hospital in history of seizures (no pleocytosis 1.8% (2/113) versus pleocytosis 4.1% (7/171), p=0.325), history of altered consciousness (no pleocytosis 24.2% (26/107) versus pleocytosis 22.1% (36/163), p=0.672), or bulging fontanelle if examined (no pleocytosis 7.8% (7/90) versus pleocytosis 7.9% (9/114), p=0.975).

| **Table 6.9 Comparison of clinical signs and laboratory parameters in infants and children with enteroviral meningitis with and without a raised CSF WBC count** | | | | | | | |
|---|---|---|---|---|---|---|---|
| | EV meningitis with a raised CSF WBC count, n=175 | | | EV meningitis without a raised CSF WBC count, n=118 | | | p-value |
| **Age (months)** ***median (IQR), n*** | **1** | **(0-2),** | **172** | **1** | **(1-2),** | **118** | **0.023** |
| **Age (days)** ***median (IQR), n*** | **44** | **(22-69),** | **172** | **31** | **(18-54),** | **118** | **0.008** |
| Time of LP after onset of symptoms (days), *median (IQR), n* | 1 | (1-2), | 172 | 1 | (1-2), | 117 | 0.553 |
| Time of LP after onset of symptoms (hours), *median (IQR), n* | 25 | (11-47), | 93 | 16 | (12-40), | 59 | 0.300 |
| **Corrected CSF WBC count[#] ($X10^6$/L),** ***median (IQR), n*** | **235** | **(69-536),** | **175** | **0** | **(0-3),** | **118** | **<0.001** |
| **CSF protein (g/L),** ***median (IQR), n*** | **0.6** | **(0.5-0.9),** | **162** | **0.4** | **(0.3-0.6),** | **107** | **<0.001** |
| **CSF glucose (mmol/L),** ***mean (95% CI), n*** | **2.7** | **(2.6-2.8),** | **169** | **3.0** | **(2.9-3.1),** | **112** | **<0.001** |
| **Blood WBC count ($X10^9$/L),** ***median (IQR), n*** | **12** | **(9-15),** | **173** | **8** | **(6-11),** | **117** | **<0.001** |
| **Blood CRP (mg/L)** ***median (IQR), n*** | **1** | **(1-6.1),** | **169** | **14** | **(7.4-25),** | **115** | **<0.001** |
| Temperature (◦C) *mean (95% CI), n* | 38.6 | (38.5-38.7), | 173 | 38.6 | (38.5-38.8), | 117 | 0.365 |
| **Heart rate (/min)** ***mean (95% CI), n*** | **161** | **(156-166),** | **172** | **173** | **(168-177),** | **117** | **<0.001** |
| **LOS (days)** ***mean (95% CI), n*** | **4.2** | **(3.8-4.6),** | **173** | **3.4** | **(3.0-3.7),** | **115** | **0.001** |

*Note: If CRP was recorded as <5 then a value of 1 was used.* [#]corrected by $500X10^6$/L RBC:1 $X10^6$/L WBC

## 6.5. Validation of the 'Bacterial Meningitis Score' in the UK-ChiMES study population

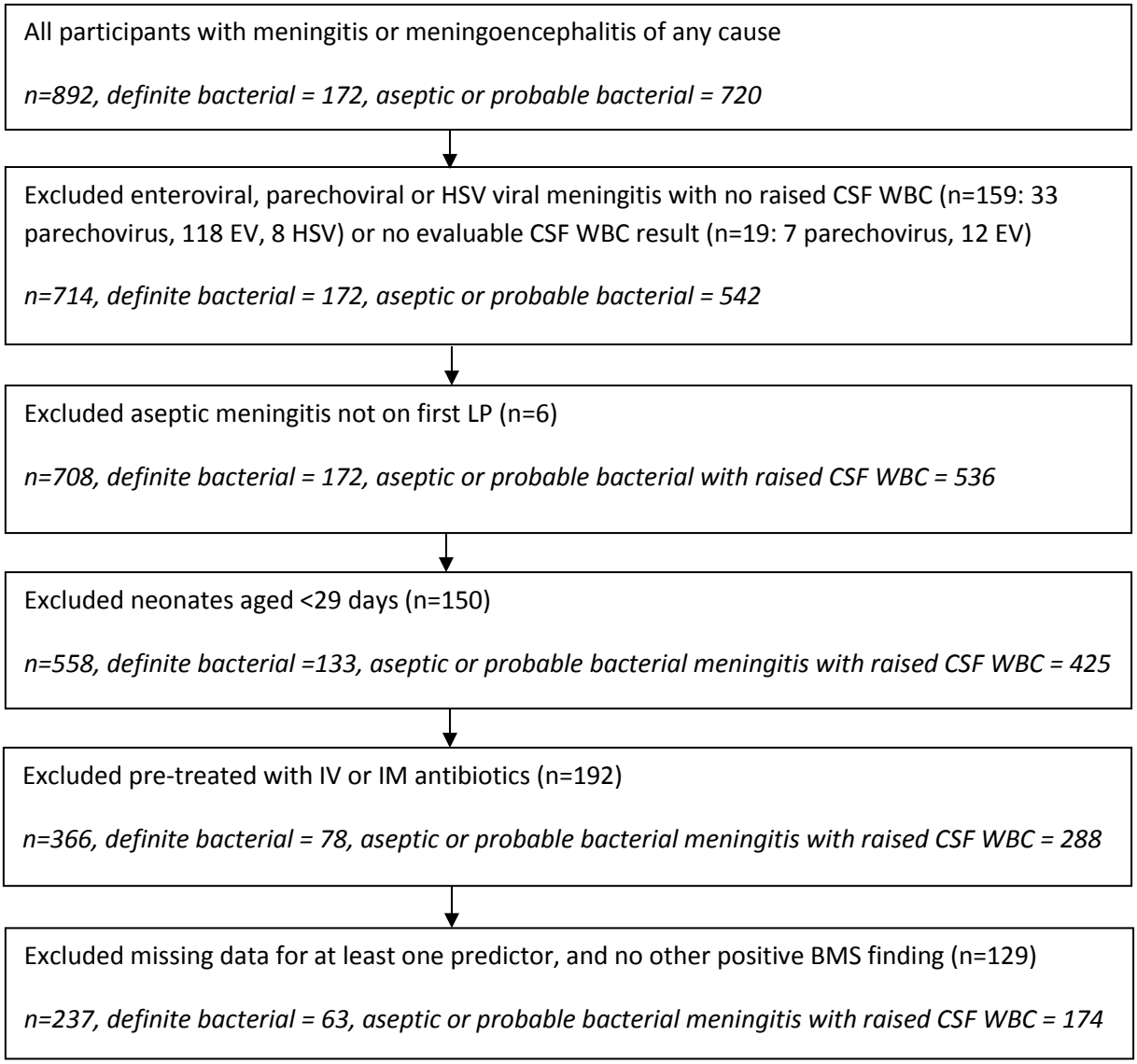
The sensitivity, specificity, negative and positive predictive values for the Bacterial Meningitis Score was calculated in 237 children aged 29 days to <16 years, who were not pre-treated with antibiotics and did not have missing data (*figure 6.5, table 6.10*). The BMS was also analysed separately for a total of 708 participants with the inclusion of neonates aged <29 days, and participants with antibiotic pre-treatment and missing data (*figure 6.5, table 6.10*). In the UK-ChiMES cohort, CSF neutrophil counts were often not recorded (*tables 6.2-6.6*) which contributed to participants with missing BMS predictors.

For included participants, seizures occurred within 29 hours of admission, or 33 hours including neonates and pre-treatment.

The sensitivity for predicting children with bacterial meningitis by having at least one positive predictor in the BMS was 100% both with and without the inclusion of neonates, if children who were pre-treated and had missing data were excluded *(table 6.10)*. If pre-treated participants were included, four children with bacterial meningitis did not have any positive predictors. These four children were all aged >28 days, and included two children with meningococcal meningitis, one child with pneumococcal meningitis, and one child with meningitis caused by *H. influenzae*. The sensitivity of the BMS reduced if participants with missing data were included *(table 6.10)*.

The specificity for predicting very low risk in children who did not have bacterial meningitis was 21.8% (95% CI 15.7-27.9%), and similarly was 20-21% if neonates and pre-treated children were included *(table 6.10)*. The negative predictive value for predicting very low risk of bacterial meningitis in children who did not have bacterial meningitis was 100% with and without inclusion of neonates, and 94-95% when pre-treated participants were included. The positive predictive value for bacterial meningitis in participants predicted as not low risk of bacterial meningitis was 31.7% (CI 28.4-35.0%), and was 68% (CI 65.2-70.2%) with inclusion of neonates, and 33% (CI 30-35.2%) including pre-treated children *(table 6.10)*.

**Figure 6.5 Participants included in validation of BMS analysis**

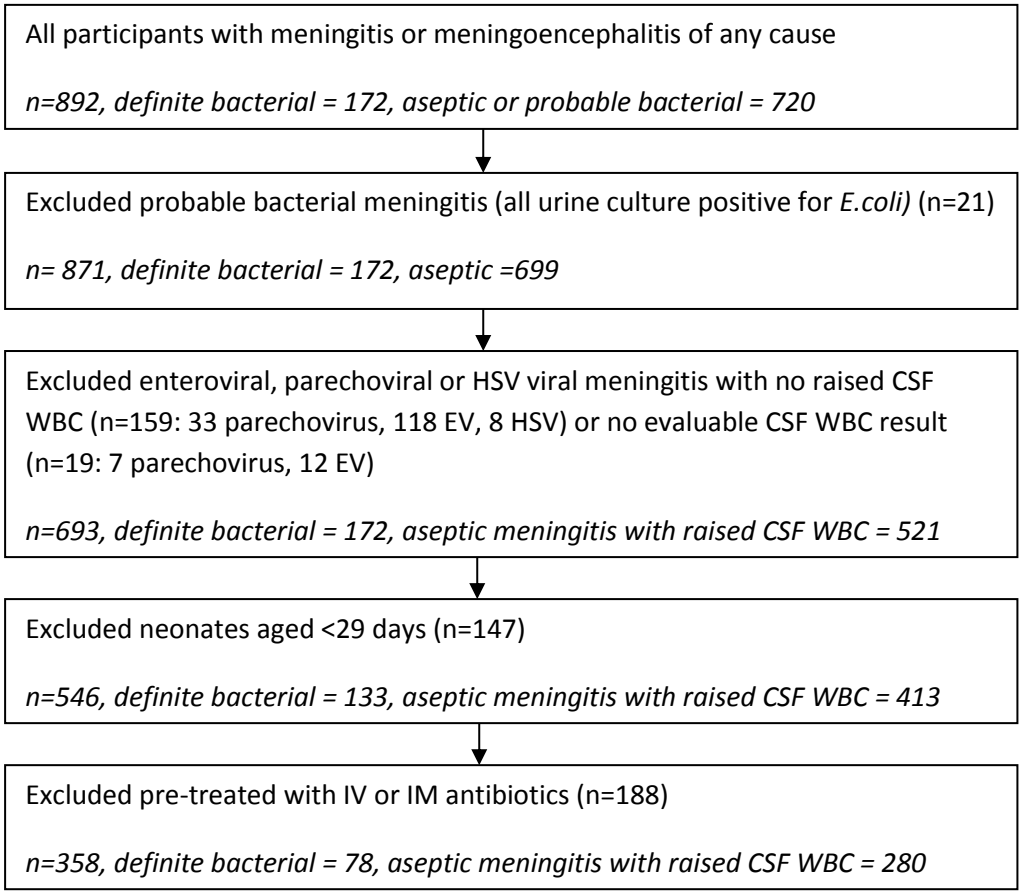


| **Table 6.10 Sensitivity, specificity, negative and positive predictive value of the BMS in the UK-ChiMES cohort** | | | | |
|---|---|---|---|---|
| | Sensitivity % (n/N) *95% CI* | Specificity % (n/N) *95% CI* | Negative Predictive Value % (n/N) *95% CI* | Positive Predictive Value % (n/N) *95% CI* |
| Children and infants aged >28 days to <16 years | | | | |
| Not pretreated, missing data excluded, n=237 | 100% (63/63) *100-100%* | 21.8% (38/174) *15.7-27.9%* | 100% (38/38) *100-100%* | 31.7% (63/199) *28.4-35.0%* |
| Not pretreated, missing data included, n=366 | 87.2% (68/78) *79.8-94.6%* | 52.8% (152/288) *49.8-55.7%* | 93.8% (152/162) *91.9-95.7%* | 33.3% (68/204) *30-36.6%* |
| Includes pretreated, missing data excluded, n=387 | 96.4% (106/110) *94.3-98.2%* | 20.9% (58/277) *18.5-23.3%* | 93.5% (58/62) *90.4-96.6%* | 32.6% (106/325) *30-35.2%* |
| Includes pretreated, missing data included, n=558 | 85.0% (113/133) *81.9-88.1%* | 48.5% (206/425) *46.1-50.9%* | 77.4% (206/226) *74.6-80.2%* | 34.0% (113/332) *31.4-36.6%* |
| All children and infants aged <16 years, including neonates | | | | |
| Not pretreated, missing data excluded, n=321 | 100% (87/87) *100-100%* | 20.9% (49/234) *18.2-23.6%* | 100% (49/49) *100-100%* | 68% (185/272) *65.2-70.2%* |
| Not pretreated, missing data included, n=474 | 87.7% (93/106) *84.5-89.9%* | 49.7% (183/368) *47.1-52.3%* | 93.4% (183/196) *91.6-95.2%* | 33.5% (93/278) *30.7-36.3%* |
| Includes pretreated, missing data excluded, n=501 | 97.2% (140/144) *95.8-98.6%* | 19.6% (70/357) *17.5-21.7%* | 94.6% (70/74) *92.0-97.2%* | 32.8% (140/427) *30.5-35.1%* |
| Includes pretreated, missing data included, n=708 | 86.6% (149/172) *84.0-89.2%* | 46.5% (249/536) *44.3-48.7%* | 91.5% (249/272) *89.8-93.2%* | 34.2% (149/436) *31.9-36.5%* |

## 6.6. Development of a new multivariable rule to predict the probability of bacterial meningitis

There were 358 children or infants aged >29 days with definite bacterial or aseptic meningitis of any cause, and no IV or IM antibiotic pre-treatment, included in the development of a new rule (*figure 4.6.*). The median age was 3 months (IQR 1-19 months) for all included children (n=358), 8 months (IQR 3-29 months) for the bacterial meningitis group only (n=78), and 2 months (IQR 1-13 months) for the aseptic meningitis group (n=280).

**Figure 6.6. Participants included in development of a multivariable rule to predict the probability of bacterial meningitis**



*6.6.1. Univariable analysis*

At univariable analysis, there were significant differences (p<0.05) between participants with bacterial and aseptic meningitis for history of fever, any respiratory symptom, vomiting, neck stiffness and altered consciousness (defined as drowsiness or loss of consciousness) (*table 6.11*), and clinical examination findings including any respiratory sign, bulging fontanelle, GCS less than 14 or 15, neck stiffness and temperature (*tables 6.12 and 6.13*). Further analysis of respiratory symptoms and signs are reported in *tables 6.15 and 6.16*. There were significant differences in CSF and blood test results on presentation to hospital between participants with bacterial and aseptic meningitis for CSF glucose, CSF:plasma glucose ratio, CSF WBC, lymphocytes, neutrophils and protein, and blood WBC, neutrophils, lymphocytes and CRP (*tables 6.13 and 6.14*).

**Table 6.11 Comparison of symptoms on history in children and infants with bacterial and aseptic meningitis**

| | Bacterial meningitis n=78 | | Aseptic meningitis n=280 | | P value |
|---|---|---|---|---|---|
| | % | n/N | % | **n/N** | |
| History of Fever | **93.5%** | **72/77** | **82.4%** | **230/279** | **0.017** |
| Respiratory symptoms | **54.0%** | **40/74** | **37.2%** | **101/271** | **0.009** |
| Rash | 23.3% | 17/73 | 27.7% | 75/271 | 0.452 |
| Vomiting | **72.6%** | **53/73** | **48.2%** | **133/276** | **<0.001** |
| Diarrhoea | 17.3% | 13/75 | 18.4% | 50/272 | 0.835 |
| Headache | 39.6% | 19/48 | 36.9% | 62/168 | 0.735 |
| Seizures <24hours | 12.3% | 9/73 | 7.8% | 21/271 | 0.218 |
| Seizures >24hours ago | 2.9% | 2/68 | 2.8% | 7/254 | 1 |
| Neck stiffness | **27.4%** | **17/62** | **13.4%** | **29/217** | **0.009** |
| Neck pain | 26.8% | 15/56 | 18.6% | 34/183 | 0.183 |
| Altered consciousness | **40.0%** | **32/72** | **24.7%** | **67/261** | **0.002** |

| Table 6.12 Comparison of categorical examination findings and laboratory results in children and infants with bacterial and aseptic meningitis | | | | | |
|---|---|---|---|---|---|
| | Bacterial meningitis n=78 | | Aseptic meningitis n=280 | | P value |
| | **%** | n/N | **%** | n/N | |
| Prolonged capillary refill time | 9.3% | 4/43 | 3.1% | 4/129 | 0.109 |
| Respiratory signs | **30.1%** | **22/73** | **15.7%** | **42/267** | **0.005** |
| Photophobia | 17.1% | 6/35 | 16.0% | 19/119 | 0.868 |
| Neck stiffness | **33.3%** | **13/39** | **16.8%** | **22/131** | **0.025** |
| Kernig's sign positive | 0% | 0/8 | 4.9% | 3/61 | 1 |
| Brudzunski's sign positive | 14.3% | 1/7 | 3.9% | 2/51 | 0.325 |
| Rash (any) | 24.6% | 16/65 | 34.8% | 87/250 | 0.119 |
| Vesicular rash | 4.2% | 1/24 | 3.0% | 4/133 | 0.569 |
| Non-blanching rash | 34.5% | 10/29 | 20.6% | 28/136 | 0.107 |
| Fontanelle bulging* | **31.0%** | **9/29** | **7.3%** | **10/137** | **<0.001** |
| GCS less than 15 | **47.4%** | **18/38** | **17.0%** | **17/99** | **<0.001** |
| GCS less than 14 | **39.5%** | **15/38** | **10.0%** | **11/99** | **<0.001** |
| Gram film positive | **44.3%** | **31/70** | **3.9%** | **10/258** | **<0.001** |

Note: *Denominator includes only children with open fontanelle able to be examined

| Table 6.13 Comparison of parametric continuous examination findings and laboratory results in children and infants with bacterial and aseptic meningitis | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Bacterial meningitis n=78 | | | Aseptic meningitis n=280 | | | |
| | mean | 95% CI of mean (1.96XSE) | n | mean | 95% CI of mean (1.96XSE) | n | p-value |
| Temperature (◦C) | **38.7** | **(38.5-38.9)** | **73** | **38.3** | **(38.2-38.4)** | **262** | **0.001** |
| Heart rate (/min) | 149 | (141-157) | 73 | 151 | (147-155) | 269 | 0.722 |
| Respiratory rate (/min) | 39.7 | (36.7-42.7) | 73 | 40.5 | (36.2-42.1) | 261 | 0.639 |
| CSF glucose (mmol/L) | **2.17** | **(1.83-2.52)** | **68** | **3.02** | **(2.94-3.09)** | **257** | **<0.001** |
| CSF:plasma glucose ratio | **0.40** | **(0.31-0.49)** | **32** | **0.56** | **(0.52-0.59)** | **109** | **0.002** |

| Table 6.14 Comparison of non-parametric laboratory results in children and infants with bacterial and aseptic meningitis | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Bacterial meningitis n=78 | | | Aseptic meningitis n=280 | | | |
| | median | IQR | n | median | IQR | n | p-value |
| Blood WBC count ($X10^9$/L) | **15** | **9-23** | **74** | **12** | **9-16** | **271** | **0.007** |
| Blood neutrophil count ($X10^9$/L) | **10** | **4-17** | **75** | **5** | **3-9** | **271** | **<0.001** |
| Blood lymphocyte count ($X10^9$/L) | **2** | **1-4** | **72** | **5** | **3-7** | **270** | **<0.001** |
| Blood CRP (mg/L) | **147.4** | **57-218** | **78** | **4** | **1-17.6** | **280** | **<0.001** |
| Corrected CSF WBC count# ($X10^6$/L) | **409** | **102-1119** | **72** | **55** | **18-184** | **280** | **<0.001** |
| CSF neutrophil number ($X10^6$/L) | **170** | **38-628** | **35** | **19** | **4-70** | **102** | **<0.001** |
| CSF lymphocyte number ($X10^6$/L) | **144** | **29-359** | **41** | **41** | **14-118** | **162** | **0.001** |
| CSF protein (g/L) | **1.2** | **0.6-2** | **65** | **0.5** | **0.3-0.8** | **251** | **<0.001** |

Note: If CRP was recorded as <5 then a value of 1 was used. #corrected by $500X10^6$/L RBC:1 $X10^6$/L WBC

*6.6.2. Multivariable logistic regression analysis*

The following nine variables which were clinically relevant, with <10% data missing, and p<0.25 at univariable analysis were included in a multivariable logistic regression analysis: history of vomiting, seizures within 24 hours, alteration in consciousness (defined as drowsiness or coma), presence of any rash on examination, temperature on arrival at hospital, blood neutrophil count, blood CRP, CSF positive Gram stain and CSF WBC count. Nine variables were identified because they fulfilled these selection criteria although the number of variables is slightly more than the 1 variable per 10 outcome events.

Respiratory symptoms and signs were not included because these variables included a range of different signs or symptoms (*tables 6.15 and 6.16*).

| **Table 6.15 Respiratory symptoms reported on history at presentation to hospital** | | |
|---|---|---|
| **SYMPTOM** | **Bacterial, n=74 with data % (n/N)** | **Aseptic, n=271 with data % (n/N)** |
| **Any respiratory symptom** | **40** | **101** |
| Fast breathing | 48% (19/40) | 36% (36/101) |
| Indrawing | 7.5% (3/40) | 9% (9/101) |
| Grunting | 28% (11/40) | 24% (24/101) |
| Apnoea | 3% (1/40) | 7% (7/101) |
| Coryza | 50% (20/40) | 47% (47/101) |
| Sore throat | 18% (7/40) | 14% (14/101) |
| Cough | 30% (12/40) | 37% (37/101) |
| Wheeze | 10% (4/40) | 8% (8/101) |
| Flu like symptoms | 8% (3/40) | 10% (10/101) |

| **Table 6.16 Respiratory signs reported on examination at presentation to hospital** | | |
|---|---|---|
| **SIGN** | **Bacterial, n=73 with data % (n/N)** | **Aseptic, n=267 with data % (n/N)** |
| **Any respiratory sign** | **22** | **42** |
| Chest recession | 27% (6/22) | 29% (12/42) |
| Grunting | 45% (10/22) | 19% (8/42) |
| Focal chest signs | 0% (0/22) | 5% (2/42) |
| Wheeze | 5% (1/22) | 5% (2/42) |
| Other | 41% (9/22) | 67% (28/42) |

A history of fever was not included because there were >10% missing data. Temperature on arrival at hospital was included although antipyretic use may have influenced this finding. Notably, temperature was not in the final model.

The blood neutrophil count was included. The area under the ROC curve was greater for neutrophils (AUC=0.645) and lymphocytes (AUC=0.712) than for WBC (AUC=0.587), and there were fewer missing data for blood neutrophils than lymphocytes. In a separate stepwise backward elimination logistic regression analysis with ten variables including both blood neutrophils and lymphocytes, blood lymphocytes was eliminated from the final model, but blood neutrophils remained.

The CSF WBC count was included. There were frequent missing data for CSF neutrophils and CSF lymphocytes. CSF glucose was not included because reporting CSF glucose without the serum glucose is an uninformative clinical finding, with a previous study showing a linear correlation between serum and CSF glucose levels,[412] therefore a CSF:plasma glucose ratio should be reported.[106] A plasma glucose was reported at time of LP for less than half of participants, therefore CSF:plasma glucose ratio was not included. CSF protein was also not included due to >10% missing data.

The full model with nine variables included 236 participants with complete data for included variables (47 bacterial, 189 aseptic). The beta coefficients, and odds ratio, with 95% confidence and p-value calculated by bootstrapping are shown in table 4.17. At a predicted probability of ≥0.5, the model predicted bacterial for 25/47 bacterial cases and 6/189 aseptic cases. The mean predicted probability in the model was 0.20, which is the

same as observed probability. The regression co-efficient for the slope of the observed outcome versus predicted probability plot was 1.052 (95%CI 0.918-1.187) (*figure 6.7*). The area under the ROC curve (c-statistic) was 0.920 (*figure 6.8*).

**Table 6.17 Table showing beta coefficient, odds ratio, with 95% confidence interval and P value calculated by bootstrapping for each independent predictor for the multivariable logistic regression analysis including nine variables.**

| Predictor | β coefficient | odds ratio (OR) | 95% CI for OR | P-value |
|---|---|---|---|---|
| Gram film positive | 1.72 | 5.58 | 1.16-54.81 | .034 |
| History of altered consciousness | 0.88 | 2.42 | 0.78-11.33 | .112 |
| History of seizure within past 24 hours | 0.71 | 2.04 | 0.25-10.82 | .356 |
| Blood neutrophils ($X10^9$/L) | 0.06 | 1.06 | 0.99-1.19 | .155 |
| Blood CRP (mg/L) | 0.02 | 1.02 | 1.01-1.03 | .001 |
| CSF WBC corrected# ($X10^6$/L) | 0.000343 | 1.000343 | 0.999852-1.001947 | .446 |
| History of vomiting | -0.19 | 0.83 | 0.21-2.65 | .712 |
| Temperature on arrival at hospital (◦C) | -0.33 | 0.72 | 0.38-1.46 | .267 |
| Any rash on examination | -0.53 | 0.59 | 0.12-1.50 | .344 |
| Constant β0 = 9.20 (-18.57-33.62, p=0.407) | | | | |

Note: #corrected by 500$X10^6$/L RBC:1 $X10^6$/L WBC

**Figure 6.7 Graph showing observed proportion of participants with bacterial meningitis compared with probability of bacterial meningitis predicted by the model with nine variables**

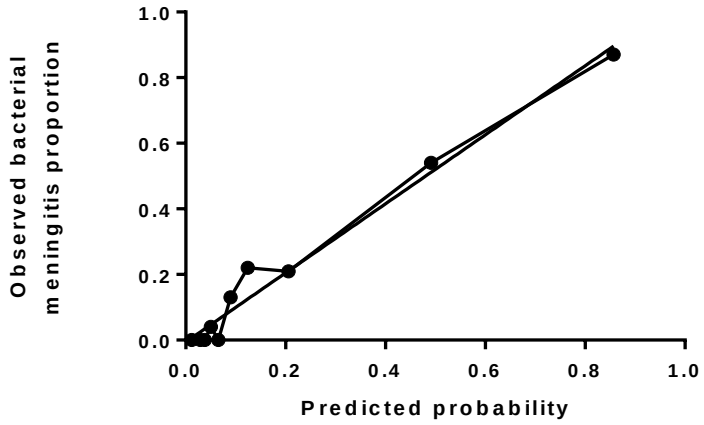
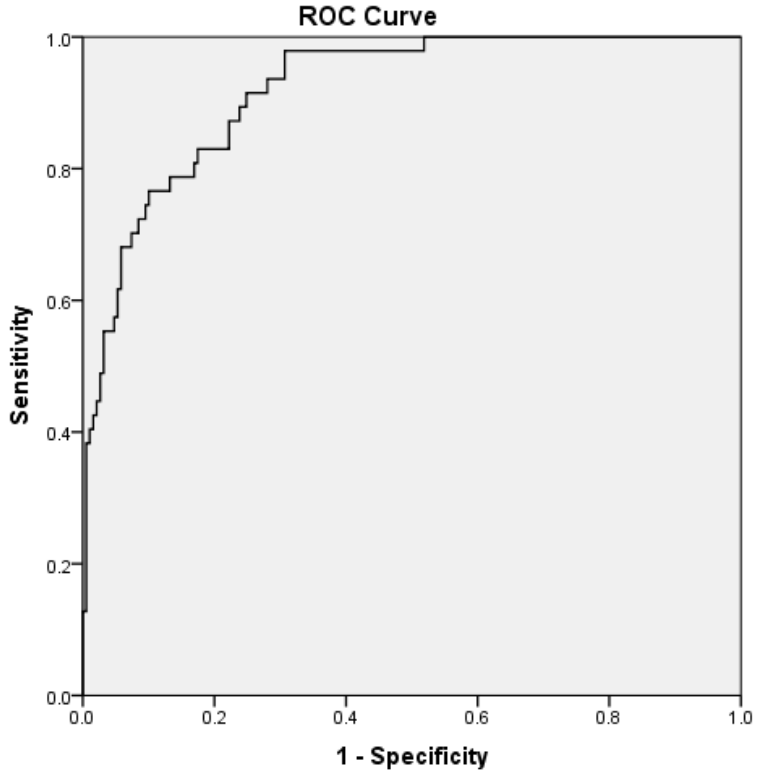


**Figure 6.8 ROC plot for predicting bacterial meningitis by the full model with nine variables**




A second model was developed by backward stepwise elimination including the nine variables in the initial model with 236 participants (47 bacterial, 189 aseptic). Four variables remained in the final model, which were Gram film (β=1.710), altered consciousness (β =0.977), blood neutrophil count (β=0.064) and CRP value (β=0.016) with constant = -3.501. A separate logistic regression analysis was then performed including only these four variables. Participants were included with complete data for all four variables, n=280 (bacterial=62, aseptic=218). The beta coefficients, and odds ratio, with 95% confidence and p-value calculated by bootstrapping are shown in *table 4.18*. At a predicted probability of 0.5, the model predicted bacterial meningitis for 38/62 bacterial cases and 9/218 aseptic cases. 100% sensitivity for predicting bacterial in bacterial cases was achieved at predicted probability cutpoint=0.06 (predicted bacterial for 62/62 bacterial and 103/218 aseptic cases). The mean predicted probability was 0.22, which is

the same as observed probability. The regression co-efficient for the slope of the observed outcome versus predicted probability plot was 1.184 (95% CI 1.004-1.364) (*figure 6.9*). The area under the ROC curve (c-statistic) was 0.916 (*figure 6.10*).

**Table 6.18 Table showing beta coefficient, odds ratio, with 95% confidence interval and P value calculated by bootstrapping for each independent predictor for the multivariable logistic regression analysis including four variables identified by backward stepwise elimination**

| Predictor | β coefficient | odds ratio (OR) | 95% CI for OR | P-value |
|---|---|---|---|---|
| Gram film positive | 2.282 | 9.793 | 3.47-36.95 | .001 |
| History of altered consciousness | .749 | 2.116 | 0.85-5.51 | .078 |
| Blood neutrophils ($X10^9$/L) | .046 | 1.047 | 0.99-1.12 | .082 |
| Blood CRP (mg/L) | .017 | 1.017 | 1.01-1.03 | .001 |
| Constant β0 = -3.395 (-4.515- -2.778, p=0.001) | | | | |

**Figure 6.9 Graph showing observed proportion of participants with bacterial meningitis compared with probability of bacterial meningitis predicted by the model with four variables**

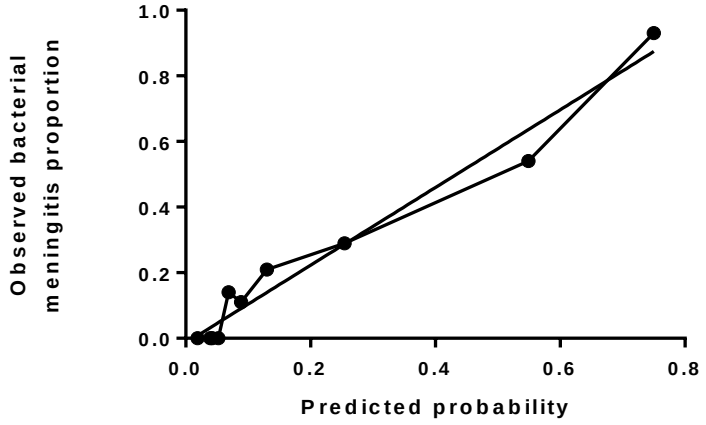
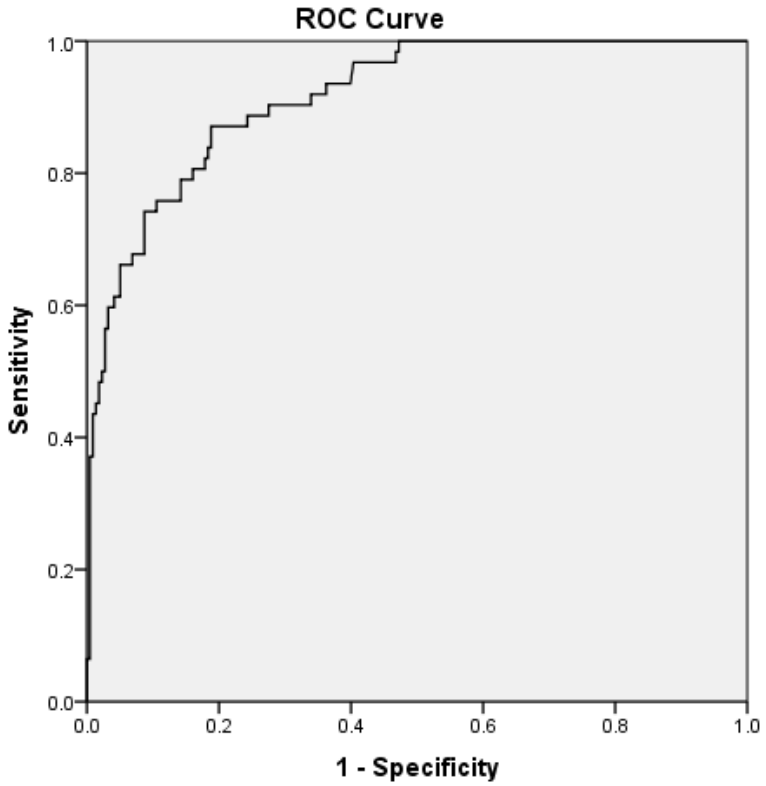


**Figure 6.10 ROC plot for predicting bacterial meningitis by the model with four variables**




A third model was developed by logistic regression analysis including the four variables identified by backward stepwise elimination and the CSF WBC count with 276 participants (58 bacterial, 218 aseptic). CSF WBC was included because CSF parameters have been used in previously developed models to predict meningitis of different aetiologies,[173] and CSF WBC was the last variable eliminated from the backward stepwise model. The beta coefficients, and odds ratio, with 95% confidence and p-value calculated by bootstrapping are shown in *table 6.19*. At a predicted probability of 0.5, the model predicted bacterial meningitis in 33/58 bacterial and 9/218 aseptic cases. 100% sensitivity for bacterial cases was only achieved at a predicted probability cutpoint of 0.06 (bacterial predicted in 58/58 bacterial and 100/218 aseptic). The mean predicted probability was 0.21, which is the same as observed probability. The regression co-efficient for the slope of the observed outcome versus predicted probability plot was 1 (95% CI 0.8907-1.11)

(*figure 6.11*). The area under the ROC curve (c-statistic) was 0.927 (*figure 6.12*). Therefore, the performance of the model with five variables was similar to the model with four variables.

**Table 6.19 Table showing beta coefficient, odds ratio, with 95% confidence interval and P value calculated by bootstrapping for each independent predictor for the multivariable logistic regression analysis including five variables**

| Predictor | β coefficient | odds ratio (OR) | 95% CI for OR | P-value |
|---|---|---|---|---|
| Gram film positive | 2.101 | 8.177 | 2.34 – 35.16 | .002 |
| History of altered consciousness | 0.680 | 1.974 | 0.78 – 5.50 | .121 |
| Blood neutrophils ($X10^9$/L) | 0.047 | 1.048 | 0.99 – 1.15 | .133 |
| Blood CRP (mg/L) | 0.016 | 1.016 | 1.01 – 1.03 | .001 |
| CSF WBC corrected# ($X10^6$/L) | 0.000418 | 1.000418 | 1.000002 – 1.002094 | .190 |
| Constant β0 = -3.522 (-4.852- -2.928, p =0.001) | | | | |

Note: #corrected by 500$X10^6$/L RBC:1 $X10^6$/L WBC

**Figure 6.11 Graph showing observed proportion of participants with bacterial meningitis compared with probability of bacterial meningitis predicted by the refined model with five variables**

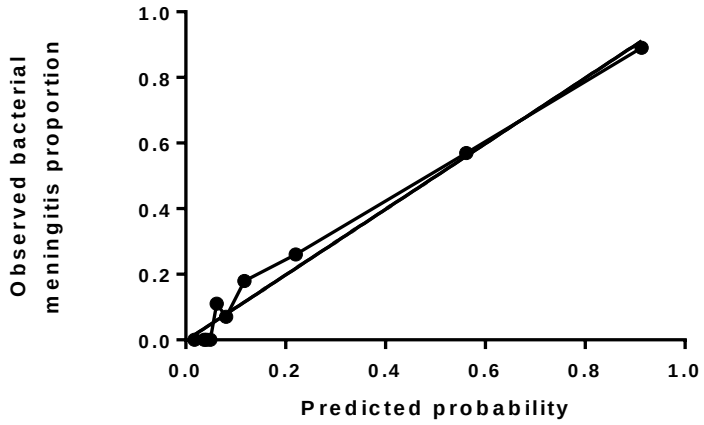
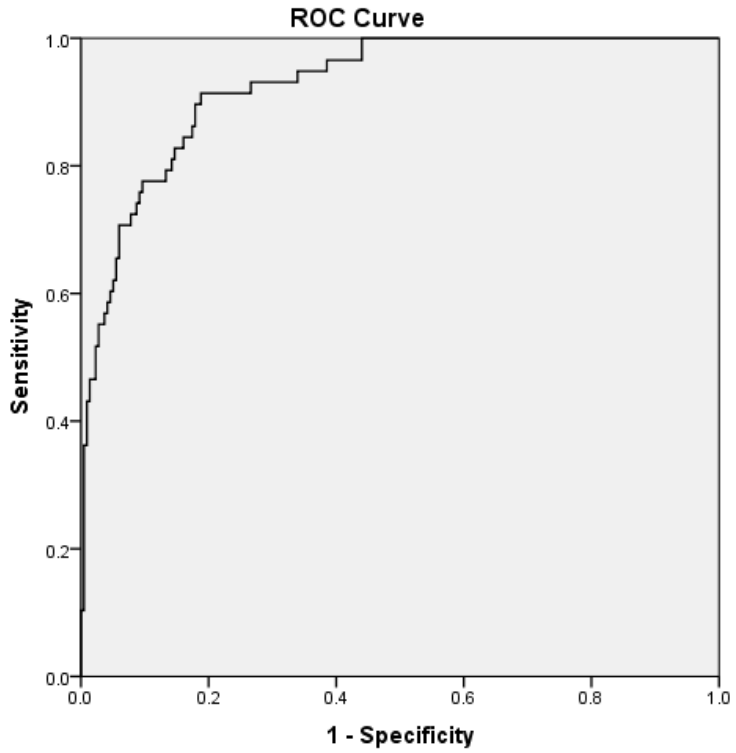


**Figure 6.12 ROC plot for predicting bacterial meningitis by the model with five variables**




Of 188 children aged >28 days who were pre-treated and therefore excluded from model development, 159 had no missing data. Considering the third model, in pre-treated children >28 days, 4% (2/50) of bacterial meningitis (sensitivity 96%) and 38.5% (42/109) of aseptic meningitis cases had a predicted probability of ≤0.06, and the c-statistic was 0.888.

Of 378 participants in the study with definite viral meningitis, 237 were aged >28 days. There were 86/237 participants aged >28 days excluded from development of the model due to no raised CSF WBC count and 12/237 excluded due to no evaluable CSF WBC count. Predicted probabilities were calculated in the third model for 67/86 participants aged >28 days with definite viral meningitis and no raised CSF WBC, who had no missing data. Notably, CSF Gram film was the most frequently missing variable. The mean

predicted probability was 0.073 (SD 0.107), and 69% (46/67) of participants with viral meningitis and no CSF pleocytosis had a predicted probability of 0.06 or lower, compared with 46% for aseptic meningitis with pleocytosis. There was one outlier with a predicted probability for bacterial meningitis of 0.88, who was an eleven-month old infant with EV meningitis, and CRP 300 mg/L, blood neutrophils 16 $X10^9$/L, corrected CSF WBC 5 $X10^6$/L, no altered consciousness and a negative CSF Gram stain.

The third model was also evaluated in 186 neonates aged <29 days (33 bacterial, 153 viral) including 63 neonates (3 bacterial, 60 viral) without a raised CSF WBC (defined in *chapter 5, section 5.2.2*), and including 49 who were pre-treated with IV or IM antibiotics. Of 231 neonates <29 days with meningitis in the study, three had probable bacterial meningitis and a further 42 had missing data for variables in the model and were therefore not included. In 186 included neonates, the mean predicted probability was 0.16, compared with observed probability of 0.18. The regression co-efficient for the slope of the observed outcome versus predicted probability plot was 1.083 (95% CI 0.8722-1.294) (*figure 6.13*). The area under the ROC curve (c-statistic) was 0.887 (*figure 6.14*). 4/107 neonates with a predicted probability ≤0.06 had bacterial meningitis, therefore the sensitivity for bacterial meningitis was 87.9% (29/33). Of aseptic meningitis cases, 69.9% (107/153) had a predicted probability ≤0.06.

With exclusion of pre-treated neonates (n=137, 25 bacterial, 112 aseptic), the mean predicted probability was 0.16, compared with observed probability of 0.18, and area under the ROC curve (c-statistic) was 0.900. At the predicted probability of ≤0.06, the

sensitivity for bacterial meningitis was 88% (22/25). Of aseptic meningitis cases, 67.9%

(76/112) had a predicted probability ≤0.06.

**Figure 6.13 Graph showing observed proportion of neonates with bacterial meningitis compared with probability of bacterial meningitis predicted by the refined model with five variables, including neonates who are pretreated and no raised CSF WBC count**

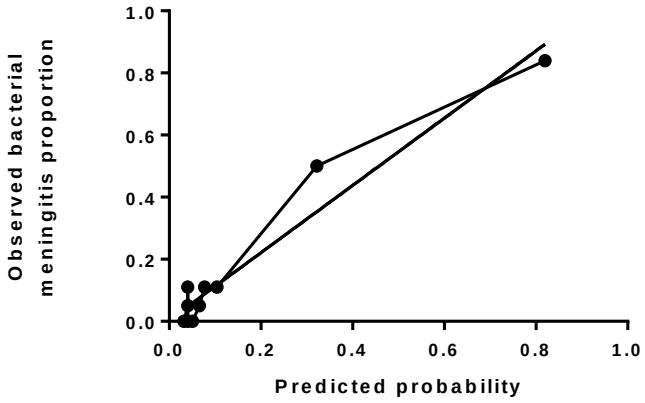
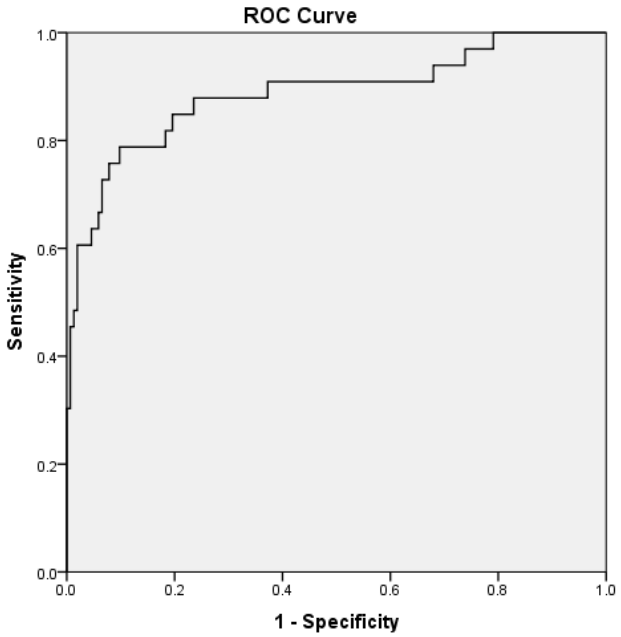



**Figure 6.14 ROC plot for predicting bacterial meningitis in all neonates by the model with five variables**



## 6.7. Discussion

Early identification of children with viral meningitis at presentation to hospital is important to avoid unnecessary antibiotics and hospital admission. In this current analysis including 892 infants and children with meningitis recruited prospectively across the UK, only 19% of cases were bacterial. A detailed analysis of clinical and laboratory features in meningitis of different aetiologies and a non-meningitis illness was described. Predictors were identified from clinical and laboratory results which are routinely collected in UK hospitals to develop a tool which could assist clinical decision making by predicting probability of bacterial meningitis, and therefore could reduce unnecessary management for children at low risk of bacterial meningitis.

Previous studies have investigated CSF and blood parameters including CSF cell counts, protein, glucose, and blood CRP and cell counts to distinguish bacterial from aseptic meningitis.[283-286] CSF results have not reliably been shown to discriminate although results of studies vary.[283-287] Studies also report variable results with blood cell counts.[268,283,284,286] A low or negative CRP has been shown to be a good predictor of non-bacterial meningitis, but studies report low specificity for raised CRP to predict bacterial meningitis.[268,283,284,289] Other previously investigated laboratory parameters include CSF neutrophil counts,[288] and CSF lactate and serum procalcitonin which are not routinely performed.[268,283,284,290,294] Although typical clinical features of meningitis are well described,[44,100,252] few studies have directly compared clinical features in bacterial and aseptic meningitis.[177,260,268,294,300] In these present data, a descriptive analysis of clinical and CSF parameters for bacterial and aseptic meningitis were consistent with previous typically described results.[44,252] Not surprisingly, children who were investigated for

suspected meningitis also presented to hospital with a similar constellation of clinical features to children with meningitis. At univariable analysis, laboratory results were better at discriminating between bacterial and aseptic meningitis than clinical features, with statistically significant results for all included CSF and blood parameters (*tables 4.13-4.14*). There were also significant differences in reported clinical features comparing bacterial and aseptic meningitis for vomiting (73% versus 48%, p<0.001), altered consciousness (40% versus 25%, p=0.002) and reduced GCS (GCS<14: 40% versus 10%, p<0.001), any respiratory symptoms (54% versus 37%, p=0.009) or signs (30 versus 16%, p=0.005), history of fever (94% versus 82%, p=0.017), temperature on arrival (38.7◦C versus 38.3◦C, p=0.001), and when reported there were also significant differences for neck stiffness and bulging fontanelle.

ICU admission rates for infants with parechoviral meningitis (10.6%) were higher than for EV meningitis (3.8%), although not as high as were reported by a recent Australian study (25%).[74] The difference may be contributed to by variation in inclusion criteria, because the Australian study included infants with any parechoviral infection,[74] not limited to suspected meningitis. Consistent with previous studies, most (81%) infants with parechoviral meningitis did not have a raised CSF WBC count.[73,74] Discussion of the comparison of enteroviral meningitis with and without pleocytosis in *chapter 7*.

Previous studies investigating clinical decision rules to differentiate bacterial and viral meningitis from clinical, blood and CSF parameters reported that the BMS had the highest sensitivity and specificity,[168,174,293-296,413] including a systematic review of bacterial meningitis prediction rules.[293] The BMS was developed in 2002,[173] and a meta-analysis

was published in 2012 including eight non-UK, validation studies, most of which were retrospective.[295] In this study, the sensitivity and negative predictive value for the BMS were 100%, both with and without inclusion of neonates, but with exclusion of children who were pre-treated with antibiotics or had missing data. However, the specificity of the BMS in this UK cohort was 22% (95% CI 15.7-27.9%), or 21% (CI 18.2-23.6%) with inclusion of neonates, which is lower than the specificity of 62.1% reported by the meta-analysis of eight previous validation studies.[295] Many children had missing data for BMS predictors, with 35% of non-pretreated children aged >28 days excluded because of missing data, most notably missing CSF neutrophil counts.

The predictors in the BMS are categorised,[173] however recommendations for development of clinical decision rules suggest not categorising continuous variables to avoid loss of information.[407,409] Nowadays, clinical decision rules can be developed for use as online calculators or as apps.[414,415] This allows clinicians to enter variables, and a predictive probability or risk score to be calculated, which also reduces the requirement to categorise continuous predictors.

Therefore, considering the low specificity and frequent missing data for the BMS in the UK population, widespread availability of devices to access online calculators or apps and that 96% (341/354) of children with confirmed EV or parechoviral meningitis received antibiotic treatment in this study (*chapter 5, section 5.3*), a new rule was developed to predict probability of bacterial meningitis, which could assist with clinical decision making. The development of the new rule used current prospectively collected data, available at presentation to hospital, including both clinical and laboratory features. To

ensure accurate comparisons between bacterial and aseptic meningitis groups, several exclusions were made for development of the rule, although further analyses were performed to assess the rule performance in these groups. Neonates aged <29 days were excluded because of previously reported differences with typical CSF parameters.[270,272,273] Children were excluded with viral meningitis without a raised CSF WBC count because they would not be identified as meningitis on initial LP results until PCR or cultures were available. Infants with probable bacterial meningitis with a positive urine *E.coli* culture were also excluded because these cases could not be accurately assigned to either group.

Although respiratory symptoms and signs were significant at univariable analysis, they were not investigated as predictors in the model, because there was substantial variation in what these represented in this current dataset. However, respiratory symptoms and signs may be useful to consider in the future as potential predictors. A history of loss of consciousness was included in the model, but may be difficult to accurately assess. GCS on arrival at hospital may be a more reliable clinical sign, and both GCS<14 and GCS<15 were significant at univariable analysis, but were frequently not reported suggesting GCS is not routinely assessed for these children at presentation to hospital in the UK. Although CSF protein and neutrophil counts were included in the BMS, these variables in addition to CSF:plasma glucose ratio were not considered in the new model because they were not adequately reported.

The first multivariable rule developed included nine variables, which was likely to be overfitted.[416] A second rule developed by backward stepwise elimination included CSF Gram film result positive, history of altered consciousness, blood neutrophil and CRP

results. A third rule was then investigated including also CSF WBC count, because CSF WBC was the last variable eliminated in the backward stepwise model, was included as a predictor in the BMS,[173] and was frequently reported in this cohort. Both the second and third models performed well. The c-statistic for the model with four variables was 0.916, and for the model with five variables was 0.927. The regression coefficients for observed versus predicted probabilities for both rules was approximately one, suggesting good discrimination. At a predicted probability cut-off of 0.06, both rules achieved 100% sensitivity for bacterial meningitis. At the 0.06 cut-off, 46% of aseptic meningitis cases were predicted as bacterial for the model with five variables, and 47% for the rule with four variables. To achieve higher specificity for bacterial meningitis, a higher predicted probability cut-off could be used. Therefore, if children with suspected meningitis and a predicted probability for bacterial meningitis in the rule of ≤0.06 were observed without antibiotics, unnecessary antibiotic management may be prevented for more than half of children with aseptic meningitis, and could also lead to resulting earlier hospital discharge. At the clinician's discretion, a higher predicted probability cut-off could also be used to assist with decision making regarding observing children off antibiotics, although at high predicted probabilities management as bacterial meningitis would be advised.

For the third rule developed including five variables, in children >28 days with viral meningitis and no raised CSF WBC count, 69% had a predicted probability of ≤0.06, and the mean predicted probability was 0.07 suggesting the rule is likely to also be applicable to these children. For both pre-treated children >28 days and all neonates <29 days, there were small numbers of participants with bacterial meningitis who had a predicted probability ≤0.06 (pre-treated >28 days n= 2/50, all neonates n= 4/33).

Interestingly, within the recently published UK NICE guideline for sepsis[417] clinical features including heart rate, respiratory rate, behaviour and temperature comprise some of the criteria used to assess risk and guide recommendations for prompt antibiotic management. In this present study, there were no significant differences in heart rate and respiratory rate for children presenting with bacterial or aseptic meningitis, there was a small but significant difference in temperature, however a history altered consciousness was included in the final model. It is feasible that the NICE sepsis guideline[417] could lead to more antibiotic use in children presenting with suspected meningitis, whereas the aim of this current study is to develop a model to reduce unnecessary antibiotic management and hospital LOS.

In the development of the clinical decision model, missing data were suspected to be missing completely at random, therefore listwise deletion should not have introduced bias.[418-420] Missing data can be missing completely at random which should result in no introduction of bias, missing at random where a missing variable may be predictable from other participant data, or missing not at random where there is some correlation between the value of the missing variable and which group they are in related to participant data that is unobserved.[418-420] In this present study, data were collected prospectively and therefore variables with frequent missing data reflect information that were not often collected in the clinical setting and were therefore reasonable to exclude. However, if there were variables with frequent missing data which may be good predictors, this could be addressed by suggesting changes in clinical practise to collect these data more often and then explore inclusion in a model.

Limitations in this analysis include high rates of missing data for some variables investigated to distinguish bacterial from aseptic meningitis, including BMS predictors. However unreported variables are likely to represent information collected and tests typically performed for children presenting with suspected meningitis on arrival at hospital in the UK. Frequent pre-treatment with antibiotics also reduced included participants numbers, but exclusion of these children was required to ensure allow accurate comparisons between groups.

*Summary*

A new clinical decision model was developed that could provide a predicted probability of bacterial meningitis on presentation to hospital, using both clinical and laboratory features. The new rule could guide clinical decision making, particularly about observing children off antibiotics who present with suspected meningitis and a low predicted probability of bacterial meningitis, which may lead to reduced unnecessary antibiotic treatment and earlier hospital discharge. The new model could be further validated in separate populations of children with meningitis.[405] Detailed clinical features of meningitis of different aetiologies were also described.

## 7. Chapter 7: Detection of enterovirus by real-time PCR of stool, serum and respiratory samples in children with suspected or confirmed viral meningitis.

### 7.1. Introduction

Most childhood aseptic meningitis is caused by enteroviruses (EV) when an aetiology is defined (*chapter 5*). Since the widespread use of PCR, it has been established that EV infection of the CNS can occur in the absence of a raised CSF WBC count, more commonly in young infants aged <1-3 months.[172,276-280] Previous studies have suggested that EV meningitis may be considered the diagnosis in children with a raised CSF WBC count and an EV-PCR positive (EV-PCR+) sample from another site, particularly if a lumbar puncture or CSF EV-PCR was not performed.[20,257,311,421] A Finnish study in adults reported that in definite or probable EV meningitis, obtaining a CSF EV-PCR+ result was less likely if the lumbar puncture was performed more than two days following onset of symptoms.[421] Therefore, in aseptic meningitis, it is of interest to investigate EV-PCR positivity from samples that are more readily obtained than CSF, for example stool and respiratory samples.

The Enterovirus R-gene® kit is a commercially available real-time PCR (RT-PCR) kit which is reported to detect 64 EV serotypes by amplification of a 146bp fragment of the highly conserved 5' non-coding region using TaqMan probes.[302,422,423] A study which evaluated the EV R-gene® kit at two hospitals in France reported that the kit detected 64/65 human EV serotypes tested from 227 EV strains.[422] The kit also detected 4/7 human rhinoviruses tested, but no non-picornaviruses.[422] Comparison of results from the EV R-gene® kit to other RT-PCR assays showed 95-97% concordance for 197 CSF samples, and 90-96%

concordance for 103 respiratory samples tested, with no confirmed positive results for samples that tested negative from comparator kits.[422] A previous study also tested stool samples with the EV R-gene® kit.[423] The kit includes a reverse transcriptase step, and an internal control which is a phage encapsulated nucleic acid sequence added to samples before extraction and therefore controls for the effectiveness of the extraction process and amplification.[302]

Early identification of EV in meningitis could reduce unnecessary hospitalisation and antibiotic treatment.[22,185,279,317,321] Few studies have investigated the frequency of isolation of EV from non-CSF sites in EV or aseptic meningitis.[311,421] Clinical differences between EV CNS infection in children with and without a raised CSF WBC count (CSF pleocytosis) are also not well described.[278,280] Improved understanding of childhood EV meningitis and the significance of isolation of EV from a non-CSF site in aseptic meningitis could assist with clinical decision making.

*Aims*

The aims of this study were:

1. To perform EV-PCR on stool, serum and respiratory samples, and investigate the proportion of EV-PCR+ samples, in children with EV meningitis or aseptic meningitis of unknown cause.

2. To investigate the time (in days or hours) following onset of symptoms that positive or negative EV-PCR results are obtained for CSF and non-CSF samples in childhood EV meningitis or aseptic meningitis.

3. To compare age, clinical and laboratory features in childhood EV meningitis with CSF pleocytosis to without CSF pleocytosis.

4. To compare clinical and laboratory features in children with EV meningitis, to children with aseptic meningitis of unknown cause who have a EV-PCR+ stool, respiratory or serum sample.

## 7.2. Methods

### *7.2.1. Participant inclusion*

Participants from the UK-CHiMES study were included in this study who fulfilled the following criteria, following tests performed at hospital sites at the discretion of the hospital physicians:

1. CSF EV-PCR+ result from hospital site OR
2. Aseptic meningitis of unknown aetiology OR
3. Normal lumbar puncture, performed for suspected meningitis

AND stool, serum, throat swab or nasopharyngeal aspirate (NPA) sample available for research analysis.

Children were excluded from the final analysis who had blood stained CSF results, CSF EV-PCR not performed at hospital site, aseptic meningitis with probable non-EV cause identified at hospital site, only an invalid EV-PCR result obtained (from non-CSF site), or a non-meningitis illness not including 'viral illness unspecified'.

For definitions of a raised CSF WBC count (CSF pleocytosis) and 'aseptic meningitis', see *chapter 5, section 2.2*.

Participants were classified into five groups determined by CSF-EV PCR results from hospital or reference laboratories, whether they had a raised CSF WBC count, and EV PCR testing of stool, throat swab, NPA or serum samples:

Group 1: CSF EV-PCR+, with pleocytosis.

Group 2: CSF EV-PCR+, no pleocytosis.

Group 3a: Aseptic meningitis of unknown cause. Stool, respiratory or serum sample/s EV-PCR+ (from enterovirus R-gene® kit or hospital site laboratory testing).

Group 3b: Aseptic meningitis of unknown cause. Stool, respiratory or serum sample/s EV-PCR-.

Group 4: Viral illness unspecified and normal lumbar puncture. Stool, respiratory or serum sample/s EV-PCR-.

*7.2.2. Clinical data collection*

Data collected into an electronic database during the UK-ChiMES study were used for analysis including results and timing of hospital laboratory tests, timing of onset of symptoms, and clinical features at presentation to hospital.

*7.2.3. Sample collection*

Both leftover samples from clinical tests performed and additional research samples were collected at hospital sites if consent was obtained including stool, throat swab, NPA and serum. All samples were frozen at -70 to -80°C as soon as possible after collection. Throat swab samples were collected with COPAN flocked universal transport media swabs.[343] Samples were transported on dry ice from hospital laboratories to the Oxford Vaccine Group laboratory, University of Oxford for analysis.

*7.2.4. Methods for RNA extraction from stool, serum, throat swab and NPA samples*

Stool, throat swab, NPA and serum samples were thawed on ice.

Stool samples were prepared according to the following procedure prior to viral RNA isolation.[424] A volume of 0.5-1ml of stool was suspended in 5ml of 0.89% sodium chloride. Centrifugation was performed at 4000 x *g* for 20 minutes. Sterile filtration of the supernatant was performed using a 0.22 µm filter. A volume of 140 µl of filtrate was used as the starting material for RNA extraction.

Samples from throat swabs and NPAs were also prepared prior to RNA extraction to avoid co-purification of cellular DNA.[425] Throat swab samples were vortexed briefly then centrifugation was performed for 10 minutes at 1500 x *g* and the supernatant was used as a starting material for RNA extraction. NPA samples were vortexed briefly and centrifugation was performed for 10 minutes at 1500 x *g*. Sterile filtration using a 0.22 µm filter was performed using the NPA supernatant, and the filtrate was used as a starting material for RNA extraction.

RNA extractions were performed according to the QIAamp Viral RNA Mini Kit spin protocol manufacturer's instructions.[425] During the procedure, RNA is adsorped onto a silica membrane and then eluted twice into a total of 80 µl of elution buffer. An internal control (10 µL), provided by the Enterovirus R-gene®[302] kit was added to samples (140µL) before extraction.[302] Reference extraction and inhibition control samples were also obtained by performing extractions of the internal control added to water. RNA extracts were stored in RNase free collection tubes. RNA extracts that were not immediately analysed by PCR were frozen at -80$^{o}$.

*7.2.5. Methods for RT-PCR*

EV reverse-transcriptase RT-PCR of RNA extracts was performed using the Enterovirus R-gene® kit, following the manufacturer's instructions,[302,422,423] with the Applied Biosystems StepOnePlus real-time PCR system.[426] The Enterovirus R-gene® real-time PCR kit[302] included:

1. enterovirus and internal control amplification premix including primers, dNTPs, amplification buffer, Taq polymerase, enteroviral probes, and probes and primers for the internal control.
2. internal control
3. reverse transcriptase
4. enterovirus positive control

The enterovirus R-gene® kit was firstly validated by performing PCR of the positive control provided at different dilutions, and water as a negative control.

Batches of samples were then analysed in a series of PCR experiments. In each PCR experiment, at least one each of the following control samples were included:

- water - negative amplification control
- internal control extracted with water - reference extraction + inhibition control (read at 560 nm which was the wavelength for the internal control fluorescent marker), negative extraction + amplification control (read at 530 nm which was the wavelength for the EV probe fluorescent marker)[302]
- enterovirus R-gene® kit positive control - positive amplification control

*7.2.6. Methods for interpretation of RT-PCR results*

The EV target was read at 530nm and internal control read at 560nm, as per manufacturer's instructions.[302] The sample was considered positive if an amplification curve was present and crossed the ΔRn 3000 point within 36 cycles. ΔRn referred to the Rn (cycle) – Rn (baseline), where Rn referred to the intensity of reporter dye normalised for non-PCR related fluorescence fluctuations.[426] Quantification was not assessed.

*7.2.7. Methods for validation of PCR results*

If the cycle number at which the amplification curve crossed the ΔRn 300 for the internal control extracted with the sample was ≤ cycle number for the internal control extracted with water +3 cycles, then it was assumed that the sample was non-inhibited and correctly extracted. If the cycle number at which the amplification curve crossed the ΔRn 300 line for the internal control extracted with the sample was > cycle number for the internal control extracted with water +3 cycles, then it was assumed that the sample was

inhibited or badly extracted. If the sample was assumed to be inhibited or badly extracted, then negative results were considered invalid, but positive results were considered valid.

EV RT-PCR using the Enterovirus R-gene® kit was repeated without re-extraction or dilution for randomly selected samples.

For five stool, serum or throat swab samples tested by Enterovirus R-gene® kit, EV-PCR results were also available and compared with tests performed at hospital laboratories.

*7.2.8. Further processing of invalid serum sample results*

Most samples with invalid results indicated by non-amplification of the internal control were further processed by a combination of repeating the PCR reaction, re-extracting RNA, and diluting samples by 1:10 and 1:20.

*7.2.9. Data analysis*

Demographic, laboratory and clinical parameters were compared between the five groups defined including age, median length of hospital stay (LOS), management with intravenous (IV) antibiotics, pre-treatment with IV antibiotics before lumbar puncture (LP), results of non-CSF EV-PCR obtained, time of LP following onset of symptoms, day of non-CSF samples taken following onset of symptoms, results of blood WBC count and CRP obtained on day 0 or 1 of admission, and clinical features including temperature,

respiratory rate, heart rate on admission to hospital, altered consciousness, rash, and history of vomiting.

A univariable analysis was performed comparing age (in days), blood WBC count, and time of LP following onset of symptoms (in hours) for EV meningitis with and without pleocytosis (groups 1 and 2). Further statistical analyses comparing features of children with EV meningitis (groups 1 and 2) to children with aseptic meningitis and a EV-PCR+ non-CSF sample (group 3a) were not performed in this dataset because of small numbers in group 3a.

### *7.2.10 Statistical methods*

Statistical analyses were performed with SPSS software. T-tests were used to compare continuous variables with normal distribution. The Wilcoxon rank-sum test was used to compare non-parametric continuous data. 95% confidence intervals and P-values were calculated. A P-value of <0.05 was considered statistically significant.

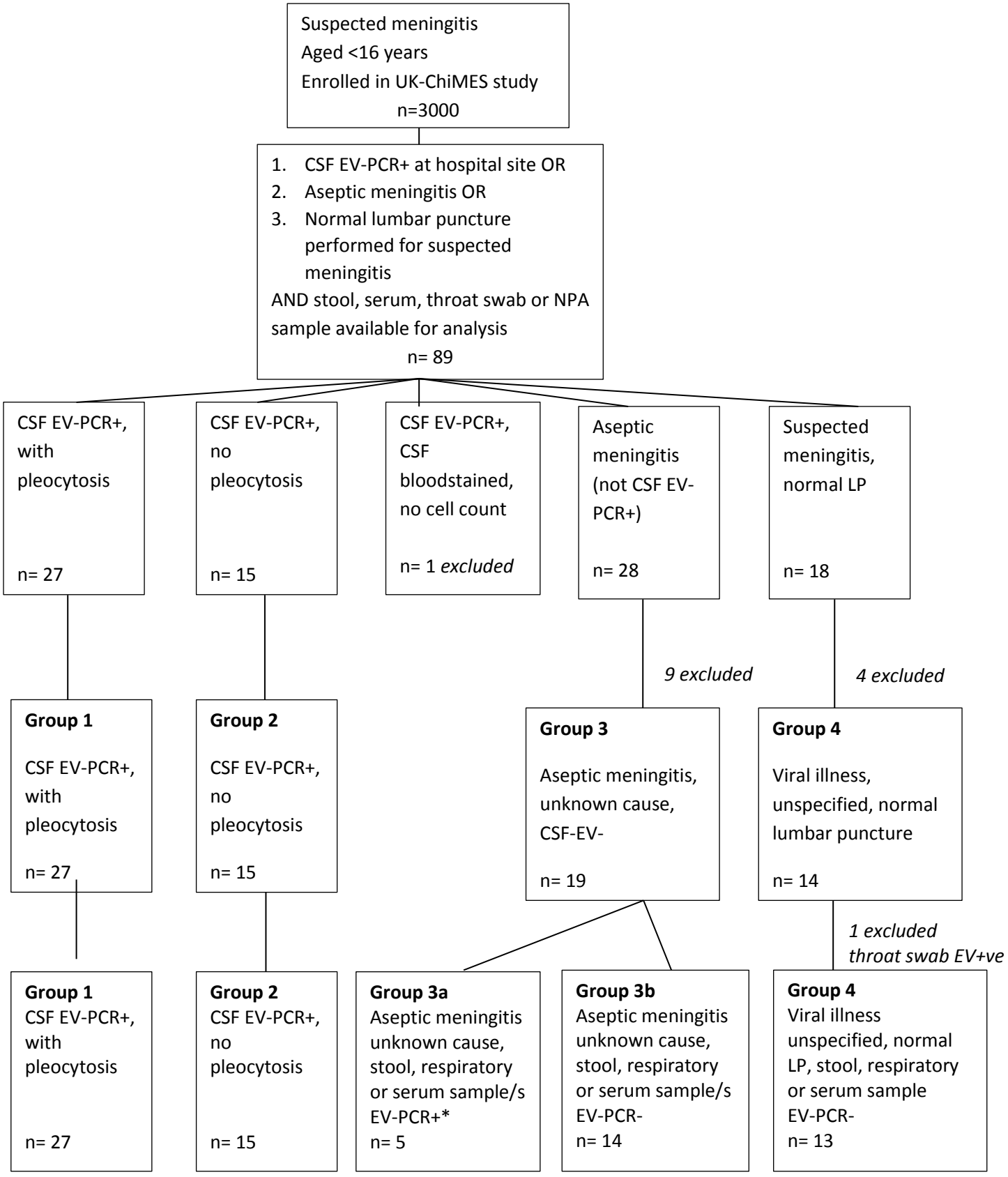
## 7.3. Results

### *7.3.1. Participant inclusion*

In the UK-ChiMES study, CSF EV-PCR was performed by the hospital site or reference laboratory for 65.1% (1764/2708) of children who had a LP performed with any available results (*chapter 5, table 5.9*).

Enterovirus R-gene® RT-PCR was performed for 56 stool, 49 serum, 33 throat swab and 3 NPA samples from 89 children included in this study, who were admitted to hospital between March 2013 and May 2015 (*figures 7.1-7.4*). The median age of participants was 1 month (range 6 days-13 years, 11 months), 57.3% (51/89) were male and 42.7% (38/89) were female. Undiluted positive samples crossed the ΔRn 3000 point between 17-32 cycles.

**Figure 7.1 Participants included in detection of EV by real-time PCR of stool, serum and respiratory samples in children with suspected or confirmed viral meningitis study**




Notes: *sample positive from either research sample Argene kit or tests performed at hospital site. Reasons for exclusions see *section 7.3.3.*

*7.3.2. Validation of PCR results*

Overall EV-PCR was repeated for twenty samples without re-extraction or dilution. All repeat samples confirmed the same result as the initial sample.

There were no invalid stool, throat swab, or NPA EV-PCR results obtained. There were 34 invalid serum samples results obtained from first PCR reaction by non-amplification of the internal control. Twenty-seven samples were further processed. Seven PCR reactions were repeated without further processing of the sample, resulting in the same results being obtained. Eight invalid serum samples were re-extracted, which resulted in one confirmed negative result and seven results remaining invalid. Six samples were diluted 1:10 which resulted in one confirmed negative result, with remaining samples invalid. Twenty-six samples were diluted 1:20 which resulted in 23 samples showing some amplification of the internal control at a high cycle number (>30 cycles), as expected. This suggests that invalid results for serum were caused by inhibitors to PCR present in the sample. However, results obtained at 1:20 dilution were classified as invalid, whether they appeared positive or negative, because the late amplification was not able to be accurately interpreted.

For five stool, serum or throat swab samples tested (positive or negative), EV-PCR results were also available from tests performed at hospital laboratories. Results from the Enterovirus R-gene® kit compared with results from tests performed at hospital site laboratories are shown in *table 7.1*. Two stool samples were positive by both Enterovirus R-gene® kit testing and hospital laboratory. One stool sample that was positive by Enterovirus R-gene® kit testing was negative at hospital site, although the actual sample

was taken separately for the study. One throat swab sample was negative by Enterovirus R-gene® kit testing, but a different swab taken at hospital site was EV-PCR positive. Information was not recorded about the day and time that the hospital throat swab was collected. An invalid result was obtained for one serum sample by Enterovirus R-gene® kit testing that was positive at hospital site.

**Table 7.1 Comparison of Enterovirus R-gene® PCR results to EV-PCR performed at hospital site**

| Participant number | Sample type | EV-PCR result from hospital site laboratory | EV-PCR result from Enterovirus R-gene® kit | Sample tested with Enterovirus R-gene® kit leftover from clinical sample or separate research sample |
|---|---|---|---|---|
| 668 | serum | positive | invalid | leftover |
| 533 | throat swab | positive | negative | swab taken specifically for study different to swab tested at hospital site |
| 336 | stool | positive | positive | separate sample |
| 1088 | stool | positive | positive | separate sample |
| 1100 | stool | negative | positive | separate sample |

*7.3.3. EV-PCR results in excluded participants*

The following children were excluded from further analysis:

- One CSF EV-PCR+ infant aged <3 months had a blood stained CSF result with CSF cell counts unable to be obtained. This infant was stool EV-PCR+ (Enterovirus R-gene®), had LOS 3 days, and received both IV antibiotics and IV aciclovir.
- Four children with aseptic meningitis and CSF EV-PCR not performed at hospital site. Two of these children were stool EV-PCR+, and throat swab EV-PCR- (Enterovirus R-gene®). The third child was both serum and throat swab EV-PCR-, and the fourth had an invalid serum EV-PCR result. None of these children were EV-PCR+ for a non-CSF sample from results of hospital site tests. Three children

were aged <3months, one was aged 1-4years, median LOS was 6 days, and all received at least one dose of IV antibiotics.

- Four children with aseptic meningitis with a probable cause identified from tests performed at hospital site, which included two participants with confirmed *E. coli* UTIs and therefore presumed *E. coli* meningitis (CSF and blood cultures negative). One child with presumed *E. coli* meningitis was both throat swab and stool EV-PCR-, the second child with presumed *E. coli* meningitis had an invalid serum result, the child diagnosed with aseptic meningits caused by Kawasaki disease had an invalid serum result, and the child diagnosed with encephalitis had a EV-PCR- throat swab.
- Three children with normal lumbar puncture results who had other defined diagnoses (two with UTI, one with sepsis). Both children with UTIs had EV-PCR- stool and throat swabs, and invalid serum results. The child with sepsis had an invalid serum result.
- Two further children whose only available non-CSF EV result was an invalid serum result (one from group 3, one from group 4).

*7.3.4. Characteristics and EV-PCR results in included participants*

Age, median length of hospital admission, management including intravenous antibiotics and pre-treatment with antibiotics before LP are shown in *tables 7.2.-7.3*. Two children in group one were reported as having both positive CSF enteroviral and parechovirus PCR results at hospital site (participants 602 and 1371). Overall, 95% (58/61) of children with aseptic meningitis, including EV meningitis, received at least one dose of IV antibiotics.

The median length of hospital admission was 3 days (IQR 2-4 days) for EV meningitis, and 5 days (IQR 3-7) for aseptic meningitis of unknown cause.

**Table 7.2 Age of participants with aseptic meningitis or suspected meningitis**

| | Age | | | |
|---|---|---|---|---|
| | <3 months % (n/N) | 3-11 months % (n/N) | 1-4 years % (n/N) | 5-14 years % (n/N) |
| Group 1: CSF EV+, with pleocytosis | 81% (22/27) | 19% (5/27) | 0% | 0% |
| Group 2: CSF EV+, no pleocytosis | 87% (13/15) | 7% (1/15) | 7% (1/15) | 0% |
| Group 3: Aseptic meningitis, unknown cause, CSF EV- | 74% (14/19) | 16% (3/19) | 5% (1/19) | 5% (1/19) |
| Group 4: Unspecified viral illness, normal LP | 57% (8/14) | 36% (5/14) | 7% (1/14) | 0% |

**Table 7.3 Median length of hospital admission and management with intravenous antibiotics for participants with aseptic meningitis or suspected meningitis**

| | LOS (median, IQR) | Received at least one dose IV antibiotics % (n/N) | Pretreated with IV or IM antibiotics prior to LP % (n/N) | Other relevamt management |
|---|---|---|---|---|
| Group 1: CSF EV+, with pleocytosis (n= 27) | 3 days (2-4.25) | 100% (27/27) | 19% (5/27) | 6 received at least 1 dose of aciclovir |
| Group 2: CSF EV+, no pleocytosis (n= 15) | 3 days (2-3) | 93% (14/15) | 47% (7/15) | 1 received IM ceftriaxone who did not receive IV antibiotics, 3 received at least 1 dose of aciclovir |
| Group 3: Aseptic meningitis, unknown cause, CSF EV- (n=19) | 5 days (3-7) | 89% (17/19) | 39% (7/18)* | 2 received at least 1 dose of aciclovir |
| Group 4: Unspecified viral illness, normal LP (n=14) | 2 days (2-3) | 100% (14/14) | 14% (2/14) | 1 child also received IM benzylpenicillin |

*Data missing for one participant

Results of Enterovirus R-gene® RT-PCR from different sites are shown in *table 7.4*. The sensitivity for stool EV-PCR positivity in CSF EV-PCR+ children was 100% (26/26, 95% CI 100-100), and the positive predictive value was 86.7% (26/30, CI 74.5-98.9). The sensitivity for respiratory sample (throat swab or NPA) EV-PCR positivity in CSF EV-PCR+ was 33.3% (5/15, CI 9.6-57.0) and positive predictive value was 83.3% (5/6, CI 53.5-113.1). 29% (4/14) of children with aseptic meningitis of unknown cause and a negative CSF EV-PCR were stool EV-PCR+.

**Table 7.4 Results of Enterovirus R-gene® RT-PCR from different sites for children with aseptic meningitis or suspected meningitis**

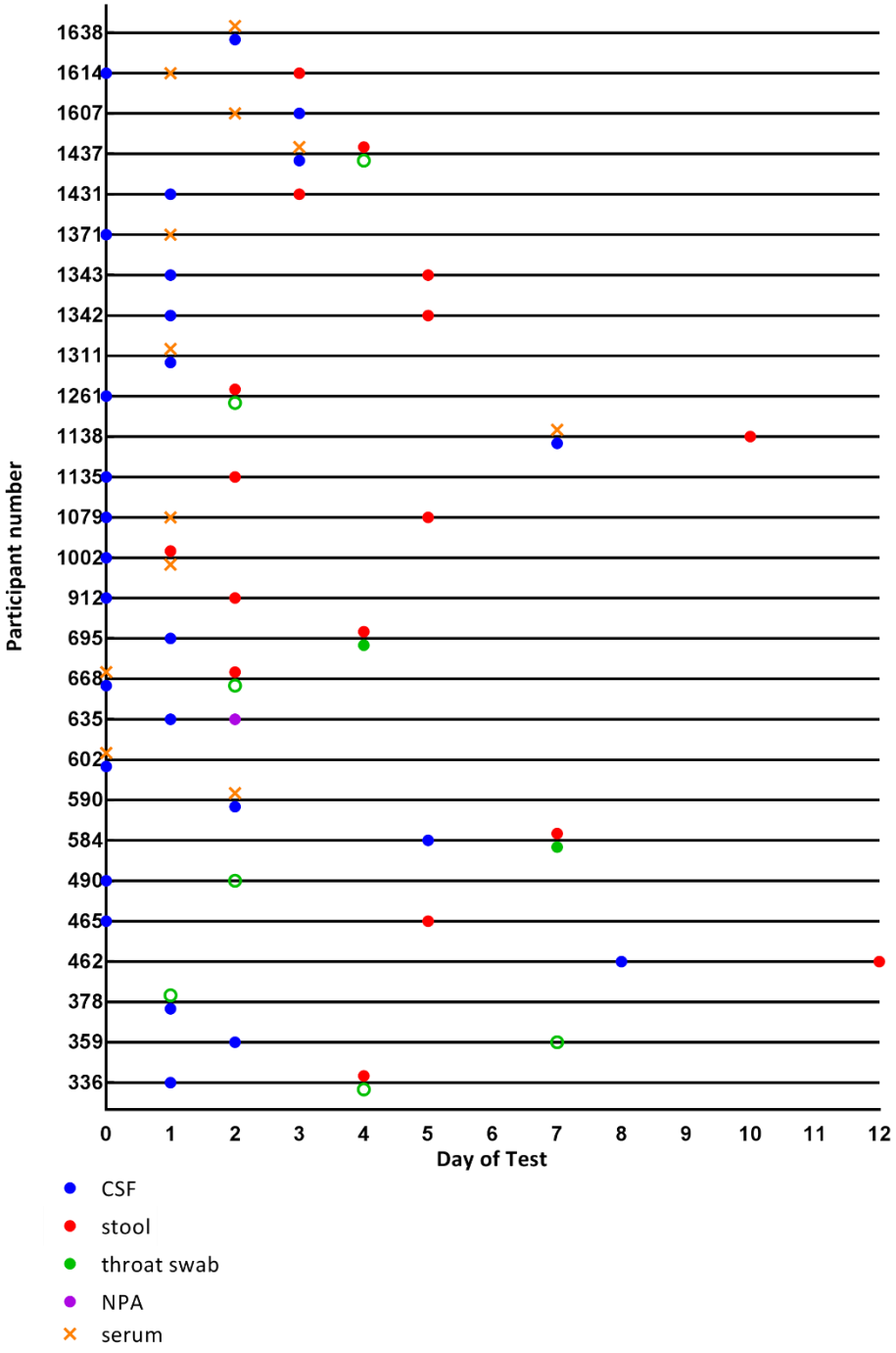
| | Percentage positive enteroviral PCR results | | | |
|---|---|---|---|---|
| | Stool % (n/N) | Throat Swab % (n/N) | NPA % (n/N) | Serum % (n/N) |
| Group 1: CSF EV+, with pleocytosis (n= 27) | 100% (17/17) | 22% (2/9) | 100% (1/1) | (0/0) |
| Group 2: CSF EV+, no pleocytosis (n= 15) | 100% (9/9) | 33% (1/3) | 50% (1 /2) | 75% (3/ 4) |
| Group 3: Aseptic meningitis, unknown cause, CSF EV- (n=19) | 29% (4/14) | 0% (0/6) | (0/0) | (0/5) |
| Group 4: Unspecified viral illness, normal LP (n=14) | 0% (0/10) | 13% (1/8) | (0/0) | 0% (0/7) |

*7.3.5. EV-PCR results and day of sample following onset of symptoms per participant*

The results of EV-PCR for CSF, stool, throat swab and NPA samples, and the day the samples were collected following onset of symptoms for each participant in groups 1-3 are shown in *figures 7.2.-7.4*. Positive stool EV-PCR samples were obtained several days following onset of symptoms, and in EV meningitis all stool samples were EV+ including samples obtained more than a week following onset of symptoms. 53% (10/19) of

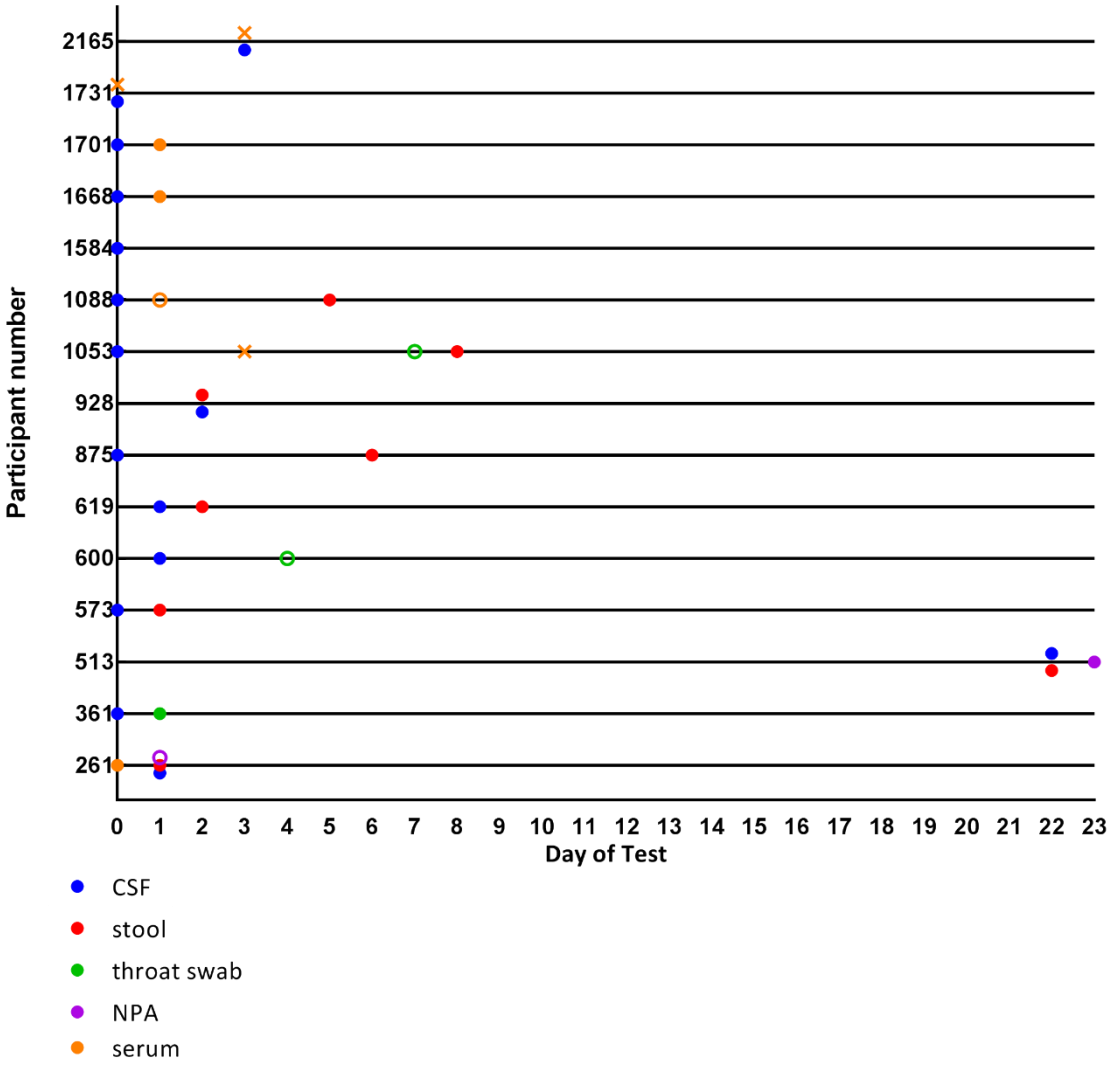
children with aseptic meningitis of unknown cause had their LP on day three or later following onset of symptoms. One child in group 4 had a positive EV throat swab on day 7 following onset of symptoms. Serum samples in group 2 were positive for one child on day 0 and two childen on day 1, and negative for one child on day 1 following onset of symptoms. Serum samples in group 3 were negative for five children, one each on days 1, 2, 3, 4 and 5 following onset of symptoms.

**Figure 7.2 Day of sample following onset of symptoms and result of PCR test from different sites for each participant, in children with confirmed EV meningitis with CSF pleocytosis (Group 1)**



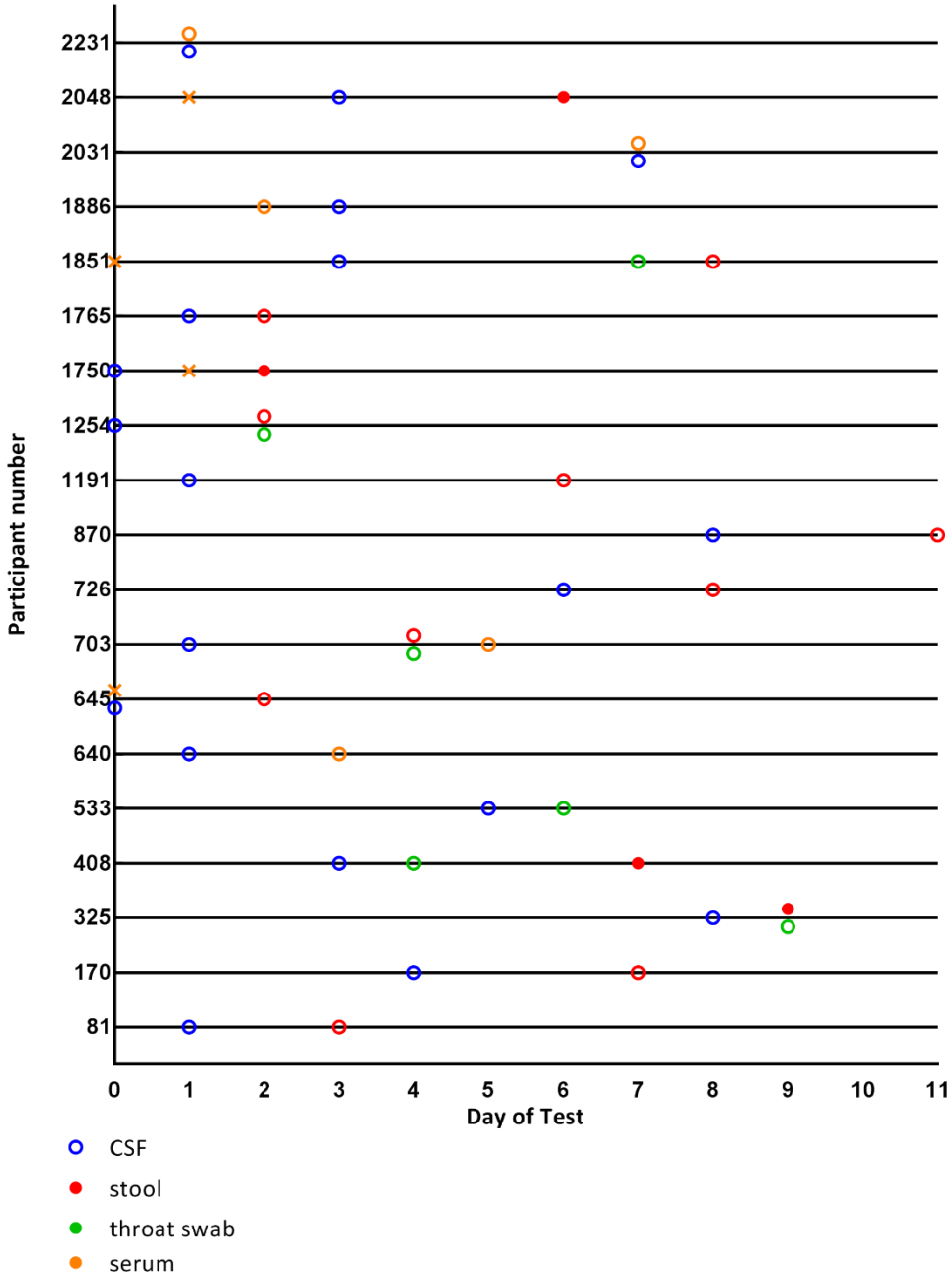

Notes: Filled circle indicates positive result, empty circle indicates negative result, x indicates invalid result. Results of Enterovirus R-gene® kit for stool and respiratory samples test, results of testing at hospital site laboratories for CSF samples.

**Figure 7.3 Day of samples following onset of symptoms and results of PCR test from different sites for each participant, in children with confirmed EV meningitis without CSF pleocytosis (Group 2)**




Notes: Filled circle indicates positive result, empty circle indicates negative result, x indicates invalid result. Results of Enterovirus R-gene® kit for stool and respiratory samples test, results of testing at hospital site laboratories for CSF samples. Participant 1584 had a stool EV-PCR+ result, day of sample missing.

**Figure 7.4 Day of sample following onset of symptoms and result of PCR test from different sites for each participant, in children with aseptic meningitis of no known cause and CSF enterovirus PCR negative (Group 3)**




Notes: Filled circle indicates positive result, empty circle indicates negative result, x indicates invalid result. Results of Enterovirus R-gene® kit for stool and respiratory samples test, results of testing at hospital site laboratories for CSF samples.

*7.3.6. Comparison of clinical and laboratory features*

Comparison of clinical and laboratory features in groups 1-4 are shown in *table 7.5*. No children in in this dataset presented with seizures, and headache was not analysed because the majority of participants were infants. The child in group 4 who had an EV-PCR+ throat swab was excluded from group 4 for comparison of clinical features, because this may represent EV infection. One child with CSF-EV- aseptic meningitis was throat swab and stool EV-PCR+ from results obtained from hospital site laboratory tests, and was therefore allocated into group 3a (*figure 7.1.*, participant 533). No further children had non-CSF EV-PCR+ results from hospital site tests that were not also positive by Enterovirus R-gene® kit.

| **Table 7.5 Comparison of clinical and laboratory features for children with EV meningitis with and without pleocytosis, aseptic meningitis of unknown cause and a non-specific viral illness** | | | | | |
|---|---|---|---|---|---|
| | **Group 1**: CSF EV+, with pleocytosis n=27 | **Group 2**: CSF EV+, no pleocytosis n=15 | **Group 3a**: Aseptic meningitis unknown cause Non-CSF sample EV+ n=5 | **Group 3b:** Aseptic meningitis unknown cause Non-CSF sample EV- n=14 | **Group 4**: Unspecified viral illness, normal LP n=13 |
| | ***median (IQR) n=*** | ***median (IQR) n=*** | ***median (IQR) n=*** | ***median (IQR) n=*** | ***median (IQR) n=*** |
| Age (months) | 1 (0-2) n=27 | 1 (0-2) n=15 | 1 (0.5-16) n=5 | 2 (1-4) n=14 | 1 (0-3) n=13 |
| Age (days) | 42 (18-68) n=27 | 34 (29-70) n=15 | 40 (31-51) n=5 | 76 (51-125) n=14 | 57 (23-107) n=13 |
| CSF WBC* ($X10^6$/L) | 302 (102-540) n=27 | 0 (0-2) n=15 | 62 (37-220) n=5 | 43 (13-204) n=14 | 0 (0-3) n=13 |
| Blood WBC† ($X10^9$/L) | 13.0 (8.9-17.2) n=26 | 9.7 (5.8-10.8) n=15 | 12.9 (10.6-17.7) n=5 | 11.7 (8.9-19.5) n=14 | 8.4 (6.1-13) n=13 |
| CRP†‡ (mg/L) | 1 (1-7) n=22 | 11 (7-27) n=15 | 12 (1-16) n=5 | 45 (1-133) n=13 | 7 (1-34) n=13 |
| Time of CSF after onset sxs (days) | 1 (0-2) n=27 | 0 (0-1) n=15 | 3 (1.5-6.5) n=5 | 1 (1-4.5) n=14 | 0 (0-2) n=13 |
| Time of CSF after onset sxs (hours) | 26.5 (11.5-56.5) n=19 | 16.3 (8.1-40.1) n=10 | 78.8 n=3 | 31.3 (15-78.4) n=7 | 24.9 (13.9-48.8) n=10 |
| CSF protein (g/L) | 0.65 (0.4-1.1) n=27 | 0.3 (0.2-0.6) n=13 | 0.8 (0.6-3.5) n=5 | 0.6 (0.5-1.3) n=13 | 0.3 (0.2-0.6) n=12 |
| CSF glucose (mmol/L) | 2.7 (2.4-3.0) n=27 | 3.05 (2.8-3.1) n=14 | 2.5 (2.1-4.2) n=5 | 3.2 (2.8-4.0) n=14 | 3.2 (3.1-3.5) n=12 |
| Temperature on arrival (°C) | 38.7 (38.2-39.1) n=27 | 38.9 (38.2-39.3) n=15 | 38.6 (37.4-39.2) n=5 | 38.7 (27.7-38.9) n=14 | 38.2 (37.5-38.9) n=13 |
| Respiratory rate (/min) | 45 (40-50) n=27 | 44 (40-58) n=15 | 42 (32-51) n=5 | 46 (40-51) n=15 | 38 (32-43) n=13 |
| Heart rate (/min) | 169 (153-188) n=27 | 170 (155-189) n=15 | 153 (134-178) n=5 | 161 (139-193) n=14 | 160 (145-173) n=13 |
| | ***% (n/N)*** | ***% (n/N)*** | ***% (n/N)*** | ***% (n/N)*** | ***% (n/N)*** |
| History of altered consciousness | 16% (4/24) | 30% (4/12) | 40% (2/5) | 7.7% (1/13) | 0% (0/13) |
| Any rash on examination | 37% (10/27) | 40% (6/15) | 40% (2/5) | 28.6% (4/14) | 16% (2/12) |
| Vomiting | 37% (10/27) | 13% (2/15) | 60% (3/5) | 57% (8/14) | 8.3% (1/12) |

Notes: *CSF WBC count was corrected for red blood cell count at a ratio of 500:1. †Obtained either on day 0 or day 1 following admission. ‡If CRP was recorded as <5, then a value of 1 was used for analysis.

Children (mostly infants) who had EV meninigits with CSF pleocytosis compared with EV meningitis without CSF pleocytosis had a higher median blood WBC count (13.0 $X10^9$/L versus 9.7 $X10^9$/L, 95% CI 0.6-7.6, p=0.02), but lower CRP (1mg/L versus 11mg/L, p=0.01). There was no significant difference comparing EV meningitis with and without pleocytosis, in age in days (42 days pleocytosis versus 34 days not pleocytosis, p=0.96), and time to LP in hours (26.5 hours pleocytosis versus 16.3 hours not pleocytosis, p=0.40). There was a significant difference in day of LP following onset of symptoms in children with EV meningitis (with or without CSF pleocytosis), compared with CSF-EV- but other site EV+ aseptic meningitis (group 3a) (median 1 day versus 3 days, p=0.049).

**7.4. Discussion**

In aseptic meningitis, no cause is identified from routine laboratory tests performed at hospital sites for many children, although enteroviruses cause the majority of cases with a defined aetiology (*chapter 5, table 5.5.*). EV infections may also cause many cases of aseptic meningitis when CSF EV-PCR has not been performed. It may be reasonable to consider that EV could also be the cause in children who are CSF EV-PCR- but have EV isolated from a non-CSF site, as has also been suggested by other small studies.[20,257,311,421] If time to making a diagnosis of probable or definite EV meningitis could be reduced, this may allow earlier cessation of IV antibiotics and earlier hospital discharge.[22,185,279,317,320,321] Notably, the majority of children included in these present data were infants, predominantly young infants aged <3months, consistent with participants with EV or aseptic meningitis in the overall UK-ChiMES study analysis (*chapter 5, table 5.5.*).

Children included in this study were admitted to hospital between March 2015 and May 2016. In the UK-ChiMES study, recruitment of children with EV meningitis peaked in the summer months during these years (*Chapter 5, figure 5.3*). In this subset of participants from the UK-ChiMES study who had EV-PCR performed of non-CSF samples by EV R-gene® kit, all children with EV meningitis (with and without CSF pleocytosis) and an available stool sample for analysis were stool EV-PCR+, and the positive predictive value of a positive stool sample for CSF-EV+ was 87%. The stool EV-PCR+ samples were obtained up to several days following reported onset of symptoms, with some positive samples collected more than a week following onset of symptoms, which could be expected because EVs may continue to be shed in stool for weeks after infection.[26] These findings suggest that stool EV-PCR is a sensitive test for diagnosing EV infection in children, and that if CSF pleocytosis is also present it could be reasonable to consider probable EV meningitis the diagnosis.

Few children with EV meningitis (with or without pleocytosis) were EV-PCR+ from an upper respiratory sample. Overall, 25% (3/12) of children with EV meningitis had a positive throat swab and 66% (2/3) had a positive NPA sample. No children with aseptic meningitis of unknown cause, and only one child who did not have meningitis had an EV-PCR+ upper respiratory sample. These data suggest that obtaining upper respiratory EV-PCR may be a less sensitive test for EV meningitis compared with stool EV-PCR, although sample size was limited.

Although the majority of the serum EV-PCR results were invalid, the serum EV-PCR+ results obtained were from samples that were collected on day 1 or 2 following onset of

symptoms, which would be consistent with viraemic stage of EV infection when virus may cross the blood brain barrier by haematogenous spread.[51,55]

These data are comparable with other small studies which also showed that stool EV-PCR was more sensitive than upper respiratory or blood PCR in EV meningitis.[311,421] A study including 76 children with EV meningitis, reported sensitivities of 98% for stool, 68% for nasopharyngeal swab and 76% for blood in EV meningitis.[311] Other studies suggested similar low sensitivities for throat swab EV-PCR samples in young infants with EV infection (41%, 13/32),[427] and adults and children with suspected EV neurological infection (26%, 16/61).[428] A further study reported a higher sensitivity for stool EV-PCR (96%, 24/25) than for CSF EV-PCR (76%, 26/34) in 34 adults with probable (defined by virus culture or antibody detection in serum, stool or throat swab, n=15) or definite (defined by virus culture or antibody detection in CSF, n=19) EV meningitis, and suggested that a presumptive diagnoses of EV meningitis could be made if stool EV-PCR+.[421] The small study in adults also reported fewer CSF EV-PCR+ results after two days following onset of symptoms, and consistent with these present data they reported no serum EV-PCR+ results and that stool samples continued to be positive more than two days following onset of symptoms.[421]

Of children with aseptic meningitis who were CSF EV- or who did not have CSF EV-PCR performed, 35% (6/17) were stool EV-PCR+ by EV R-gene® kit, but none were serum or throat swab EV-PCR+. Two of these children did not have CSF EV-PCR performed at hospital site, and the remaining four were CSF EV- but had no other possible cause defined from hospital site investigations. One further child with CSF EV- aseptic

meningitis was stool and throat swab EV-PCR+ at hospital site laboratory. Although these data are limited by sample size, the infants and children who were CSF EV-PCR- and stool EV-PCR+ presented with generally similar clinical features to children who were CSF EV+ including vomiting, rash, fever and altered consciousness.

There was a trend, which was statistically significant (p=0.049) for later lumbar puncture following onset of symptoms for children with aseptic meningitis who were CSF EV- but stool EV+ (group 3a), compared with children who were CSF EV+ (groups 1 and 2). There may be a lower yield for obtaining CSF EV-PCR+ results in EV meningitis if the lumbar puncture is delayed, consistent with findings reported by a small study in adults.[421]

Of children with aseptic meningitis who were CSF-EV- and had no EV+ sample obtained from a non-CSF site (group 3b), there was a trend to higher median CRP compared with any other groups, and 53% of children with CSF EV- aseptic meningitis overall (groups 3a and 3b) had a LP day three or later following onset of symptoms or later (*figure 7.4.*). In the UK-ChiMES study overall (*chapter 5, section 5.3.5.)*, median hospital LOS for aseptic meningitis of unknown cause was longer than EV or parechoviral meningitis. These findings suggest that some of these children may have either represented partially treated bacterial meningitis or been treated for possible bacterial meningitis as no causative organism was identified.

Children, most of whom were young infants, with confirmed EV meningitis with CSF pleocytosis had a higher median peripheral WBC count compared with EV meningitis without pleocytosis ($13X10^9$/L versus $9.7X10^9$/L, p=0.02) but lower CRP (1mg/L versus

11mg/L, p=0.01). There was a non-statistically significant trend to earlier lumbar puncture and younger age for participants without CSF pleocytosis. Analysis of the full UK-ChiMES dataset also indicated that children with EV meningitis with CSF pleocytosis compared to without CSF pleocytosis had a higher median peripheral WBC count ($12X10^9$/L versus $8X10^9$, p=<0.001) but lower CRP (1mg/L versus 14 mg/L, p=<0.001) (*chapter 6, section 6.4.*). In the full UK-ChiMES dataset, the finding of younger age for children without pleocytosis was statistically significant (44 days with CSF pleocytosis, versus 31 days without CSF pleocytosis, p=0.008), however although there was also a trend to earlier LP in children without CSF pleocytosis, this was not significant (*chapter 6, section 6.4.*). Children with CSF pleocytosis had a slightly longer LOS compared to without pleocytosis (4.2 days versus 3.4 days, p=0.001) in the full dataset (*chapter 6, section 6.4.*).

These findings are consistent with other small studies, which have demonstrated that the absence of pleocytosis in EV meningitis is more common in young infants aged <1-3 months, although definitions for CSF pleocytosis vary between studies.[172,276-280] One small study reported absence of pleocytosis in 56% of neonates aged <1 month compared with 25-35% of children aged 1 month to 19 years.[279] A small study from New Zealand reported that 37% of infants ≤2 months compared with 13% of children aged 2 months to 18 years had no pleocytosis.[276] A larger study from the USA which included 257 samples from children ≤18 years reported that pleocytosis was absent in EV meningitis in 30% of infants ≤2 months, compared with only 2% aged >2 months to ≤18 years.[277] A retrospective study from South Korea of 390 children and infants with EV meningitis demonstrated a decreasing proportion of absence of pleocytosis with age from 77% of infants aged 0-27 days, to 6% of children aged 5-18 years.[280] Another retrospective study

found that pleocytosis was absent in 41% of infants aged <29 days, and 10% at 57-90 days.[278] A retrospective study from Canada reported absence of pleocytosis in 32% (10/31) of infants aged <30 days, 21% (4/19) aged 30-60 days and 4.4% aged ≥61 days to ≤18 years (8/182).[172] It is possible that CSF pleocytosis in EV CNS infections is less common in younger infants because of immaturity of the immune system;[278,280] and although not a significant finding, children earlier in an illness may not yet have developed an inflammatory response to EV infection, which would be supported by the lower peripheral WBC in children without CSF pleocytosis, although median blood CRP was higher.

Although a recent small study[73] suggested that CSF EV-PCR+ without CSF pleocytosis was not indicative of central nervous system (CNS) disease unless specific neurological signs or abnormal neuroimaging were present, other studies either define this as meningitis or suggest it is consistent with enteroviral infection of the CNS.[20,278,280] *Harvala et al.* suggested that if the viral load is higher in serum than in CSF, then the CSF-PCR+ result was due to 'leaking' of RNA into the CSF compartment.[73] In the UK-CHiMES study, there was no significant difference in history of seizures or altered consciousness, or a bulging fontanelle on examination, in children with EV meningitis with or without CSF pleocytosis (*chapter 6, section 6.4*), and signs of meningeal irritation were infrequently reported. Regardless of whether the illness is defined as meningitis or not, these children have EV infection.

Consistent with findings from *chapter 5, section 5.3.*, almost all children with aseptic meningitis (including EV meningitis) received IV antibiotics (95%, 58/61), and 32% (19/60)

received IV or IM antibiotics prior to LP. Pre-treatment with antibiotics would particularly impact diagnostic accuracy for children in group 3, although no bacterial PCRs were positive in this group. The majority of participants were young infants presenting with fever, and therefore management with IV antibiotics would have been reasonable to treat the possibility bacterial meningitis or infection until culture and PCR results were available.

This study had several limitations. The major limitation of this study was small sample size, which affected whether further statistical analyses could be performed. Only available samples were tested for each participant, therefore every child did not have all samples tested. It is feasible that if all non-CSF samples were available for all children, other EV-PCR+ results may have been obtained, which may have affected results. Children who were pre-treated with IV or IM antibiotics may have represented pre-treated bacterial meningitis. There were two inconsistencies with EV R-gene® kit results compared with hospital laboratory EV-PCT results, although the samples tested were collected separately.

Many serum sample results tested by EV R-gene® kit in these experiments were invalid, likely caused by inhibitors to PCR present in the sample or reaction, because following dilution (1:10 and 1:20) late amplification of the internal control occurred for many samples. Mechanisms by which inhibition of PCR occurs include degradation of RNA or DNA, and inhibition of polymerase or reverse transcriptase enzymes.[429] Inhibitors that can be present in clinical specimens include haemoglobin, heparin, IgG, lactoferrin and myoglobin.[429] Stool samples may also contain PCR inhibitors[429] although this was not a

concern in these experiments. Examples of PCR inhibition introduced during a PCR reaction include the presence of nucleases which degrade RNA or DNA or by PCR components adsorbing to reaction tubes.[429] In these experiments, a nucleic acid extraction process was included, and invalid samples were diluted which however also resulted in both dilution of inhibitors and decreased PCR sensitivity.[429]

Summary

In childhood enteroviral meningitis, all stool samples obtained were EV-PCR positive and EV was isolated from stool samples many days following onset of symptoms. Although EV is not always isolated from CSF, the finding of an EV-PCR positive sample from another site could support the diagnosis of probable EV meningitis, and influence clinical decision making. Children with CSF EV infection who presented with CSF pleocytosis had higher blood WBC, but lower CRP, and were slightly older than those without CSF pleocytosis.

# 8. Chapter 8: Health related quality of life and short-term outcomes following childhood viral and bacterial meningitis

## 8.1. Introduction

Most childhood meningitis nowadays is aseptic or viral *(chapter 5)*. Findings from limited studies reporting outcomes following viral meningitis vary, with some studies reporting that although acute neurological complications occur including seizures, raised intracranial pressure and abnormal mental state, there are no permanent neurological sequelae.[74,262,264,331,332] In contrast, other studies document ongoing neuropsychological, motor, developmental and cognitive sequelae, particularly if viral meningitis occurred as a young infant.[333-336] Outcomes following bacterial meningitis are well-described and include deafness, epilepsy, motor impairment and other neuropsychological and cognitive sequelae.[118,324-326327,328]

The World Health Organisation defines quality of life (QOL) as 'an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns. It is a broad ranging concept affected in a complex way by the person's physical health, psychological state, personal beliefs, social relationships and their relationship to salient features of their environment.'[430] Health-related quality of life (HR-QOL)could be defined as 'how health affects QOL', although definitions vary.[431] An alternative definition proposed by *Calman et al*[432], and discussed by *Carr et al,*[433] is that health related QOL is 'the gap between our expectations of health and our experience of it'.[432,433] *Carr et al* suggested that

perception of health varies between individuals and within an individual over time.[433] There is probably variation in what is measured by health-related QOL questionnaires.[431]

The Infant-Toddler Quality of Life Questionnaire (ITQOL) was designed to assess health-related QOL for children aged 2 months to <5 years.[345,434,435] Both 47-item and 97-item versions are available, which measure the same concepts. The 97-item ITQOL was previously validated in 717 Dutch children, and results compared between a random sample of the general population and children with respiratory disease.[345] The study reported that the ITQOL was a feasible tool with good parent response rates, and results discriminated well between children with and without chronic medical conditions and with high or low medical consumption, although there were some inconsistencies with test-retest reliability and a ceiling effect was reported for some items.[345] The ITQOL results obtained were generally comparable with another preschool QOL questionnaire assessed.[345] The short form version (47 item) was developed following the Dutch validation study.[345] The 47- item ITQOL (ITQOL-SF47) includes 8 multi-item and 3 single-item scales including overall health, physical abilities, growth and development, bodily discomfort, temperament and moods, overall behaviour, general health perceptions, change in behaviour, parent emotional and time impact, and family cohesion. Items are scored by parents on 4-5 point Likert-type scales, and then transformed to a scale from 0 (worst health) to 100 (best health).[338,436] Some behaviour and change in health items are not relevant for infants.[338]

The aims of this study were to assess outcomes following bacterial and aseptic meningitis, or a non-meningitis illness for children aged <16 years recruited to the UK-ChiMES study including:

1. Neurological sequelae and other complications at discharge and 3 months following discharge from hospital
2. Health-related quality of life assessed by ITQOL questionnaire at hospital discharge, 6, 12 and 18 months following discharge for children aged <2 years

## 8.2. Methods

Outcomes were analysed for children aged <16 years with confirmed meningitis and a non-meningitis control group, recruited to the UK-ChiMES study from December 2012-June 2016 across 31 UK hospitals (see *general methods chapter 2* and *definitions chapter 5, section 5.2.2*). Outcomes were assessed at hospital discharge and 3 months post-discharge. For children aged <2 years, parents completed the 47-item Infant-Toddler Quality of Life (ITQOL) questionnaire at discharge, 6, 12 and 18 months following discharge (*see chapter 2, table 2.4*).[345] Demographic data for included children were analysed.

### *8.2.1. Methods for analysis of outcomes at discharge and at 3 months following discharge*

Short-term outcome data were collected at discharge, including neurological sequelae. Medium term outcomes were assessed at 3 months post-discharge by a brief parent interview by phone call, or during a hospital clinical appointment. At 3 months post-discharge, the parent interview included questions about whether the child was back to

normal, concerns about sleep, behaviour, attention, school function, daily activities, and concerns about ongoing symptoms, neurological, visual and hearing sequelae. The proportion of children who received a hearing test following discharge and results when available were also reported, which is the standard of care for bacterial meningitis in the UK,[106] but there are varying practises and no clear guidelines for viral meningitis. A subgroup analyses of general and neurological outcomes was also performed for children with meningitis caused by the following specific bacterial or viral aetiologies.

*8.2.2. Methods for analysis of ITQOL questionnaires*

ITQOL-SF47 questionnaires were completed by families for children aged <2 years prior to discharge from hospital (baseline), and posted to participating families for children who were aged <2 years at 6, 12 and 18 months following discharge. Paper questionnaires were returned by post and entered on the Openclinica database by coordinating centres.

The ITQOL-SF47 questionnaire was analysed according to methods described by the publisher.[436] Concepts were scored as missing if more than half of the items for that concept were missing.[436] Multi-item scales analysed were physical abilities, overall growth and development, bodily discomfort or pain, temperament and moods, overall behaviour, general health perceptions, parent emotional impact and parent time impact. Single-item scales analysed were overall health, change in health and family cohesion. Analysis included all available questionnaires at each timepoint regardless of whether a questionnaire was completed at discharge or not.

ITQOL results at discharge, 6, 12 and 18 months following discharge were compared to a non-meningitis control group, in children with bacterial meningitis, probable bacterial (*E. coli*) meningitis, enteroviral meningitis, parechoviral meningitis, probable enteroviral meningitis, other viral meningitis, aseptic meningitis of unknown cause, and possible meningitis (for definitions see c*hapter 5, section 5.2.2*).

### *8.2.3. Statistical methods*

Statistical analyses were performed with SPSS software. Mean transformed ITQOL scores for each scale out of 100, standard deviations, and 95% confidence intervals were calculated. Two sided independent-samples t-tests were calculated to compare mean scores for each ITQOL scale to the control group at each timepoint. Chi-squared tests, or Fisher's exact tests if any expected numbers were <5, were used to compare general and neurological outcomes for children with meningitis of different aetiologies to the control group of children. P-values were calculated with a cut-off for significance of <0.05.

## 8.3. Results

### *8.3.1. Demographics of participants included in outcome analysis*

Outcomes were analysed for 2119 out of 2754 children aged <16 years with suspected or confirmed meningitis described in *chapter 6, section 6.3.1* (*table 8.2*). Demographic data are shown in *table 8.1*.

| **Table 8.1 Demographic features of 2119 children included in analysis of major sequelae** | |
|---|---|
| Age at admission median (IQR, range) | 1 month (IQR 1-6, range 0-190 months) |
| Sex | |
| *male* | 1227 (57.9%) |
| Ethnicity | |
| *white* | *1635* |
| *mixed* | *131* |
| *Asian or Asian British (included Chinese)* | *187* |
| *Black* | *67* |
| *Other* | *68* |
| *Unknown* | *31* |

**Table 8.2 Number of children with outcome data at discharge or three months post discharge in children with meningitis of different aetiologies and the non-meningitis control group**

| | Discharge, %, n/N | Three months post discharge, %, n/N | Total discharge, 3 months post discharge, or both %, n/N |
|---|---|---|---|
| Meningococcal | 92%, 47/51 | 73%, 37/51 | 96%, 49/51 |
| Pneumococcal | 87%, 46/53 | 64%, 34/53 | 96%, 51/53 |
| GBS | 84%, 26/31 | 74%, 23/31 | 90%, 28/31 |
| *E. coli* | 86%, 19/22 | 55%, 12/22 | 95%, 21/22 |
| Other bacterial | 87%, 13/15 | 67%, 10/15 | 87%, 13/15 |
| probable *E. coli* | 86%, 18/21 | 67%, 14/21 | 95%, 20/21 |
| Enterovirus | 92%, 280/305 | 74%, 226/305 | 98%, 299/305 |
| Parechovirus | 96%, 47/49 | 76%, 37/49 | 100%, 49/49 |
| probable EV | 85%, 11/13 | 92%, 12/13 | 100%, 13/13 |
| other viral | 79%, 19/24 | 50%, 12/24 | 88%, 21/24 |
| unknown aseptic | 94%, 262/280 | 74%, 206/280 | 98%, 273/280 |
| possible meningitis | 89%, 98/110 | 65%, 72/110 | 96%, 106/110 |
| not meningitis (control) | 93%, 1115/1199 | 72%, 867/1199 | 98%, 1176/1199 |
| TOTAL | 92%, 2001/2173 | 71%, 1562/2173 | 98%, 2119/2173 |

*8.3.2. Outcomes reported at discharge and 3 months post-discharge*

At discharge, all outcomes analysed were reported more frequently ($p<0.05$) following bacterial meningitis compared with the non-meningitis control group (*table 8.3.*). For all bacterial meningitis, reduced mobility was the most frequently reported outcome (15%),

and was reported by 34% following pneumococcal meningitis. Parent report of any new or worse hearing impairment since illness was reported most often following pneumococcal (21%) and meningococcal (19%) meningitis. There were no statistically significant differences between outcomes reported at discharge following viral meningitis caused by EV or HPeV (all reported 0-1%) compared with the non-meningitis control group (*table 8.3*).

Sequelae at 3 months post-discharge were more common following bacterial than viral meningitis (*table 8.4*). Although there was a statistically significant difference in seizures between children with bacterial meningitis and the control group at discharge (9% vs 1%, p=<0.001), at 3 months post-discharge there was no difference in reported seizures (bacterial 4% versus control 5%, p=1.000). The difference was due to resolution of seizures in three children with pneumococcal meningitis, but outcomes were not reported at 3 months for eight children with bacterial meningitis who had seizures at discharge (four with GBS and four with pneumococcal meningitis). There were 25% of children who had bacterial meningitis reported as 'not back to normal' compared with 9% in the control group (p<0.001), and 10% with EV or parechoviral meningitis (p=0.751 compared with control group). For bacterial meningitis, sleep (21% vs 7%, p=<0.001), behaviour (17% vs 3%, p=<0.001), attention (9% vs 2%, p=0.002), and daily activities (7% vs 2 %, p=0.020) were all reported more frequently as not having returned to normal compared with the control group (*table 8.4.*).

At 3 months post-discharge, reduced mobility was reported by 26% of children who had bacterial meningitis, including 43% of both pneumococcal (6/14) and GBS (3/7)

meningitis. Further details available for children with pneumococcal meningitis and reduced mobility included a 9-month old child (age at admission) with reported concerns about left sided weakness and temporarily not crawling after illness, and a 41-month old child with new delayed motor milestones. For the child with meningococcal meningitis and reduced mobility, there was concern about a right hemiplegia. The three participants with GBS meningitis and reduced mobility were all infants <3 months at admission, one of whom was reported as having slight weakness right arm. Four children with possible meningitis who were reported as having reduced mobility all had meningococcal bacteraemia, and one had pneumococcal bacteraemia, including a one-year old with meningococcal bacteraemia who had reduced left hand movement, and a 14-month old with meningococcal bacteraemia who had joint pain and movement restriction.

For viral meningitis, the only domains that were significantly different to the control group at 3 months post-discharge were reduced mobility (8% vs 2%, p=0.033), and hearing impairment (7% vs 1%, p=0.025) (*table 8.4.*). The four children with EV meningitis and impaired mobility included an 8-month old infant who had previously crawled and had to re-learn to crawl after the illness, and two young infants aged <1 month and one child aged 15 months at admission with further details not specified. A one-month old infant with parechoviral meningitis was reported as unable to use their left leg properly at 3 months following admission.

Reduced mobility was reported for one child (aged 12 years) with HSV meningoencephalitis, and was not otherwise specified. The children with unknown aseptic meningitis and reduced mobility included a one-month old infant with reported

right arm weakness, a 7-year old with mild left sided weakness, and an 11-month old also receiving chemotherapy with reported muscle weakness.

Of children who had hearing test results recorded, abnormal results were most frequently reported following pneumococcal meningitis (35%), and meningococcal meningitis (15%) (*table 8.5*). Overall, there was parent report of concern about abnormal hearing in 40% of bacterial meningitis, including 27% (3/11) of GBS (although all recorded hearing test results for infants who had GBS meningitis were normal) and one infant with *E. coli* meningitis, who had profound right sided sensorineural hearing loss at a hearing test.

There was parent report of concern about hearing impairment at 3 months post-discharge for four infants who had EV meningitis, three of whom had hearing test results recorded. Hearing test results for two infants indicated mild hearing loss, and results for the third infant indicated a right sided hearing deficit requiring further follow-up. The two children with possible meningitis and abnormal hearing tests both had meningococcal bacteraemia.

The three children with pneumococcal meningitis and parent report of visual impairment (not otherwise specified) were aged 7, 13 and 27 months.

**Table 8.3 Outcomes reported at hospital discharge in children with meningitis of different aetiologies compared with the non-meningitis control group (new or worse since illness)**

| | | Headache | Seizures | Speech[+] | Anxiety | Reduced mobility | Memory[+] | Visual[+] | Hearing[+] |
|---|---|---|---|---|---|---|---|---|---|
| **bacterial** | % | **4%** | **9%** | **5%** | **4%** | **15%** | **3%** | **6%** | **13%** |
| | *p** | *0.017* | *<0.001* | *0.006* | *0.008* | *<0.001* | *0.044* | *<0.001* | *<0.001* |
| meningococcal | % | 6% | 2% | 0% | 8% | 5% | 4% | 3% | 19% |
| N=47 | n/N | 2/36 | 1/46 | 0/28 | 2/24 | 2/40 | 1/26 | 1/33 | 5/27 |
| pneumococcal | % | 3% | 18% | 14% | 4% | 34% | 4% | 16% | 21% |
| N=46 | n/N | 1/31 | 8/44 | 4/29 | 1/24 | 14/41 | 1/23 | 5/32 | 6/28 |
| GBS | % | 6% | 15% | 0% | 0% | 0% | 0% | 0% | 6% |
| N=31 | n/N | 1/16 | 4/26 | 0/11 | 0/13 | 0/14 | 0/12 | 0/17 | 1/16 |
| *E. coli* | % | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| N=19 | n/N | 0/11 | 0/19 | 0/8 | 0/8 | 0/11 | 0/7 | 0/9 | 0/11 |
| other | % | 0% | 0% | 0% | 0% | 17% | 0% | 0% | 0% |
| N=13 | n/N | 0/9 | 0/13 | 0/8 | 0/5 | 2/12 | 0/7 | 0/12 | 0/9 |
| **EV or HPeV** | **%** | **1%** | **1%** | **0%** | **0%** | **0%** | **0%** | **0%** | **1%** |
| | *p** | *0.646* | *0.542* | *1.000* | *1.000* | *0.692* | *1.000* | *0.209* | *0.198* |
| enterovirus | % | 1% | 0% | 0% | 0% | 1% | 0% | 1% | 1% |
| N=280 | n/N | 2/165 | 1/274 | 0/120 | 0/101 | 1/195 | 0/107 | 1/179 | 1/160 |
| parechovirus | % | 0% | 2% | 0% | 0% | 0% | 0% | 0% | 0% |
| N=47 | n/N | 0/33 | 1/47 | 0/17 | 0/15 | 0/33 | 0/17 | 0/34 | 0/30 |
| **not meningitis** | **%** | **1%** | **1%** | **0%** | **0%** | **1%** | **0%** | **0%** | **0%** |
| N=1115 | n/N | 5/726 | 13/1085 | 3/601 | 1/496 | 7/792 | 1/514 | 0/804 | 0/770 |
| probable bacterial | % | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| N=18 | n/N | 0/11 | 0/18 | 0/13 | 0/10 | 0/16 | 0/11 | 0/14 | 0/12 |
| probable EV | % | 0% | 0% | 33% | 14% | 38% | 17% | 0% | 0% |
| N=11 | n/N | 0/8 | 0/11 | 3/9 | 1/7 | 3/8 | 1/6 | 0/8 | 0/7 |
| other viral | % | 19% | 0% | 8% | 8% | 25% | 8% | 7% | 0% |
| N=19 | n/N | 3/16 | 0/18 | 1/13 | 1/12 | 4/16 | 1/13 | 1/14 | 0/13 |
| unknown aseptic | % | 9% | 3% | 3% | 2% | 11% | 4% | 3% | 2% |
| N=262 | n/N | 17/192 | 7/255 | 6/177 | 3/151 | 23/205 | 7/161 | 6/194 | 4/173 |
| possible meningitis | % | 4% | 7% | 2% | 2% | 14% | 2% | 0% | 3% |
| N=98 | n/N | 3/72 | 7/94 | 1/62 | 1/52 | 11/79 | 1/55 | 0/73 | 2/61 |

Note *p-value is comparison to non-meningitis control group, [+]reported impairment, EV=enterovirus, HPeV=parechovirus

| Table 8.4 Outcomes three months post-discharge in children with meningitis of different aetiologies compared with the non-meningitis control group | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Not back to normal | Sleep[#] | Behaviour[#] | Attention[#] | School function[#] | Daily activities[#] | Headache | Seizures | Speech[+] | Anxiety | Reduced mobility | Memory[+] | Visual[+] | Hearing[+] |
| **bacterial** | % | **25%** | **21%** | **17%** | **9%** | **23%** | **7%** | **17%** | **4%** | **19%** | **20%** | **26%** | **0%** | **9%** | **40%** |
| | *p** | *<0.001* | *<0.001* | *<0.001* | *0.002* | *0.155* | *0.020* | *0.003* | *1.000* | *<0.001* | *0.003* | *<0.001* | *1.000* | *0.032* | *<0.001* |
| meningococcal | % | 11% | 17% | 11% | 4% | 0% | 0% | 10% | 0% | 10% | 11% | 10% | 11% | 0% | 50% |
| N=37 | n/N | 3/28 | 5/29 | 3/28 | 1/27 | 0/7 | 0/24 | 1/10 | 0/10 | 1/10 | 1/9 | 1/10 | 1/9 | 0/8 | 5/10 |
| pneumococcal | % | 38% | 27% | 26% | 13% | 30% | 12% | 20% | 7% | 46% | 40% | 43% | 10% | 21% | 53% |
| N=34 | n/N | 11/29 | 8/30 | 8/31 | 4/30 | 3/10 | 3/26 | 2/10 | 1/14 | 6/13 | 4/10 | 6/14 | 1/10 | 3/14 | 9/17 |
| GBS | % | 25% | 26% | 22% | 11% | 100% | 9% | 17% | 10% | 0% | 25% | 43% | 0% | 0% | 27% |
| N=23 | n/N | 5/20 | 5/19 | 4/18 | 2/18 | 1/1 | 1/11 | 1/6 | 1/10 | 0/6 | 1/ 4 | 3/7 | 0/3 | 0/4 | 3/11 |
| *E. coli* | % | 27% | 20% | 0% | 10% | 0% | 0% | 14% | 0% | 0% | 0% | 0% | 0% | 0% | 14% |
| N=12 | n/N | 3/11 | 2/10 | 0/10 | 1/10 | 0/3 | 0/7 | 1/7 | 0/7 | 0/6 | 0/5 | 0/6 | 0/5 | 0/5 | 1/7 |
| other | % | 25% | 0% | 13% | 0% | 100% | 20% | 33% | 0% | 0% | 0% | 0% | 0% | 0% | 50 |
| N=10 | n/N | 2/8 | 0/8 | 1/8 | 0/7 | 1/1 | 1/5 | 1/3 | 0/4 | 0/2 | 0/2 | 0/2 | 0/1 | 0/2 | 1/ 2 |
| **EV or HPeV** | **%** | **10%** | **9%** | **3%** | **1%** | **10%** | **3%** | **8%** | **3%** | **4%** | **0%** | **8%** | **7%** | **3%** | **7%** |
| | *p** | *0.751* | *0.196* | *0.899* | *0.324* | *1.000* | *0.496* | *0.099* | *0.530* | *0.307* | *0.585* | *0.033* | *0.062* | *0.328* | *0.025* |
| enterovirus | % | 11% | 11% | 3% | 1% | 12% | 2% | 9% | 3% | 4% | 0% | 8% | 0% | 2% | 6% |
| N=226 | n/N | 21/188 | 19/177 | 6/175 | 1/158 | 3/26 | 3/130 | 4/45 | 2/70 | 2/45 | 0/30 | 4/52 | 0/33 | 1/59 | 4/65 |
| parechovirus | % | 4% | 0% | 3% | 0% | 0% | 6% | 0% | 0% | 0% | 0% | 14% | 0% | 17% | 13% |
| N=37 | n/N | 1/28 | 0/30 | 1/29 | 0/22 | 0/3 | 1/17 | 0/6 | 0/8 | 0/6 | 0/5 | 1/7 | 0/5 | 1/6 | 1/8 |
| **not meningitis** | **%** | **9%** | **7%** | **3%** | **2%** | **11%** | **2%** | **3%** | **5%** | **2%** | **3%** | **2%** | **1%** | **1%** | **1%** |
| N=867 | n/N | 66/698 | 44/674 | 22/677 | 13/649 | 16/149 | 9/525 | 5/189 | 12/234 | 3/179 | 5/149 | 4/197 | 1/155 | 3/216 | 3/220 |
| probable bacterial | % | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| N=14 | n/N | 0/11 | 0/11 | 0/11 | 0/10 | 0/1 | 0/10 | 0/2 | 0/2 | 0/1 | 0/1 | 0/1 | 0/1 | 0/2 | 0/2 |
| probable EV | % | 27% | 27% | 18% | 27% | 50% | 20% | 25% | 20% | 0% | 100% | 0% | 100% | 0% | 25% |
| N=12 | n/N | 3/11 | 3/11 | 2/11 | 3/11 | 3/6 | 2/10 | 1/ 4 | 1/5 | 0/3 | 2/2 | 0/4 | 2/2 | 0/5 | 1/ 4 |
| other viral | % | 50% | 36% | 45% | 36% | 44% | 30% | 25% | 33% | 22% | 33% | 11% | 38% | 38% | 22% |
| N=12 | n/N | 6/12 | 4/11 | 5/11 | 4/11 | 4/9 | 3/10 | 2/8 | 3/9 | 2/9 | 3/9 | 1/9 | 3/8 | 3/8 | 2/9 |
| unknown aseptic | % | 20% | 10% | 12% | 8% | 15% | 5% | 18% | 7% | 5% | 7% | 14% | 7% | 11% | 6% |
| N=206 | n/N | 34/172 | 17/166 | 20/163 | 12/158 | 10/67 | 7/130 | 10/57 | 5/73 | 3/57 | 3/45 | 9/63 | 4/54 | 7/65 | 4/69 |
| possible meningitis | % | 25% | 19% | 17% | 8% | 13% | 17% | 14% | 3% | 13% | 19% | 21% | 9% | 21% | 7% |
| N=72 | n/N | 16/63 | 12/63 | 11/63 | 5/61 | 3/23 | 9/53 | 4/28 | 1/31 | 3/24 | 4/21 | 6/28 | 2/23 | 6/29 | 2/30 |

*comparison to non-meningitis group, [#]reported not back to normal, [+]reported impairment, EV=enterovirus, HPeV=parechovirus. Outcomes are new or worse since illness.

| Table 8.5 Results of hearing tests obtained by three months discharge in children with meningitis of different aetiologies and the non-meningitis control group, excluding pre-existing hearing impairment | | | | | | |
|---|---|---|---|---|---|---|
| | **Hearing assessment obtained % (n/N)** | **Number with conclusive result N** | Normal % (n) | Unilateral sensorineural loss % (n) | Bilateral sensorineural loss % (n) | Non-conductive hearing loss not otherwise specified % (n) |
| meningococcal | 70% (26/37) | **20** | 85% (17) | 5% (1) | 5% (1) | 5% (1) |
| pneumococcal | 84% (26/31) | **20** | 65% (13) | 5% (1) | 30% (6) | 0 |
| GBS | 77% (17/22) | **16** | 100% (16) | 0 | 0 | 0 |
| *E. coli* | 75% (9/12) | **8** | 87.5% (7) | 12.5% (1) | 0 | 0 |
| other bacterial | 22% (2/9) | **1** | 100% (1) | 0 | 0 | 0 |
| probable *E. coli* | 31% (4/13) | **4** | 100% (4) | 0 | 0 | 0 |
| enterovirus | 35% (74/212) | **68** | 96% (65) | 15% (1) | 0 | 29% (2) |
| parechovirus | 31% (11/35) | **9** | 89% (8) | 0 | 0 | 11% (1) |
| probable EV | 17% (2/12) | **1** | 100% (1) | 0 | 0 | 0 |
| other viral | 18% (2/11) | **2** | 100% (2) | 0 | 0 | 0 |
| unknown aseptic | 38% (72/191) | **60** | 98% (59) | 0 | 0 | 2% (1) |
| possible meningitis | 47% (31/66) | **27** | 92.6% (25) | 3.7% (1) | 3.7% (1) | 0 |
| not meningitis | 56% (44/785) | **37** | 97% (36) | 0 | 3% (1) | 0 |

*8.3.3. Demographics of children aged <2years included in quality of life analysis*

Demographic data for 863 out of 1914 children aged <2 years, with suspected or confirmed meningitis described in *chapter 6, section 6.3.1.* with at least one ITQOL questionnaire completed by parents are reported in *table 8.6*, and diagnoses for included children are reported in *table 8.7*.

| **Table 8.6 Demographics of 863 children aged <24 months with at least one ITQOL questionnaire completed** | |
|---|---|
| Age at admission median (IQR) | 1 month (0-3 months) |
| Sex | |
| *male* | 58.7% (507) |
| Ethnicity | |
| *white* | *718* |
| *mixed* | *57* |
| *Asian or Asian British (included Chinese)* | *43* |
| *Black* | *13* |
| *Other* | *20* |
| *Unknown* | *12* |

| **Table 8.7 Number of children with an ITQOL questionnaire completed at each timepoint in children with meningitis of different aetiologies and the non-meningitis control group** | | | | | |
|---|---|---|---|---|---|
| | Discharge %, n/N | 6 months %, n/N | 12 months %, n/N | 18 months %, n/N | At least one timepoint completed, n |
| bacterial | 37%, 50/136 | 28%, 36/128 | 18%, 22/119 | 7%, 6/91 | 64 |
| probable *(E.coli)* | 29%, 6/21 | 33%, 7/21 | 15%, 3/20 | 16%, 3/19 | 10 |
| enterovirus | 42%, 123/294 | 43%, 125/293 | 22%, 64/288 | 16%, 44/282 | 163 |
| parechovirus | 46%, 22/48 | 52%, 25/48 | 40%, 19/47 | 17%, 8/47 | 31 |
| probable EV | 56%, 5/9 | 75%, 6/8 | 43%, 3/7 | 0%, 0/7 | 7 |
| other viral | 50%, 6/12 | 36%, 4/11 | 30%, 3/10 | 0%, 0/8 | 6 |
| unknown aseptic | 34%, 67/195 | 27%, 52/192 | 16%, 29/184 | 16%, 26/166 | 82 |
| possible meningitis | 38%, 31/81 | 45%, 36/80 | 29%, 21/73 | 17%, 10/61 | 44 |
| not meningitis (control) | 31%, 344/1118 | 25%, 275/1087 | 14%, 142/1024 | 9%, 81/929 | 456 |
| **Total** | **34%, 654/1914** | **30%, 566/1868** | **17%, 306/1772** | **11%, 178/1610** | **863** |

*8.3.4. Results of infant-toddler quality of life questionnaires*

Results of ITQOL questionnaires by domain, in children aged <2 years with meningitis of different aetiologies compared at each timepoint with the control group are reported in *figures 8.1.-8.11*. ITQOL results by meningitis aetiology are also reported in *tables 8.8-8.16*.

In young children, quality of life scores across several domains were lower following bacterial meningitis compared with a non-meningitis illness (*table 8.9*), including significant differences at 12 months for growth and development (mean score 86 vs 92, p=0.033), discomfort or pain (56 vs 69, p=0.010), temperament and moods (71 vs 77, p=0.040) and parent emotional impact (76 vs 86, p=0.037).

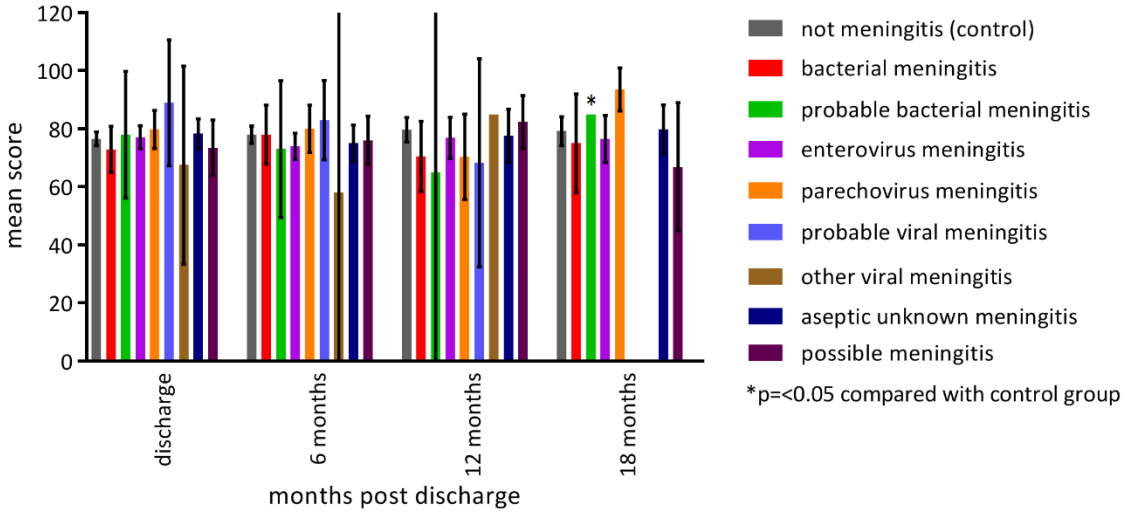
Parent emotional impact scores were lower (p<0.05) at discharge for meningitis of several aetiologies (bacterial (mean score 47), enteroviral (61), probable viral (52), other viral (16), aseptic unknown (60)) and possible meningitis (mean score 49), compared with the non-meningitis control group (mean score 67) (*figure 8.9*).

For enteroviral meningitis, parent time-impact scales at discharge (mean score 71 vs 78, p=0.021) and general health perceptions at 6 months (58 vs 63, p=0.009) were also lower than the control group. Although there were fewer low scores than for bacterial meningitis, overall behaviour scores for EV meningitis at 18 months post-discharge were lower than the control group (72 vs 77, p=0.031). For parechoviral meningitis, the only significant finding was a lower mean discomfort and pain score at discharge (42 vs 53, p=0.018) compared with the control group.

For children who had possible meningitis, which is a broad group including some children who had meningococcal bacteraemia, lower scores compared with the control group were observed for general health perceptions at discharge (mean score 55 vs 63, p=0.020) and 6 months (56 vs 63, p=0.018). However, temperament and mood scores for possible meningitis were higher at 6 months post-discharge than the control group (79 vs 72, p=0.002). There were few participants with completed questionnaires for probable bacterial, probable viral, other viral meningitis.

Across diagnoses there appeared to be a trend to increasing scores from discharge to follow-up timepoints for several domains including discomfort and pain, temperament and moods, change in health, parent time-impact and parent emotion-impact scores (figures *8.4, 8.5, 8.8-10*.).

**Figure 8.1 Overall health quality of life mean scores with 95% confidence intervals in children aged <2 years with meningitis compared with a control group**




Note: for probable bacterial meningitis at 18 months, n=3

**Figure 8.2 Physical abilities quality of life mean scores with 95% confidence intervals in children aged <2 years with meningitis compared with a control group**

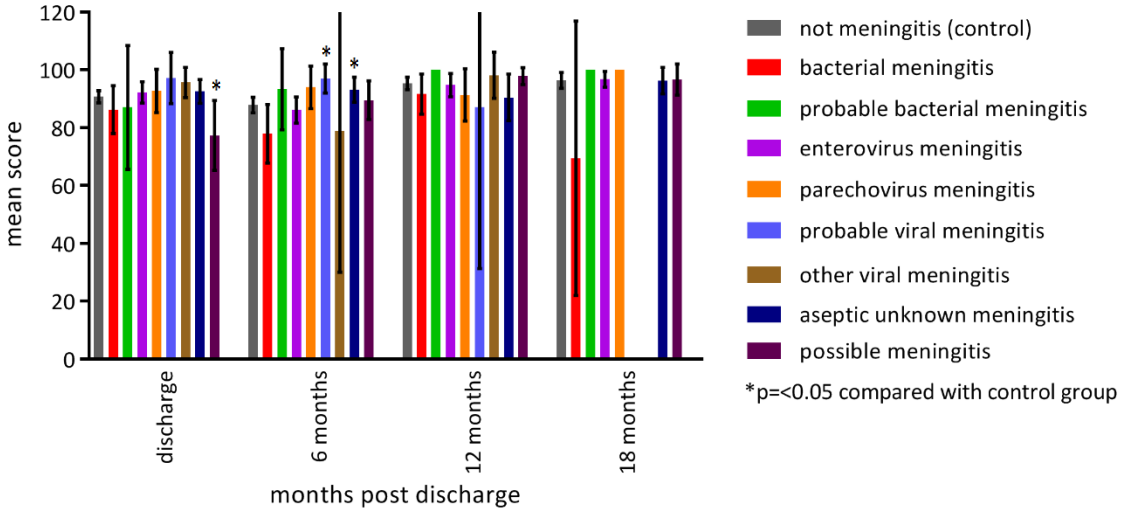


**Figure 8.3 Growth and development quality of life mean scores with 95% confidence intervals in children aged <2 years with meningitis compared with a control group**

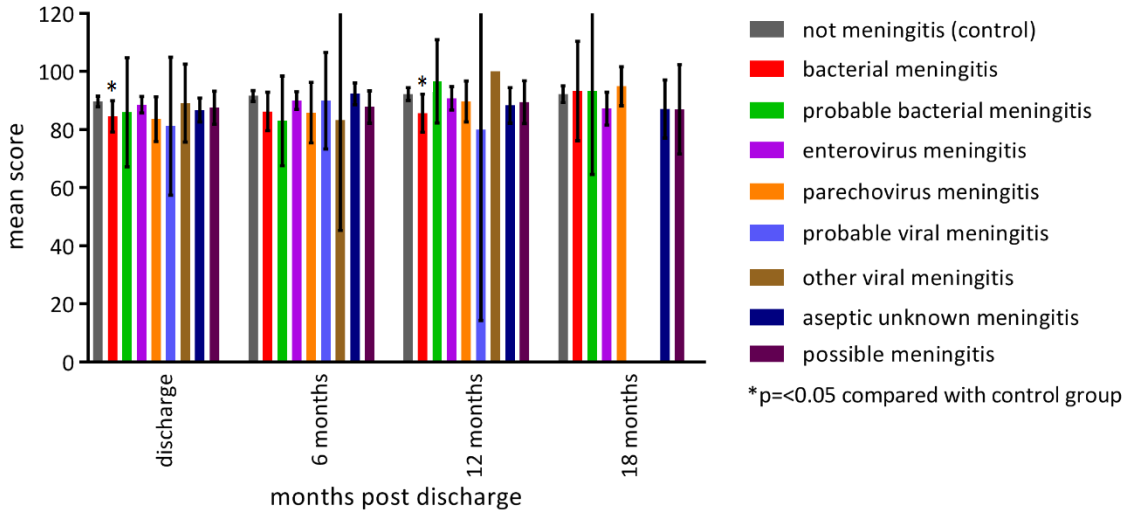
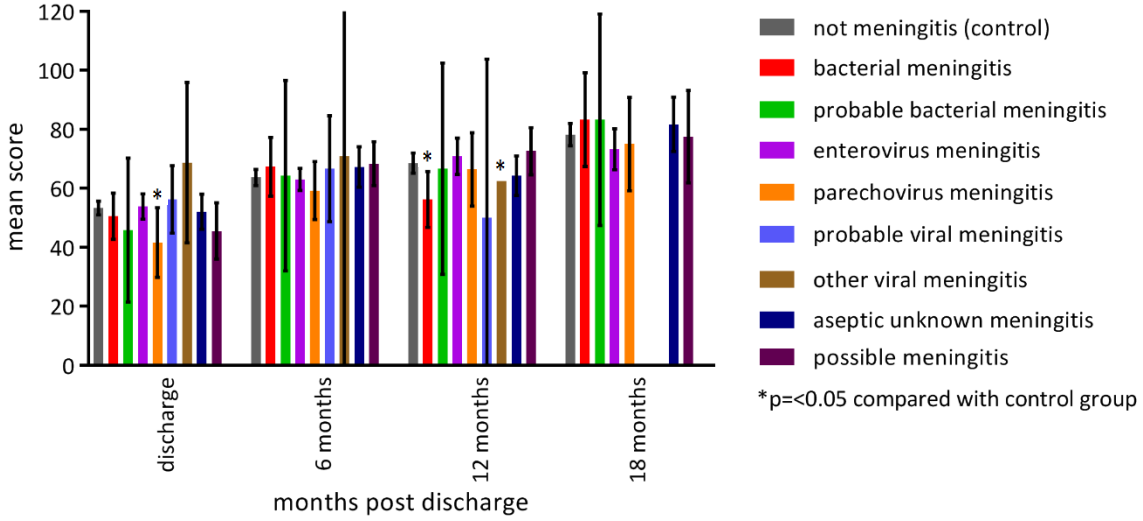



**Figure 8.4 Discomfort and pain quality of life mean scores with 95% confidence intervals in children aged <2 years with meningitis compared with a control group**




Note: for other viral meningitis at 12 months, n=3

**Figure 8.5 Temperament and moods quality of life mean scores with 95% confidence intervals in children aged <2 years with meningitis compared with a control group**

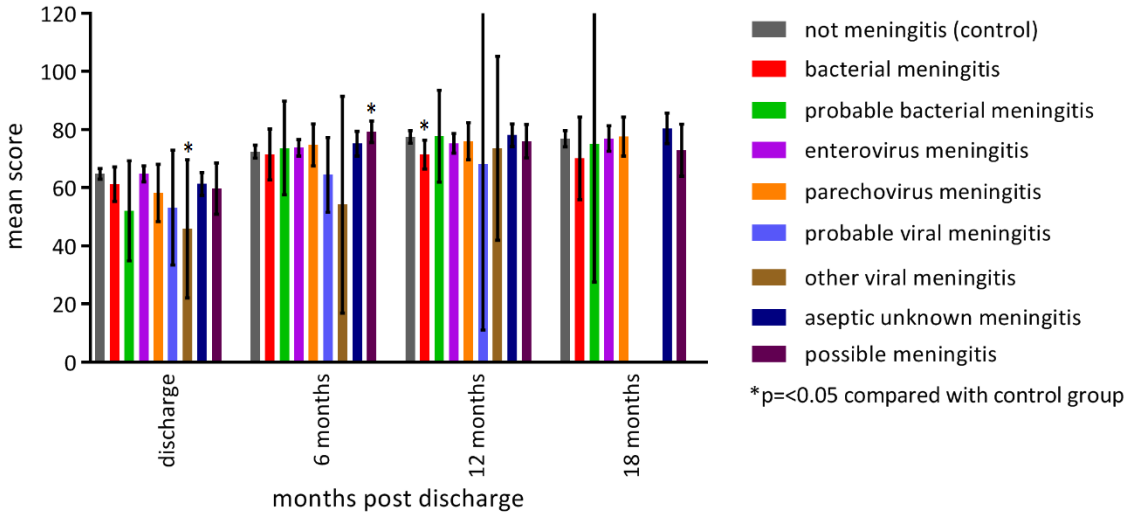



**Figure 8.6 Overall behaviour quality of life mean scores with 95% confidence intervals in children aged <2 years with meningitis compared with a control group**

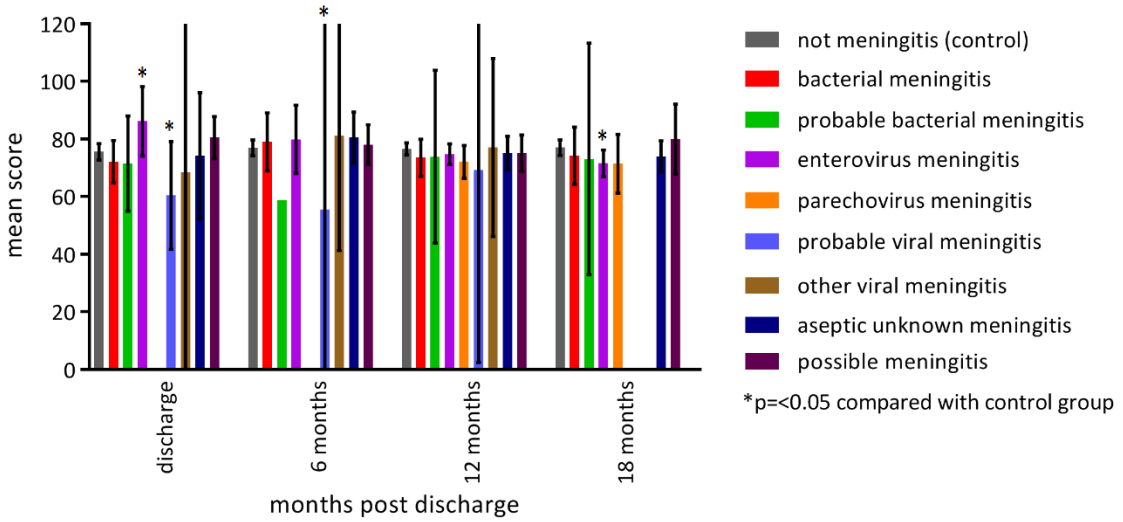


**Figure 8.7 General health perceptions quality of life mean scores with 95% confidence intervals in children aged <2 years with meningitis compared with a control group**

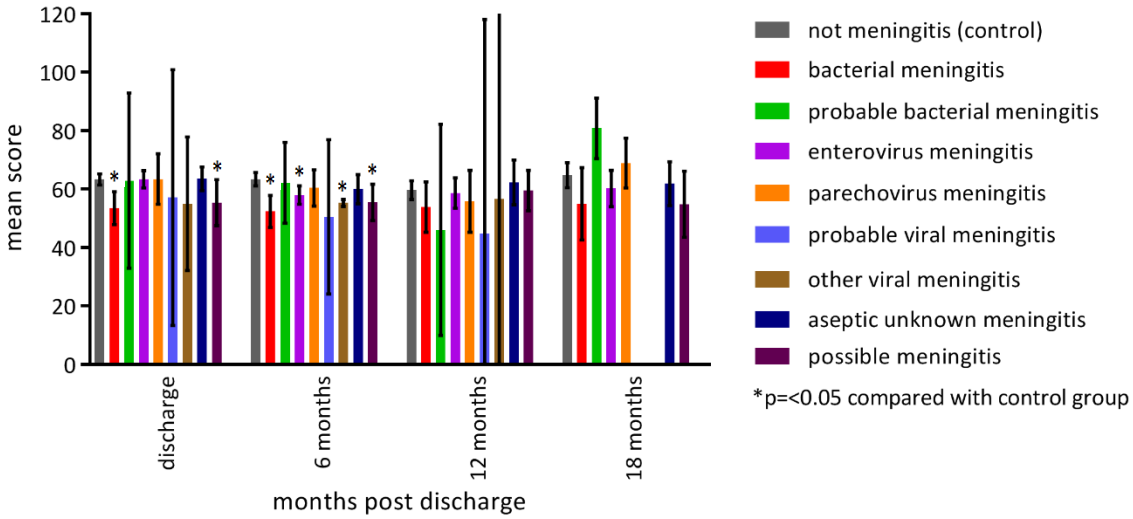
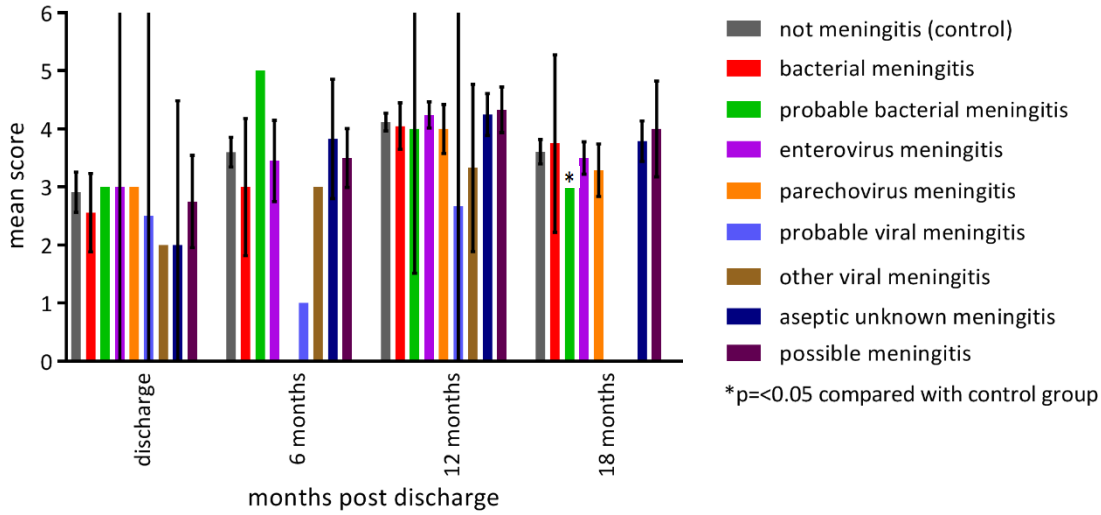



**Figure 8.8 Change in health quality of life mean scores with 95% confidence intervals in children aged <2 years with meningitis compared with a control group**




Note: for probable bacterial meningitis at 18 months, n=3

**Figure 8.9 Parent emotional impact quality of life mean scores with 95% confidence intervals in children aged <2 years with meningitis compared with a control group**

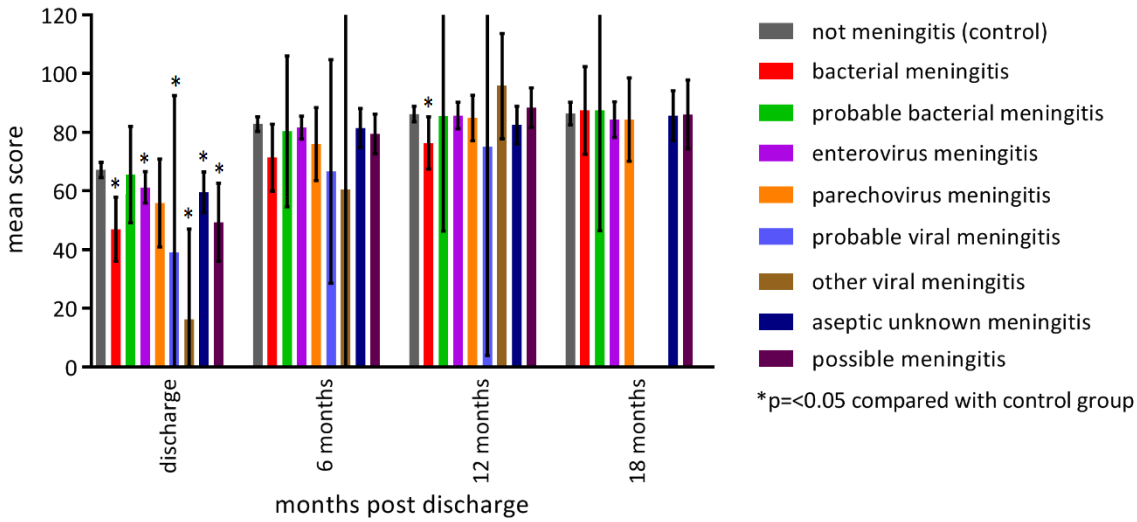



**Figure 8.10 Parent time impact quality of life mean scores with 95% confidence intervals in children aged <2 years with meningitis compared with a control group**

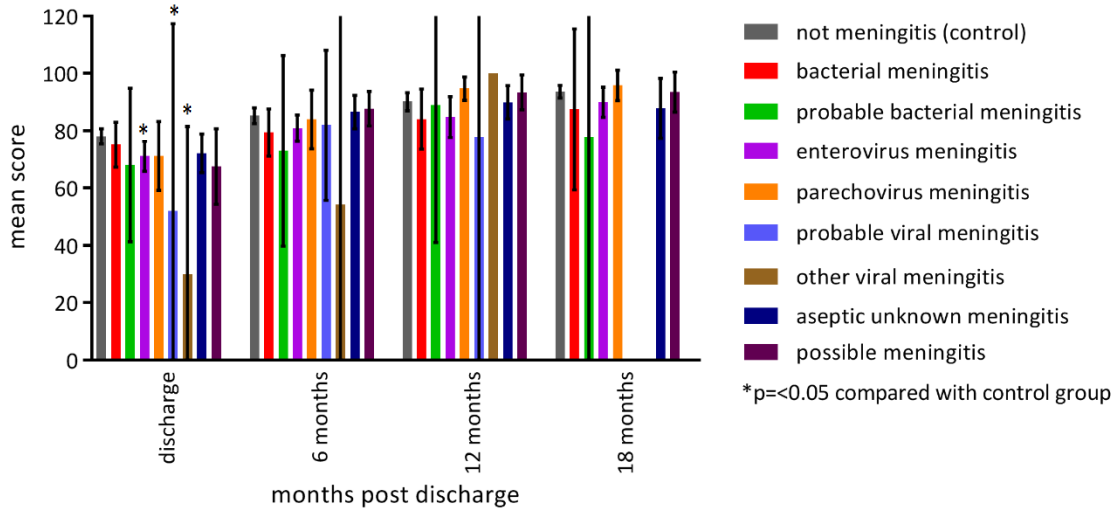
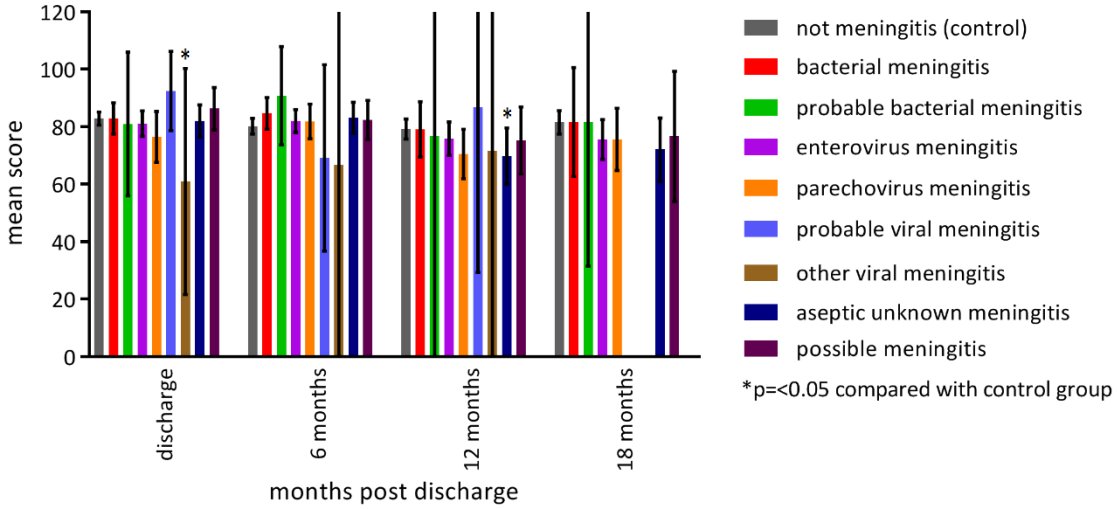


**Figure 8.11 Family cohesion quality of life mean scores with 95% confidence intervals in children aged <2 years with meningitis compared with a control group**



| Table 8.8 Infant-toddler quality or life (ITQOL) mean scores in children aged <2 years with a non-meningitis diagnosis (control group) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Discharge | | | 6 months | | | 12 months | | | 18 months | | |
| | mean | SD | n | mean | SD | n | mean | SD | n | mean | SD | n |
| overall health | 76.59 | 21.98 | 324 | 78 | 23 | 238 | 79.71 | 21.75 | 105 | 79.25 | 21.16 | 73 |
| physical abilities | 90.71 | 17.87 | 274 | 87.87 | 22.44 | 265 | 95.38 | 12.98 | 142 | 96.40 | 12.20 | 79 |
| overall growth and development | 89.73 | 16.14 | 309 | 91.60 | 14.99 | 247 | 92.31 | 13.25 | 141 | 92.22 | 12.94 | 81 |
| discomfort or pain | 53.36 | 21.61 | 335 | 63.66 | 21.85 | 248 | 68.57 | 20.67 | 142 | 78.24 | 17.20 | 81 |
| temperament and moods | 64.81 | 16.95 | 333 | 72.42 | 17.96 | 255 | 77.48 | 13.01 | 142 | 76.90 | 12.74 | 81 |
| overall behaviour | 75.55 | 10.42 | 54 | 76.94 | 11.49 | 70 | 76.59 | 12.37 | 139 | 77.03 | 12.11 | 80 |
| general health perceptions | 63.36 | 17.65 | 329 | 63.40 | 18.53 | 251 | 59.67 | 19.06 | 139 | 64.79 | 19.40 | 80 |
| change in health | 2.91 | 1.01 | 35 | 3.60 | 0.96 | 57 | 4.12 | 0.91 | 138 | 3.61 | 0.93 | 77 |
| parent emotional impact | 67.23 | 23.80 | 327 | 82.86 | 19.93 | 246 | 86.27 | 15.88 | 142 | 86.42 | 17.25 | 81 |
| parent impact - time scale | 78.08 | 23.80 | 325 | 85.25 | 22.77 | 270 | 90.14 | 18.98 | 142 | 93.66 | 9.91 | 81 |
| family cohesion | 82.89 | 21.03 | 332 | 80.22 | 21.68 | 247 | 79.22 | 20.94 | 141 | 81.56 | 18.26 | 80 |

| **Table 8.9 Infant-toddler quality or life (ITQOL) mean scores in children aged <2 years with bacterial meningitis compared with the non-meningitis control group** | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | discharge | | | | 6 months | | | | 12 months | | | | 18 months | | | |
| | mean | SD | n | p value | mean | SD | n | p value | mean | SD | n | p value | mean | SD | n | p value |
| overall health | 73.0 | 27.4 | 49 | .380 | 78 | 24 | 24 | .941 | 70.5 | 25.8 | 20 | .095 | 75.0 | 13.7 | 5 | .660 |
| physical abilities | 86.3 | 26.3 | 41 | .304 | 77.9 | 29.5 | 35 | .061 | 91.7 | 15.7 | 22 | .227 | 69.4 | 45.2 | 6 | .205 |
| overall growth and development | **84.6** | **18.3** | **47** | **.049** | 86.3 | 17.7 | 30 | .074 | **85.7** | **14.8** | **22** | **.033** | 93.3 | 16.3 | 6 | .842 |
| discomfort or pain | 50.5 | 27.4 | 49 | .407 | 67.3 | 27.1 | 31 | .391 | **56.3** | **21.4** | **22** | **.010** | 83.3 | 15.1 | 6 | .483 |
| temperament and moods | 61.3 | 20.5 | 48 | .255 | 71.5 | 24.3 | 32 | .790 | **71.4** | **11.2** | **22** | **.040** | 70.1 | 13.5 | 6 | .215 |
| overall behaviour | 72.1 | 12.2 | 13 | .305 | 79.1 | 12.0 | 8 | .623 | 73.5 | 14.6 | 22 | .294 | 74.2 | 8.0 | 5 | .604 |
| general health perceptions | **53.5** | **19.3** | **48** | **<.001** | **52.4** | **15.0** | **31** | **.002** | 53.9 | 19.5 | 22 | .190 | 55.0 | 9.9 | 5 | .268 |
| change in health | 2.6 | 0.9 | 9 | .337 | 3.0 | 1.4 | 8 | .127 | 4.0 | 0.9 | 22 | .737 | 3.8 | 1.0 | 4 | .772 |
| parent emotional impact | **47.0** | **36.9** | **46** | **.001** | 71.4 | 31.1 | 31 | .053 | **76.4** | **20.0** | **22** | **.037** | 87.5 | 14.3 | 6 | .882 |
| parent impact - time scale | 75.2 | 26.6 | 47 | .442 | 79.4 | 24.2 | 36 | .152 | 84.1 | 23.6 | 22 | .180 | 87.5 | 26.7 | 6 | .599 |
| family cohesion | 82.9 | 18.3 | 46 | .989 | 84.7 | 15.1 | 31 | .150 | 79.1 | 21.8 | 22 | .979 | 81.7 | 18.1 | 6 | .989 |

Note: P-value compared with non-meningitis control group

| **Table 8.10 Infant-toddler quality or life (ITQOL) mean scores in children aged <2 years with probable bacterial (E. coli) meningitis compared with the non-meningitis control group** | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | discharge | | | | 6 months | | | | 12 months | | | | 18 months | | | |
| | mean | SD | n | p value | mean | SD | n | p value | mean | SD | n | p value | mean | SD | n | p value |
| overall health | 78.00 | 17.54 | 5 | .887 | 73 | 19 | 5 | .591 | 65.00 | 49.50 | 2 | .355 | **85.00** | **0.00** | **3** | **.023** |
| physical abilities | 87.04 | 20.39 | 6 | .620 | 93.33 | 13.33 | 6 | .554 | 100.00 | 0.00 | 3 | .540 | 100.00 | 0.00 | 3 | .613 |
| overall growth and development | 86.04 | 17.86 | 6 | .580 | 83.04 | 16.71 | 7 | .138 | 96.67 | 5.77 | 3 | .572 | 93.33 | 11.55 | 3 | .884 |
| discomfort or pain | 45.83 | 23.27 | 6 | .399 | 64.29 | 34.93 | 7 | .942 | 66.67 | 14.43 | 3 | .874 | 83.33 | 14.43 | 3 | .615 |
| temperament and moods | 52.08 | 16.40 | 6 | .069 | 73.69 | 17.46 | 7 | .854 | 77.78 | 6.36 | 3 | .969 | 75.00 | 19.09 | 3 | .803 |
| overall behaviour | 71.48 | 6.66 | 3 | .508 | 58.75 | | 1 | | 73.88 | 12.06 | 3 | .707 | 73.06 | 16.18 | 3 | .582 |
| general health perceptions | 62.90 | 24.17 | 5 | .954 | 62.19 | 14.95 | 7 | .864 | 46.11 | 14.56 | 3 | .224 | 80.83 | 4.17 | 3 | .158 |
| change in health | 3.00 | | 1 | | 5.00 | | 1 | | 4.00 | 1.00 | 3 | .828 | **3.00** | **0.00** | **3** | **<.001** |
| parent emotional impact | 65.63 | 15.69 | 6 | .870 | 80.36 | 27.82 | 7 | .746 | 85.42 | 15.73 | 3 | .927 | 87.50 | 16.54 | 3 | .915 |
| parent impact - time scale | 68.06 | 25.50 | 6 | .308 | 73.02 | 35.96 | 7 | .168 | 88.89 | 19.25 | 3 | .910 | 77.78 | 38.49 | 3 | .549 |
| family cohesion | 81.00 | 20.12 | 5 | .842 | 90.83 | 16.25 | 6 | .235 | 76.67 | 40.41 | 3 | .923 | 81.67 | 20.21 | 3 | .992 |

Note: P-value compared with non-meningitis control group

| **Table 8.11 Infant-toddler quality or life (ITQOL) mean scores in children aged <2 years with enteroviral meningitis compared with the non-meningitis control group** | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | discharge | | | | 6 months | | | | 12 months | | | | 18 months | | | |
| | mean | SD | n | p value | mean | SD | n | p value | mean | SD | n | p value | mean | SD | n | p value |
| overall health | 77.12 | 20.98 | 113 | .822 | 74 | 23 | 102 | .085 | 76.88 | 24.29 | 48 | .471 | 76.53 | 23.84 | 36 | .547 |
| physical abilities | 92.26 | 16.68 | 81 | .488 | 86.12 | 24.89 | 119 | .494 | 94.74 | 15.88 | 63 | .765 | 96.77 | 8.93 | 43 | .861 |
| overall growth and development | 88.62 | 15.26 | 113 | .526 | 90.02 | 16.09 | 109 | .369 | 90.79 | 15.99 | 63 | .479 | 87.27 | 18.66 | 44 | .084 |
| discomfort or pain | 53.82 | 23.76 | 121 | .844 | 63.07 | 19.85 | 110 | .808 | 70.83 | 24.49 | 63 | .496 | 73.30 | 22.81 | 44 | .175 |
| temperament and moods | 64.80 | 15.29 | 119 | .995 | 73.73 | 15.11 | 110 | .503 | 75.33 | 13.39 | 63 | .280 | 76.94 | 14.24 | 43 | .989 |
| overall behaviour | **86.19** | **9.72** | **5** | **.032** | 79.89 | 18.63 | 12 | .604 | 74.73 | 14.38 | 64 | .346 | **71.55** | **15.21** | **43** | **.031** |
| general health perceptions | 63.35 | 16.27 | 119 | .994 | **57.97** | **16.68** | **109** | **.009** | 58.68 | 20.77 | 63 | .739 | 60.25 | 20.11 | 43 | .225 |
| change in health | 3.00 | 1.41 | 2 | .909 | 3.45 | 1.04 | 11 | .659 | 4.24 | .86 | 58 | .374 | 3.50 | .89 | 42 | .532 |
| parent emotional impact | **61.27** | **29.28** | **117** | **.049** | 81.66 | 20.42 | 108 | .604 | 85.74 | 17.97 | 64 | .833 | 84.38 | 20.06 | 44 | .551 |
| parent impact - time scale | **71.15** | **28.80** | **117** | **.021** | 80.96 | 25.53 | 123 | .097 | 84.77 | 28.58 | 64 | .173 | 89.96 | 17.20 | 44 | .195 |
| family cohesion | 81.09 | 24.56 | 119 | .445 | 81.98 | 20.70 | 106 | .479 | 75.87 | 22.96 | 63 | .307 | 75.58 | 22.45 | 43 | .113 |

Note: P-value compared with non-meningitis control group

| **Table 8.12 Infant-toddler quality or life (ITQOL) mean scores in children aged <2 years with parechoviral meningitis compared with the non-meningitis control group** | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | discharge | | | | 6 months | | | | 12 months | | | | 18 months | | | |
| | mean | SD | n | p value | mean | SD | n | p value | mean | SD | n | p value | mean | SD | n | p value |
| overall health | 79.77 | 14.92 | 22 | .358 | 80 | 19 | 23 | .757 | 70.38 | 24.28 | 13 | .152 | 93.57 | 8.02 | 7 | .081 |
| physical abilities | 92.74 | 12.45 | 13 | .687 | 94.00 | 15.73 | 20 | .117 | 91.37 | 18.68 | 19 | .235 | 100.00 | 0.00 | 8 | .409 |
| overall growth and development | 83.69 | 16.49 | 20 | .106 | 85.91 | 23.43 | 22 | .275 | 89.74 | 14.57 | 19 | .433 | 95.00 | 8.02 | 8 | .554 |
| discomfort or pain | **41.67** | **26.02** | **21** | **.018** | 59.24 | 22.68 | 23 | .356 | 66.45 | 25.70 | 19 | .683 | 75.00 | 18.90 | 8 | .615 |
| temperament and moods | 58.25 | 21.64 | 21 | .092 | 74.75 | 16.75 | 23 | .551 | 76.01 | 13.27 | 19 | .644 | 77.60 | 8.01 | 8 | .879 |
| overall behaviour | | | 0 | | | | 0 | | 72.08 | 11.86 | 19 | .136 | 71.41 | 12.22 | 8 | .214 |
| general health perceptions | 63.45 | 18.93 | 21 | .982 | 60.38 | 14.72 | 24 | .439 | 55.86 | 22.04 | 19 | .423 | 68.93 | 9.26 | 7 | .579 |
| change in health | 3.00 | | 1 | | | | 0 | | 4.00 | .88 | 19 | .603 | 3.29 | .49 | 7 | .157 |
| parent emotional impact | 55.94 | 32.03 | 20 | .136 | 76.04 | 29.41 | 24 | .277 | 84.87 | 16.05 | 19 | .719 | 84.38 | 17.03 | 8 | .750 |
| parent impact - time scale | 71.25 | 25.72 | 20 | .216 | 84.00 | 24.76 | 25 | .795 | 94.74 | 8.43 | 19 | .300 | 95.83 | 6.30 | 8 | .545 |
| family cohesion | 76.50 | 18.99 | 20 | .185 | 81.88 | 14.20 | 24 | .610 | 70.53 | 17.86 | 19 | .086 | 75.63 | 12.94 | 8 | .373 |

Note: P-value compared with non-meningitis control group

| **Table 8.13 Infant-toddler quality or life (ITQOL) mean scores in children aged <2 years with probable enteroviral meningitis compared with the non-meningitis control group** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | discharge | | | | 6 months | | | | 12 months | | | |
| | mean | SD | n | p value | mean | SD | n | p value | mean | SD | n | p value |
| overall health | 89.00 | 17.46 | 5 | .210 | 83 | 13 | 6 | .603 | 68.33 | 14.43 | 3 | .371 |
| physical abilities | 97.22 | 5.56 | 4 | .468 | **97.04** | **4.80** | **6** | **.003** | 87.04 | 22.45 | 3 | .279 |
| overall growth and development | 81.25 | 14.93 | 4 | .297 | 90.00 | 15.81 | 6 | .796 | 80.00 | 26.46 | 3 | .121 |
| discomfort or pain | 56.25 | 7.22 | 4 | .790 | 66.67 | 17.08 | 6 | .738 | 50.00 | 21.65 | 3 | .126 |
| temperament and moods | 53.13 | 12.44 | 4 | .170 | 64.44 | 12.27 | 6 | .281 | 68.06 | 22.95 | 3 | .223 |
| overall behaviour | **60.42** | **7.53** | **3** | **.017** | **55.57** | **16.87** | **2** | **.012** | 69.31 | 26.94 | 3 | .686 |
| general health perceptions | 57.08 | 27.51 | 4 | .483 | 50.50 | 21.28 | 5 | .125 | 44.72 | 29.56 | 3 | .186 |
| change in health | 2.50 | .71 | 2 | .574 | 1.00 | | 1 | | 2.67 | 2.08 | 3 | .351 |
| parent emotional impact | **39.06** | **33.61** | **4** | **.020** | 66.67 | 36.37 | 6 | .056 | 75.00 | 28.64 | 3 | .233 |
| parent impact - time scale | **52.08** | **41.04** | **4** | **.032** | 81.94 | 24.95 | 6 | .726 | 77.78 | 38.49 | 3 | .634 |
| family cohesion | 92.50 | 8.66 | 4 | .363 | 69.17 | 30.89 | 6 | .223 | 86.67 | 23.09 | 3 | .544 |

Note: P-value compared with non-meningitis control group

| **Table 8.14 Infant-toddler quality or life (ITQOL) mean scores in children aged <2 years with other viral meningitis compared with the non-meningitis control group** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | discharge | | | | 6 months | | | | 12 months | | | |
| | mean | SD | n | p value | mean | SD | n | p value | mean | SD | n | p value |
| overall health | 67.50 | 32.52 | 6 | .321 | 58 | 39 | 2 | .195 | 85.00 | | 1 | |
| physical abilities | 95.65 | 4.97 | 6 | .500 | 78.89 | 30.71 | 4 | .430 | 98.15 | 3.21 | 3 | .713 |
| overall growth and development | 89.17 | 12.81 | 6 | .932 | 83.33 | 15.28 | 3 | .343 | 100.00 | 0.00 | 3 | .319 |
| discomfort or pain | 68.75 | 25.92 | 6 | .086 | 70.83 | 31.46 | 3 | .574 | **62.50** | **0.00** | **3** | **.001** |
| temperament and moods | **45.83** | **22.67** | **6** | **.007** | 54.17 | 15.02 | 3 | .081 | 73.61 | 12.73 | 3 | .611 |
| overall behaviour | 68.58 | 22.04 | 2 | .372 | 81.04 | 4.42 | 2 | .618 | 77.08 | 12.44 | 3 | .946 |
| general health perceptions | 55.00 | 21.78 | 6 | .253 | **55.28** | **0.48** | **3** | **<.001** | 56.67 | 30.14 | 3 | .790 |
| change in health | 2.00 | | 1 | | 3.00 | | 1 | | 3.33 | .58 | 3 | .142 |
| parent emotional impact | **16.25** | **24.84** | **5** | **<.001** | 60.42 | 53.16 | 3 | .541 | 95.83 | 7.22 | 3 | .301 |
| parent impact - time scale | **30.00** | **41.50** | **5** | **<.001** | 54.17 | 45.90 | 4 | .269 | 100.00 | 0.00 | 3 | .371 |
| family cohesion | **61.00** | **31.70** | **5** | **.022** | 66.67 | 31.75 | 3 | .285 | 71.67 | 36.86 | 3 | .543 |

Note: P-value compared with non-meningitis control group

**Table 8.15 Infant-toddler quality or life (ITQOL) mean scores in children aged <2 years with aseptic meningitis of unknown aetiology compared with the non-meningitis control group**

| | discharge | | | | 6 months | | | | 12 months | | | | 18 months | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mean | SD | n | p value | mean | SD | n | p value | mean | SD | n | p value | mean | SD | n | p value |
| overall health | 78.28 | 20.61 | 64 | .570 | 75 | 21 | 45 | .405 | 77.61 | 21.21 | 23 | .673 | 79.80 | 20.49 | 25 | .910 |
| physical abilities | 92.55 | 15.37 | 56 | .474 | **93.15** | **15.46** | **51** | **.043** | 90.48 | 20.83 | 28 | .239 | 96.28 | 11.33 | 26 | .966 |
| overall growth and development | 86.79 | 16.18 | 62 | .192 | 92.35 | 13.03 | 49 | .746 | 88.39 | 15.76 | 28 | .168 | 87.12 | 24.83 | 26 | .323 |
| discomfort or pain | 52.12 | 23.86 | 65 | .677 | 67.25 | 24.21 | 50 | .299 | 64.29 | 17.25 | 28 | .305 | 81.73 | 22.70 | 26 | .409 |
| temperament and moods | 61.32 | 15.65 | 64 | .127 | 75.17 | 15.03 | 50 | .312 | 78.10 | 9.95 | 28 | .814 | 80.45 | 12.95 | 26 | .222 |
| overall behaviour | 74.14 | 17.70 | 5 | .786 | 80.50 | 12.39 | 10 | .366 | 75.13 | 15.25 | 29 | .578 | 74.05 | 13.34 | 26 | .290 |
| general health perceptions | 63.57 | 16.55 | 66 | .930 | 60.01 | 17.45 | 49 | .237 | 62.32 | 19.71 | 28 | .506 | 61.88 | 17.74 | 24 | .513 |
| change in health | 2.00 | 1.00 | 3 | .141 | 3.83 | .98 | 6 | .569 | 4.25 | .93 | 28 | .481 | 3.79 | .83 | 24 | .397 |
| parent emotional impact | **59.52** | **27.79** | **63** | **.023** | 81.50 | 23.59 | 50 | .671 | 82.54 | 16.82 | 29 | .256 | 85.68 | 20.15 | 24 | .859 |
| parent impact - time scale | 72.14 | 26.82 | 64 | .075 | 86.54 | 20.95 | 52 | .705 | 89.94 | 15.17 | 29 | .958 | 87.85 | 24.69 | 24 | .271 |
| family cohesion | 82.00 | 22.79 | 65 | .758 | 83.10 | 19.14 | 50 | .384 | **69.83** | **25.76** | **29** | **.036** | **72.08** | **26.04** | **24** | .106 |

Note: P-value compared with non-meningitis control group

| **Table 8.16 Infant-toddler quality or life (ITQOL) mean scores in children aged <2 years with possible meningitis compared with the non-meningitis control group** | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | discharge | | | | 6 months | | | | 12 months | | | | 18 months | | | |
| | mean | SD | n | p value | mean | SD | n | p value | mean | SD | n | p value | mean | SD | n | p value |
| overall health | 73.50 | 25.53 | 30 | .468 | 76 | 24 | 34 | .489 | 82.35 | 17.78 | 17 | .636 | 66.88 | 26.45 | 8 | .129 |
| physical abilities | **77.35** | **30.65** | **27** | **.034** | 89.56 | 19.39 | 35 | .673 | 97.83 | 6.41 | 21 | .397 | 96.67 | 7.50 | 10 | .946 |
| overall growth and development | 87.59 | 15.04 | 29 | .492 | 87.79 | 15.92 | 34 | .169 | 89.52 | 16.12 | 21 | .383 | 87.00 | 21.50 | 10 | .470 |
| discomfort or pain | 45.56 | 25.93 | 31 | .114 | 68.38 | 21.37 | 34 | .237 | 72.62 | 17.51 | 21 | .395 | 77.50 | 21.89 | 10 | .901 |
| temperament and moods | 59.72 | 23.58 | 30 | .256 | **79.26** | **10.56** | **34** | **.002** | 76.04 | 12.31 | 20 | .642 | 72.92 | 12.46 | 10 | .352 |
| overall behaviour | 80.48 | 7.92 | 7 | .234 | 78.05 | 11.43 | 13 | .749 | 75.11 | 13.79 | 21 | .615 | 79.96 | 17.02 | 10 | .494 |
| general health perceptions | **55.39** | **21.14** | **30** | **.020** | **55.50** | **18.03** | **35** | **.018** | 59.52 | 15.11 | 21 | .973 | 54.83 | 15.87 | 10 | .123 |
| change in health | 2.75 | .50 | 4 | .752 | 3.50 | .80 | 12 | .747 | 4.33 | .86 | 21 | .307 | 4.00 | 1.15 | 10 | .231 |
| parent emotional impact | **49.38** | **35.71** | **30** | **.012** | 79.46 | 19.68 | 35 | .346 | 88.44 | 14.38 | 20 | .564 | 86.11 | 15.24 | 9 | .959 |
| parent impact - time scale | 67.50 | 35.24 | 30 | .117 | 87.73 | 17.87 | 36 | .530 | 93.42 | 12.60 | 19 | .466 | 93.52 | 9.11 | 9 | .968 |
| family cohesion | 86.33 | 19.69 | 30 | .389 | 82.43 | 19.64 | 35 | .569 | 75.25 | 24.79 | 20 | .440 | 76.67 | 29.47 | 9 | .638 |

Note: P-value compared with non-meningitis control group

## 8.4. Discussion

Health related quality of life following viral and bacterial meningitis in young children aged <2 years has not been previously described. For young children, quality of life scores across several domains were lower in children with bacterial meningitis than a control group of children admitted to hospital with an acute non-meningitis illness, measured by the Infant Toddler Quality of Life questionnaire. However, at discharge parent emotion impact scores were lower in both bacterial meningitis and aseptic meningitis of several aetiologies compared with the non-meningitis control group. For enteroviral meningitis, lower QOL scores compared with the control group were also observed for limited domains until 18 months post-discharge. Short-term sequelae were more common following bacterial than viral meningitis, however there were reported parental concerns about reduced mobility and hearing impairment in some infants following enteroviral meningitis.

These data reported parental concern about short-term sequelae, in contrast with previous published papers which have generally reported long-term outcomes by more objective measures.[118,326-328] Of children with bacterial meningitis at three months post-discharge, 25% were reported as not back to normal, and all areas of functioning were reported more frequently as not being back to normal compared with the control group, apart from school function. For bacterial meningitis, headache, seizures, reduced mobility, anxiety, and impaired speech, memory, vision or hearing were all reported more frequently at discharge compared with the control group. By three months post discharge, all sequalae continued to be more frequently reported compared with control group apart from seizures. Although higher rates of reported outcomes may be expected

in these present data due to different study design, similarities to previous studies for outcomes following meningitis of different aetiologies were observed.[118,326-328] Reduced mobility was reported for 43% of children following pneumococcal meningitis, but only one child (10%) following meningococcal meningitis. A previous meta-analysis of long term outcomes following bacterial meningitis at all ages, also reported motor deficit more frequently following pneumococcal (5.8%) then meningococcal (1%) meningitis,[118] although a recent Canadian study reported motor deficit at discharge for 4.1% of children who survived IMD.[327]

Seizures were reported following bacterial meningitis in 9% of children at discharge and 4% (one child following pneumococcal and one child following GBS meningitis) at 3 months following discharge. The meta-analysis of bacterial meningitis outcomes reported a similar frequency of seizures (3.7%) for all cause bacterial meningitis, although more frequent following pneumococcal (2.5%) than meningococcal (0.5%) meningitis.[118] Other studies of outcomes following childhood meningococcal disease also report relatively low frequencies of seizures for any MenB disease (2%),[326] and any IMD (0.7%).[327]

Concern about new onset or worsened visual disturbance was reported by parents in three children (21%) following pneumococcal meningitis. The meta-analysis of long term outcomes reported visual disturbance for 1.1% of people at all ages following pneumococcal meningitis,[118] with the higher frequency of concern in these present data likely contributed to by the subjective nature of the parent interview.

Reduced mobility was reported for 3/7 (43%) and seizures by 1/10 (10%) of infants following GBS meningitis. A study reporting long term follow-up for 43 survivors of GBS meningitis in infancy, also suggested substantial concerns about motor impairment following GBS meningitis, with cerebral palsy or spasticity reported in 11.6% and seizure disorder reported in 16.3% of survivors in childhood.[328]

NICE guidance recommends a hearing test for all children who have bacterial meningitis 4-6 weeks following discharge,[252] but practice is more varied following viral meningitis. Hearing tests were obtained for only 72% (80/111) of children within three months following bacterial meningitis, with 81% of tests providing conclusive results. It is possible that hearing tests may have been obtained at >3 months following bacterial meningitis for more children, however these data highlight a need to improve clinical follow-up of these children.[106] Frequency of any SNHL was highest following pneumococcal meningitis (bilateral SNHL 30% (6/20), unilateral SNHL 5% (1/20)), and although hearing threshold levels were not available to allow comparison with other studies, the meta-analysis of bacterial meningitis outcomes also reported highest rates of hearing loss following pneumococcal meningitis (7.5%).[118] Abnormal non-conductive hearing results were reported for 15% of children (3/20) following meningococcal meningitis, compared with 2.6% reported by the meta-analysis at all ages,[118] 7.4% reported previously following any childhood IMD,[327] and 6% reported previous following childhood MenB disease.[326] Interestingly all available hearing results following GBS meningitis were reported as normal, although the previous study of long term follow-up of GBS meningitis reported bilateral SNHL for 9.3% of children.[328]

Hearing tests were also obtained for 34% (85/247) of children following EV or parechoviral meningitis, with abnormalities reported for three infants following EV and one infant following parechoviral meningitis. Although results of further hearing testing would be required to assess the significance of these findings, this suggests that obtaining hearing tests may require consideration in infants following EV meningitis.

Although there are few studies reporting outcomes following childhood viral meningitis, most available literature suggests no ongoing neurological sequalae.[74,262,264,330-332] However, a few small studies have suggested limited concerns about neurocognitive and language sequelae.[333-335] A study published in 1996 including 16 children who had EV meningitis in infancy, reported some subtle differences in language development.[333] An earlier study published in 1975 of 19 children following EV infection reported lower mean IQ scores and concerns about language development for children who had EV infection as infants, compared with controls.[335] A further study also published in 1975 reported low IQ and spasticity for 2/15 children following neonatal meningoencephalitis caused by Coxsackie B5.[334] In this present study, the parent interview at three months post-discharge included several general questions about the parent's perspective of the child's functioning, followed by more specific questions about sequelae. The frequencies of parent report of not returning to normal was similar in children who had viral meningitis cause by EV or parechovirus (10%) compared with the control group of acute non-meningitis illnesses (9%), including no significant differences in reported abnormal sleep, behaviour, attention, school function, and daily activities, which suggests that parents perceive that children recover well from viral meningitis. However, at three months post discharge following viral meningitis caused by EV or parechovirus, there were significant

differences compared with the control group in reported concern about reduced mobility, as well as hearing impairment as discussed above. Although not statistically significant, compared with the control group, new or worse headache was also reported in 9% (4/45) of children who had EV meningitis at 3 months following discharge, compared with 3% in the control group. Ongoing follow-up would be necessary to further define these concerns.

Following parechoviral meningitis, there was one infant (14%, 1/7) with reported reduced mobility due to difficulties using one leg. Two recent studies including very small numbers of participants have reported higher rates of developmental delays or neurological sequelae following parechoviral infection during infancy (32-63%),[217,336] although an Australian case series of 118 infants with parechoviral infections reported motor delay or abnormal tone in only four infants.[74]

Studies previously reporting health-related quality of life following meningitis in young children are scarce. A recent UK study investigated quality of life in 109 children following any laboratory confirmed MenB disease with questionnaires completed a median of 134 days following illness compared with retrospective completion of the questionnaires recalling the worst day of illness.[437] The questionnaire included sequelae, a visual analogue scale, and a quality of life questionnaire (EQ-5DY) which is not validated for children aged <8 years and was only completed for 17% of infants.[437] They reported very low QOL on the worst day of illness, with the majority of children returning to close to 'perfect health' scores within 6 months.[437] Few further studies have reported HR-QOL for school aged children following meningitis. One study from Scotland reported HR-QOL

at mean 8 years following meningitis (majority bacterial), and found that HR-QOL was significantly poorer compared with a healthy control population as measured by PedsQL questionnaire completed by parents, including significantly lower emotional, social, school functioning and fatigue scores.[325] A further study investigated HR-QOL in Dutch school aged children 4-10 years following non-*H. influenzae* type b bacterial meningitis using two questionnaires completed by parents, and reported decreased HR-QOL particularly for general health perceptions, emotions and self-esteem in survivors of bacterial meningitis compared with a reference population.[438]

In this study, lower quality of life scores compared with the control group were most often observed following bacterial meningitis, including for several domains at 12 months post-discharge. The parent emotional impact scores were lower at discharge compared with the control group for both bacterial meningitis and several aseptic meningitis groups, which might suggest that parents perceive the diagnosis and management of any meningitis illness as significant. However, the low parent emotional impact scores did not persist for aseptic meningitis groups at follow-up timepoints. For enteroviral meningitis, parent time-impact scales at discharge and general health perceptions at 6 months were also lower than the control group. Although there were fewer low scores than for bacterial meningitis, overall behaviour scores for EV meningitis at 18 months post-discharge were lower than the control group which may further support the need for more detailed long-term follow-up of neuropsychological and cognitive outcomes.

Contemporary, detailed outcome data are required to drive cost-effectiveness analyses[439] for consideration of implementation of vaccines into national immunisation schedules.

Studies specifically reporting outcomes following MenB disease[326,437] were required to develop a cost-effectiveness model[439] prior to the implementation of the 4CMenB vaccine in the routine UK infant vaccine schedule. These present data provide current prospectively collected knowledge of outcomes and quality of life across bacterial and viral meningitis aetiologies, and therefore will be vital to inform future priorities for prevention strategies. Extensive knowledge about outcomes are also necessary to improve understanding of appropriate clinical follow-up, and support for children and families, following bacterial or viral meningitis.

The main limitation of this study was partial return rates for postal questionnaires, and completion of the three-month interview, contributed to by difficulties contacting families. Not all young children included in the QOL analysis had a discharge questionnaire completed, and questionnaires were not completed at every timepoint for each child. Long term data and further detailed knowledge about the nature of reported outcomes would also enable further definition of sequelae following meningitis. However, there are few studies to assess both quality of life and outcomes following both childhood aseptic and bacterial meningitis, and the inclusion criteria for this prospective study allowed the formation of a highly relevant control group for comparisons.

In conclusion, this study provided a unique opportunity to improve understanding of outcomes following childhood viral meningitis, and to assess health-related quality of life following bacterial and viral meningitis in young children, which were not previously well defined in the literature. These findings will inform further research strategies, particularly to further define long term neuropsychological and cognitive outcomes for

children following viral meningitis, which could inform clinical guidelines for appropriate support of these children, and priorities for prevention, for example development of new vaccines. Detailed knowledge about outcomes following bacterial meningitis was also necessary to further inform cost-effectiveness analyses for development and implementation of preventative strategies including new vaccines, and improve understanding of optimal follow-up and support in the clinical setting.

# 9. Chapter 9. Conclusions

## 9.1. Introduction

Previous studies reported reductions in microbiologically confirmed cases of childhood bacterial meningitis in the UK following the implementation of different vaccination programmes.[110,113,127,12812,107,109,148-150] Contemporary knowledge about historical changes in epidemiology and the current aetiology of all-cause childhood meningitis, how to reliably distinguish between bacterial and aseptic meningitis at presentation to hospital, and outcomes particularly following viral meningitis, was needed to inform research priorities for prevention, decision-making about immunisation programmes, and guidelines for clinical management to improve the care of children with bacterial and aseptic meningitis.

The aims of this study were to investigate the retrospective epidemiology of childhood bacterial and viral meningitis in the UK over five decades, which then provided context for an in-depth analysis from a multicentre UK-wide prospective study of the current causes of childhood meningitis, a comparison of clinical and laboratory features and investigation of clinical decision rules to distinguish bacterial and aseptic meningitis, a study of enteroviral PCR positivity from non-CSF sites in suspected and confirmed EV meningitis, and analysis of short term outcomes and health-related quality of life following childhood meningitis.

**9.2. The retrospective epidemiology of childhood meningitis in the UK from hospital admissions datasets**

*(references [169,339])*

Analysis of hospital admissions datasets in England from 1968-2011 demonstrated reductions in childhood meningitis caused by *H. influenzae*, *N. meningitidis,* and *S. pneumoniae*, predominantly due to the success of vaccine programmes. There was a decrease in admissions for *H. influenzae* meningitis in children aged <15 years following the 1992 introduction of routine Hib vaccination, from 6·72/100 000 (95% confidence interval 6.18-7.26) children in 1992 to 0·39/100 000 (0.26-0.52) in 1994. Hospital admissions for meningococcal disease increased during the 1990s, and peaked at 34·54 (33·30-35·78) admissions/100 000 children <15 years in 1999, and then reduced to 12·40/100 000 (11·68-13·12) in 2011, after the MenC vaccine was introduced in 1999. Admissions for pneumococcal meningitis also increased from the 1990s, and reached 4·45/100 000 (4.0-4.9) children in 2006, and then reduced to 2·03/100 000 (1.74-2.32) in 2011, following the 2006 introduction of the pneumococcal conjugate vaccine.

However, substantial numbers of bacterial meningitis cases still occurred in England. Bacterial meningitis remains a severe illness with substantial morbidity and mortality.[100,118,324,351] Therefore, early identification and management, and ongoing cost-effective strategies for prevention of these bacterial cases remains vital. Interestingly, hospital admission rates for meningococcal disease and pneumococcal meningitis in England were higher than were reported by microbiological surveillance data when direct comparisons were possible, although there were similar post-vaccination disease

trends.[110,149] The higher rates reported in the hospital admissions datasets warrants consideration when defining epidemiology imperative to decision making about bacterial meningitis vaccination programmes.

The reduction in bacterial meningitis admissions that occurred during the past two decades highlighted that most childhood meningitis cases nowadays in England were likely to be viral. Analysis of retrospective hospital admissions for viral meningitis in England indicated that although there was a decline in viral meningitis admission rates during the late 1980s from 13.5/100 000 children aged <15 per year (95% confidence interval 13.0-14.0) in 1968-1985 to 5.2 per 100 000 (5.1-5.3) in 1989-2011, which may have been driven by the MMR vaccination, this only occurred in children aged 1-14 years. In contrast, viral meningitis hospital admission rates in infants increased in recent years, and reached 70.0/100 000 (63.7-76.2) in 2011, caused by increased admission rates in young infants aged ≤90 days. Since 2000, admissions with a specified viral aetiology also increased, likely contributed to by improved diagnostic capabilities.

Considering declining bacterial meningitis cases, it has become increasingly challenging for clinicians to identify the few children with a bacterial aetiology for meningitis promptly on arrival at hospital, whilst avoiding unnecessary management for children with viral aetiologies. These data indicated substantial median length of hospital admissions of 3-4 days in children and infants with viral meningitis, and previous studies from other countries reported that the majority of children with viral meningitis received intravenous antibiotic management.[172,186,312]

### 9.3. The current aetiology of childhood meningitis by analysis of a prospective UK-wide multicentre cohort study

The vast changes in childhood meningitis epidemiology during the past two decades demonstrated in the historical hospital admissions data confirmed the need to understand the current aetiology of childhood meningitis in the UK. Contemporary data were needed to accurately define aetiology. The UK-Childhood Meningitis and Encephalitis cohort Study (UK-ChiMES) prospectively recruited children aged <16 years admitted to hospital with suspected or confirmed meningitis from December 2012 to June 2016. Analysis of data for 2754 out of 3003 children recruited to the study showed that only 19.3% (172/892) of meningitis cases had a confirmed bacterial cause. There was no cause identified for a substantial proportion (38.9%, 280/720) of aseptic meningitis cases from tests performed at hospital sites, with previous studies also reporting that frequently no cause is identified in aseptic meningitis.[172,177-179] More than half of all meningitis cases occurred in young infants aged <3 months (60%). Considering bacterial meningitis, *S. pneumoniae* (31% of cases) caused slightly fewer cases compared with *N. meningitidis* (29% of cases), in contrast to a recent paper reporting laboratory confirmed data during an earlier period from 2004-11, which found that *N. meningitidis* was the most common cause.[104] In neonates, Group B Streptococcus was the cause of bacterial meningitis in 53% of infants aged <29 days and 42% of infants aged <3 months. *E. coli* was the second most frequent cause of bacterial meningitis in young infants, accounting for 21% of cases aged <3 months. Most viral meningitis was caused by enteroviruses, comprising 81% (305/378) of all definite viral meningitis cases, 83% of which occurred in young infants <3 months. There were also 49 parechovirus meningitis cases, 94% of which occurred in young infants <3 months. The inclusion of children with suspected

meningitis, who did not have confirmed meningitis allowed the development of a non-meningitis control group for further analyses in the thesis. These data confirmed that the great majority of childhood meningitis is not bacterial, and support the concept that although ongoing optimisation of vaccination programmes for remaining bacterial cases is important, research into prevention of the most common current causes of childhood meningitis should also be prioritised, including ongoing research into vaccinations for Group B Streptococcus and enteroviruses.

There was longer median LOS in children with meningitis of unknown aetiology than in EV or parechoviral meningitis (6 days versus 3 days, p=<0.001). The vast majority of children with EV meningitis (96%, 292/305), parechoviral meningitis (100%, 49/49) and aseptic meningitis of unknown aetiology (92%, 258/280) received at least one dose of IV or IM antibiotics. Interestingly, 26% of children with no aetiology defined did not have CSF-EV PCR performed, and a greater proportion of children pre-treated before LP had no cause identified (37% versus 29%, p=0.016). These data suggest that further optimising the investigation of children with meningitis, and development of improved diagnostic tests, could lead to increased aetiological attribution and reduced unnecessary antibiotic management and LOS. A clinical decision rule to assist clinicians with decision making prior to results of diagnostic testing becoming available could also lead to improved management of both bacterial and viral meningitis.

### 9.4. Clinical features and investigation of clinical decisions rules to distinguish bacterial and aseptic meningitis in children by analysis of a prospective UK-wide multicentre cohort study

Early identification of children and infants with viral meningitis could avoid unnecessary antibiotic management and reduce hospital LOS. Clinical findings and results of laboratory tests performed soon after arrival at hospital were analysed for 2173 prospectively recruited children with meningitis of different aetiologies, meningitis of unknown cause, and a non-meningitis control group.

A pre-existing clinical decision rule, the 'Bacterial Meningitis Score',[173] was assessed in children with meningitis and a raised CSF WBC count, who were recruited to the prospective cohort study. Interestingly the sensitivity and negative predictive value of the BMS were 100% both with and without inclusion of neonates, a group who have been excluded from previous validation studies.[295] However, many children in the present cohort (35% of non-pretreated children >28 days) had missing data for BMS predictors. The specificity (22% excluding neonates) was also lower than was reported by BMS validation studies from other countries,[295] which suggested the need for a new clinical decision rule relevant to the current UK population.

The study to investigate the development of a new multivariable clinical decision model to predict probability of bacterial meningitis included children with a raised CSF WBC (WBC>5 $X10^6$/L), aged >28 days, who had not been pre-treated with antibiotics. Variables were selected for inclusion in the model that were clinically relevant, with <10% data missing, and $p<0.25$ at univariable analysis. A model was developed by logistic regression

with backward stepwise elimination, and continuous variables were kept continuous. Both the model developed by backward elimination including CSF Gram film positive result, history of altered consciousness, blood neutrophil count and blood CRP, and a further model which also included CSF WBC, performed well although validation in a separate population is recommended for clinical decision rules.[405] The model could be used as an online calculator or app and provides a predicted probability of bacterial meningitis, which could be used to assist clinical decision making.

### 9.5. Detection of enterovirus by real-time PCR of non-CSF samples in children with suspected or confirmed viral meningitis.

The most common cause of meningitis in the UK-ChiMES study was enteroviruses. Establishing the diagnosis of EV meningitis early could avoid unnecessary hospital LOS and antibiotic management.[22,28,185,257,279,313-319] Previous studies have suggested that in children with a raised CSF WBC count and an EV-PCR positive (EV-PCR+) sample from another site, EV meningitis could be considered the diagnosis.[20,257,311,421] Therefore EV-PCR positivity from non-CSF samples was investigated in a subset of children (predominantly young infants) recruited to the UK-ChiMES study with suspected or confirmed non-bacterial meningitis. The Enterovirus R-gene® kit[302,422] was used to perform real time PCR on stool, throat swab, NPA and serum samples, following an RNA extraction process.[425] In this study, all children (100%, 26/26) with EV meningitis and an available stool sample were stool EV-PCR+, but fewer upper respiratory samples were positive (33%, 5/15). Stool EV-PCR+ results were obtained for many days following onset of symptoms. Of children with aseptic meningitis of unknown cause, 35% (6/17) were

stool EV-PCR+ by EV R-gene® kit,[302,422] but none were serum or throat swab EV-PCR+. Although sample size was small, children who were CSF EV-PCR-, but non-CSF site EV-PCR+ had longer time from onset of symptoms to LP, compared with CSF EV-PCR+ children (median 3 days versus 1 day, p=0.049), which suggested that a finding of EV positivity from a non-CSF site may indicate EV meningitis particularly if LP is delayed and could therefore also assist clinical decision making about observing children off antibiotics.

It has been previously established that EV meningitis can occur in the absence of a raised CSF WBC count, which has been increasingly recognised since the widespread use of PCR.[172,276-280] In this study, children (mostly young infants) with EV meningitis with a raised CSF WBC compared to without a raised CSF WBC had a higher median peripheral WBC count, were younger, but had lower blood CRP. This present study was able to compare clinical features in a substantial number of prospectively recruited infants and children with EV meningitis with (n=175) and without (n=118) a raised CSF WBC count, and further research comparing outcomes for these children will also further improve understanding of this reasonably recently described clinical presentation.

**9.6. Short term outcomes and quality of life following childhood meningitis**

Outcomes following viral meningitis are poorly defined in the literature,[74,262,264,331-336] although outcomes following bacterial meningitis are well-described.[118,324-326327,328] Short term outcomes were analysed at discharge and three months' post-discharge in 2119 children, and health related quality of life was assessed by the Infant Toddler Quality of

Life questionnaire[345] at discharge, and 6, 12 and 18 months following discharge in 863 young children aged <2 years, who were recruited to the UK-ChiMES study with meningitis, or with suspected meningitis but a final non-meningitis diagnosis.

Short-term sequelae were reported frequently following bacterial meningitis compared with a control group, including headache, seizures, reduced mobility, anxiety, and impaired speech, memory, vision or hearing. For some infants following enteroviral meningitis, there were reported parental concerns about reduced mobility and hearing impairment. Outcomes were also analysed by pathogen.

Quality of life scores across several domains were lower for young children with bacterial meningitis than the non-meningitis control group. At discharge, parent emotional impact scores were lower for both bacterial meningitis and aseptic meningitis of several aetiologies compared with a control group. For enteroviral meningitis, parent time-impact scores at discharge, general health perceptions at 6 months, and overall behaviour scores at 18 months post-discharge were also lower than for the control group.

These findings suggest that there may be subtle sequelae following viral meningitis, and more long-term knowledge is still needed about neuropsychological and cognitive outcomes following childhood viral meningitis to inform clinical guidelines for appropriate support of these children, and priorities for prevention. Detailed knowledge about outcomes following bacterial meningitis will further inform guidelines for clinical follow-up and cost-effectiveness analyses for the development and implementation of new vaccines.

### 9.7. Considerations for future analyses and studies

A key contribution of these data are the detailed analyses of outcomes following bacterial and aseptic meningitis, although the outcome analyses are a secondary objective within the UK-ChiMES study. Future analyses of outcome questionnaire results by bacterial pathogen would provide further important data for consideration in vaccine implementation cost-effectiveness studies. Furthermore, comparison of outcomes in children with enteroviral meningitis with and without CSF pleocytosis, including quality of life and neuropsychological outcomes, would improve understanding of whether these clinical presentations represent different disease entities. Further quality of life and neuropsychological questionnaires obtained during the UK-ChiMES study to be analysed in future studies are shown in *table 2.5.*

Development of a new clinical decision rule to distinguish bacterial and aseptic meningitis was an objective of the UK-ChiMES study. In future analyses, consideration could be given to designing a separate clinical decision rule to distinguish children with bacterial meningitis from all children presenting to hospital with suspected meningitis regardless of whether the final diagnosis is aseptic meningitis. In such a separate analysis, it may be possible to include only clinical features and results of blood tests available prior to obtaining a lumbar puncture. Development of such a rule would allow inclusion of the large cohort of children recruited who presented with suspected meningitis, but did not have a final diagnosis of meningitis. From a clinical practise perspective, a different rule designed to distinguish the small number of children with bacterial meningitis from all children presenting with suspicion of meningitis, would be of value nowadays in an era in

which only a small proportion of children have a serious bacterial infection of the many children who present to hospital with febrile illnesses.

The analysis in this thesis excluded 8.3% of children recruited to the UK-ChiMES study due to incomplete data. The majority of these excluded participants could be included in the final study analysis. In this thesis, exclusions were made in the analyses of clinical features and clinical decision rules to ensure accuracy of defined groups which also resulted in reduced numbers. Further consideration could be given to exclusion criteria in future analyses.

Considering the UK-ChiMES study, aspects of the study design that may have enhanced findings include consideration of narrower inclusion criteria to allow more focus on definite meningitis cases. Use of different inclusion criteria may have allowed more resource to be focussed on collection of outcome data in children with definite meningitis. However, inclusion of only children with meningitis is unlikely to have changed the number of participants with definite meningitis recruited at hospital sites, and inclusion of children with suspected meningitis also allowed the development of a control group for comparison of outcomes.

Future studies in UK-ChiMES include further diagnostic testing of selected CSF samples obtained, disease mechanisms and genomic studies, which will also provide valuable knowledge to further improve understanding and care of children who present to hospital with suspected meningitis.

### 9.8. Summary

In this study, analysis of historical hospital admissions from the 1960s to 2011 demonstrated radical changes in the epidemiology of childhood bacterial and aseptic meningitis in England, particularly during the last two decades of the study. Admission rates decreased for childhood bacterial meningitis of different aetiologies and for aseptic meningitis in children aged >1 year, however admission rates for aseptic meningitis in infants have increased in recent years. These changes reflect the effect of different vaccine programmes, advances in microbiological diagnostics, and there may be some contribution from changes in clinical and coding practise. These retrospective data provided a framework for an in-depth analysis from 2012-2016 of the current aetiology of childhood meningitis from a prospective cohort study across 31 UK hospital sites.

Analyses from the largest prospective study to provide contemporary knowledge about childhood meningitis in the UK confirmed that the great majority of meningitis nowadays is aseptic, with most viral meningitis occurring in young infants and most bacterial meningitis occurring in young children. These data highlight the continued importance of prevention of the remaining bacterial meningitis cases including ongoing research into Group B Streptococcus vaccines, whilst considering new strategies for prevention of viral meningitis particularly research into enteroviral vaccines. Very few infants or children with aseptic or confirmed viral meningitis did not receive intravenous or intramuscular antibiotics, and median hospital length of stay for confirmed enteroviral or parechoviral meningitis was three days, but longer if no aetiology was identified. Therefore, early identification of viral meningitis, which currently comprises most childhood meningitis, is important to prevent unnecessary hospital length of stay and antibiotic management.

Optimising investigations to promptly confirm aetiology, and development of new diagnostic tests would assist with early identification. Clinical decision rules to assist with decision making before diagnostic test results become available could also improve management of both bacterial and viral meningitis.

To investigate how to distinguish viral and bacterial meningitis early in the illness, a detailed analysis was performed of clinical and laboratory features on admission to hospital of meningitis of different aetiologies from the prospective UK-wide study. The previously developed Bacterial Meningitis Score[173] was assessed in this UK cohort, although data were frequently missing for BMS predictors. A new clinical decision model was therefore developed to provide a predicted probability of bacterial meningitis, which performed well in this current dataset and could be used as an online tool or app to assist clinical decision making, for example about observing children off antibiotics who present with suspected meningitis and a low predicted probability of bacterial meningitis.

A further study was also performed of EV-PCR positivity from non-CSF sites in suspected or confirmed aseptic meningitis, and although included numbers were limited, the study found that in EV meningitis all children with available stool samples were stool EV-PCR positive up to several days following onset of illness, and 35% of children with aseptic meningitis of unknown aetiology were stool EV-PCR+. Children with aseptic meningitis of unknown aetiology, but a stool EV-PCR+ result and CSF EV-PCR- result had their LPs later than children who were CSF EV+ suggesting that performing stool EV-PCR may support making a diagnosis of probable EV meningitis particularly if LP delayed, and may also assist with clinical decision making.

Analysis of short-term outcomes and health related quality of life following childhood meningitis, including outcomes following viral meningitis which are not well described in the literature, indicated that although sequelae and lower QOL scores occur more frequently following bacterial than following aseptic meningitis, some parental concerns about sequelae and lower QOL scores compared with a control group were reported following EV meningitis. These data are important to continue to inform cost-effectiveness strategies for prevention, and clinical guidelines about follow-up and support for these children.

These data including detailed knowledge of the current aetiology of childhood meningitis in the context of substantial historical changes in epidemiology, extensive analysis of clinical and laboratory features of meningitis of different aetiologies and investigation of a clinical decision model using contemporary prospective data, and the study of outcomes and quality of life following meningitis, could inform priorities for research and vaccine implementation, and clinical guidance particularly regarding identification and management of suspected viral meningitis cases, and ultimately improve the care of children with meningitis.

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