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ACUTE ENCEPHALITIS IN CHILDHOOD: Clinical Characteristics and Outcome - with Special Reference to Tick-Borne Encephalitis

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ABSTRACT

Acute encephalitis is relatively uncommon but potentially devastating. The prognosis varies from complete recovery to severe sequelae or death. The diagnosis is difficult to establish and the etiology often remains unclear. Furthermore, the long-term prognosis of acute encephalitis in children is poorly described and prognostic markers in the acute phase are lacking. In this thesis, the aim was to characterize acute encephalitis in childhood in regard to etiology, clinical presentation and long-term outcome. Another aim was to study biomarkers in CSF of children with TBE and to identify markers predicting the long-term outcome.

Methods: We retrospectively studied the medical records of all children with acute encephalitis at Astrid Lindgren Children´s hospital in Stockholm during 2000–2004 (study I). This cohort was followed-up 3-8 years later using 2 questionnaires grading persisting symptoms and every day functioning. Seventy-one children were eligible for the follow-up (study II). In study III, 55 children with TBE in 2004-2008 were evaluated 2-7 years later. Two questionnaires were used grading persisting symptoms and executive functioning in everyday life. General cognitive ability was also investigated in a subgroup, using WISC-IV. In study IV, cytokines, chemokines and markers of neuronal damage were analyzed in CSF samples from 37 children with TBE in 2004-2010, using a multiplex assay.

Results: The etiology of childhood encephalitis was dominated by TBE, EV, VZV, influenza virus and RSV, but remained unknown in 52% of cases. Fever and encephalopathy were present in a majority of the children, seizures or focal neurological deficits were seen in 40%. EEG-examinations were pathological in 90%, and 55% of the children with acute encephalitis had pleocytosis. There were no mortalities, but 60% of the children had persisting symptoms at the time of discharge. In the long-term follow-up, persisting symptoms were reported by 54%, both in children with and without symptoms at discharge. Cognitive problems and personality changes were the dominating complaints. No other prognostic marker than the severity of the encephalitis leading to admission to the PICU was found. All children with encephalitis who made a full recovery did so within 6 to 12 months. In the acute phase of TBE, fever and headache were present in nearly all children, whereas seizures and focal neurological findings were less frequently seen. Nearly half of the children with TBE were classified as meningitis and the clinical picture were in these cases often unspecific and symptoms of CNS involvement vague. All children with TBE had pleocytosis. At follow-up, 69% experienced persistent problems; headache, fatigue and cognitive problems dominated. On more detailed tests of cognitive functions, problems with working memory and executive functioning were seen both in children with mild and with severe symptoms in the acute phase. In TBE, children with problems at follow-up had significantly higher levels of IFN-γ, IL-4, IL-6 and IL-8 in CSF than children with a good outcome. Markers of neuronal damage did not discriminate between children with good or poor outcome.

Conclusion: We conclude that the etiology of encephalitis among Swedish children is at large the same as in other European countries endemic for TBE. EEG examination and analysis of CSF are essential for diagnosing CNS involvement, especially since CNS symptoms may be vague. Persisting symptoms were present in a substantial number of children at long-term follow-up, cognitive problems, headache, personality changes and fatigue dominated. The clinical picture in the acute phase cannot be used to predict outcome. In TBE, high levels of IFN-γ, IL-4, IL-6 and IL-8 in CSF might indicate a risk of incomplete recovery. All children with acute encephalitis or TBE should be offered follow-up evaluations in order to detect long-term sequelae.

LIST OF SCIENTIFIC PAPERS

I. **Fowler A**, Stodberg T, Eriksson M, Wickstrom R. Childhood encephalitis in Sweden: etiology, clinical presentation and outcome. Eur J Paediatr Neurol. 2008; 12(6):484–490.

II. **Fowler A**, Stodberg T, Eriksson M, Wickstrom R. Longterm outcomes of acute encephalitis in childhood. Pediatrics. 2010; 126: e828-35.

III. **Fowler A**, Forsman L, Eriksson M, Wickstrom R. Tick-borne encephalitis carries a high risk of incomplete recovery in children. J Pediatr. 2013; 163(2):555-60.

IV. **Fowler A**, Ygberg S, Bogdanovic G, Wickstrom R. Biomarkers in CSF in children with TBE – association with long-term outcome. Manuscript

CONTENTS

LIST OF ABBREVIATIONS

ADEM	Acute disseminated encephalomyelitis
AHLE	Acute hemorrhagic leucoencephalopathy
APC	Antigen Presenting Cells
BBB	Blood Brain Barrier
BRIEF	Behavior Rating Inventory of Executive Function
CEP	California Encephalitis Project
CI	Confidence Interval
CMV	Cytomegalovirus
CNS	Central nervous system
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CT	Computer Tomography
DC	Dendritic cells
EBV	Epstein Barr Virus
EEG	Electroencephalography
EV	Enterovirus
GCS	Glasgow Coma Scale
HHV-6	Human Herpes Virus 6
HSE	Herpes Simplex Encephalitis
HSV	Herpes Simplex Virus
JEV	Japanese Encephalitis Virus
MMR	measels, mumps, rubella
MRI	Magnetic Resonance Imaging
NK-cells	Natural Killer cells
NMDAr	N-methyl-D-aspartate receptor
PICU	Pediatric Intensive Care Unit
PLEDs	Periodic Lateralized Epileptiform Discharges
RHIFUQ	Rivermead head injury follow-up questionnaire
RPQ	Rivermead Post concussion symptoms questionnaire
RR	Relative Risk
RSV	Respiratory Syncytial Virus
TBE	Tick-borne encephalitis
TBEV	Tick-borne encephalitis Virus
TBI	Traumatic brain injury
VZV	Varicella Zoster virus
WBC	White blood cells
WISC-IV	Wechsler Intelligence Scale for Children, 4th edition
WM	Working memory
WNV	West Nile Virus

1 INTRODUCTION

Infections in the central nervous system (CNS) are relatively uncommon but potentially devastating. The outcome often relies on prompt start of antibacterial and antiviral treatment. However, identifying a child with a CNS infection is not always easy. Every day pediatricians meet children at the pediatric emergency department presenting with complaints such as fever, headache, nausea, lethargy, feeding difficulties or seizures. Symptoms that are unspecific and seen in a number of conditions as well as in CNS infections. A challenge is, that although CNS infections are relatively rare, the symptomatic picture compatible with a potential CNS infection is not. Acute CNS infections are most often caused by bacteria or virus, but can also be caused by fungi and parasites or be mediated by autoimmune mechanisms. Seriuos bacterial CNS infections such as meningitis casued by Heamofilus influenza or Streptococcus pneumoniae have become more rare in many countries after the introduction of childhood immunizations directed at these pathogens. However, a feared condition with high mortality and morbidity, if left untreated, is encephalitis caused by herpes simplex virus, type 1 (HSV-1). In CNS infections, the etiology varies between geographical regions as well as with age and remains unknown in a large proportion of cases. [1-4] Already in 1924, aseptic meningoencephalitis was described by pediatrican Arvid Wallgren and it became known as Wallgrens disease. The difficulties in confirming the etiology were discussed in many papers and it still remains a challenge, even today. [5]

The long-term outcome after encephalitis in childhood varies from death or severe sequelae to full recovery. The prognosis seems to vary to some extent with the etiology and agents that cause necrosis or vasculitis often have a worse outcome, e.g. HSV-1. The long-term outcome after encephalitis caused by other etiologies is less well investigated. Moreover, the outcome measures used in studies on childhood encephalitis are usually death or severe sequelae and mild to moderate symptoms are seldom described. In adults, neuropsycological sequelae are often observed following CNS infections and whether this is also the case in children needs to be elucidated further. [6, 7]

Currently, there are no prognostic markers, other than some etiologies (e.g. HSV-1), that can predict the risk of developing severe illness or incomplete recovery after acute encephalitis. Furthermore, the pathogenesis such as viral entry into the CNS and the mechanism behind the neuronal damage is often poorly understood. Many cases of acute encephalitis where the etiology is identified are caused by common viruses that most of us are exposed to frequently during our lifetime. Why some individuals develop severe complications, such as encephalitis, when encountering these viruses is unknown, but genetic differences in our immune responses have been suggested.

This thesis will focus mainly on acute encephalitis in the pediatric population and will address the etiology, clinical picture and outcome after encephalitis in childhood in Sweden, with a special reference to encephalitis caused by Tick-borne encephalitis (TBE) virus.

1.1 ACUTE ENCEPHALITIS IN CHILDHOOD

A CNS infection can involve different anatomical regions and is referred to accordingly, *meningitis* - involving the meninges, *encephalitis* - involving the brain parenchyma, *myelitis* – involving the spinal cord, *radiculitis* – involving the nerve roots and *neuritis* – involving peripheral nerves. There is often an overlapping involvement of several areas and terms such as meningoencephalitis or encephalomyelitis are sometimes used. Most cases of acute encephalitis are attributed to be viral, but other infectious causes have to be considered. Also, many non-infectious differential diagnoses give rise to symptoms that resembles those seen in acute encephalitis. For example, trauma, intoxication, intracranial tumor, stroke or metabolic disorders with acute incompensation during infection can cause symptoms similar to those seen in encephalitis.

1.1.1 Clinical symptoms

In many children with CNS infection, a prodromal period of infectious symptoms from the upper respiratory tract or gastrointestinal tract precede the onset of CNS symptoms [8-11]. In the case of encephalitis caused by enterovirus (EV) or TBE the clinical course is often biphasic. Most children with acute CNS infection will present with fever or a recent history of fever, but not all. [8, 10-12] If there is no rise of body temperature at presentation, the history of a recent infection can easily be missed if a thorough history is not taken. The symptoms in acute encephalitis are to a certain degree age specific with more unspecific symptoms in young children. Together with fever, other frequently reported symptoms in CNS infections are headache and nausea, seen in infections both of bacterial and viral etiology, as well as in meningitis and encephalitis. [8-11] Nuchal rigidity and sensitivity to light or sound are sometimes seen and will strengthen the suspicion of CNS infection. However, absence of e.g. nuchal rigidity cannot be used to rule CNS infection out since it is often missing in children. In infants, a tense and bulging fontanelle can be a symptom of increased intracranial pressure, but is also an unspecific sign. [13, 14]

To differentiate between isolated meningitis and encephalitis at the first assessment is often not possible. In the case of encephalitis, involvement of the brain parenchyma is usually more profound, compared to in aseptic meningitis. A varying degree of encephalopathy is often present with depressed consciousness, disorientation or behavioral changes. Focal neurological deficits are sometimes seen with hemiparesis, ataxia, change in muscular tone, tremor or dysphasia, often mimicking symptoms of intracranial vascular lesions or tumor cerebri. Seizures are often seen in the acute phase of encephalitis in children. In a study of young children, aged 2-23 months with herpes simplex encephalitis (HSE) all developed seizures at some point during the acute phase [15]. Whereas in encephalitis caused by other etiologies, such as TBE, seizures are more seldom seen [16]. The symptoms of encephalitis are often unspecific and there is not one single test or examination that on its own can confirm the diagnosis. Therefore, taking a thorough medical history and clinical examination of the child is very important. The younger the child, the more difficult it is to establish the degree of CNS involvement. [13, 15, 17] For example, lethargy, irritability or feeding problems might be the main

symptoms of CNS infection in an infant, but can likewise turn out to be due to dehydration, low energy intake, pain or high fever. It has been shown that even in severe encephalitis, symptoms might initially be unspecific with no obvious neurological symptoms and with normal cerebrospinal fluid (CSF) findings at onset of the disease [17].

Some viruses have a predilection of infecting certain anatomical regions in the CNS. Depending on the localization of the lesions, the symptoms will vary. For example, involvement of the cerebellum will result in ataxia or hypotonia, lesions in cerebral cortex might present with hemiparesis of the contralateral extremities. Limbic encephalitis is often dominated by psychiatric symptoms, memory problems and seizures. In the case of brainstem involvement, the autonomic regulation may be affected with changes of heart rate, blood pressure and breathing pattern. It is therefore important to thoroughly and repeatedly evaluate vital parameters and the level of consciousness since there is a risk of rapid deterioration.

1.1.2 Diagnostic considerations

As mentioned, a thorough clinical evaluation of symptoms and medical history is essential in a child with suspected encephalitis. A broad approach is often needed initially to cover several, potentially dangerous, differential diagnoses. Laboratory work-ups should aim at finding treatable conditions and rule out other medical causes, such as hypoglycemia, electrolyte disturbances, metabolic disorders and intoxications, as well as trying to establish the etiology of a possible CNS infection. Lumbar puncture should be performed early unless there is a strong suspicion of raised intracranial pressure. Pleocytosis with mononuclear dominance is often seen, but normo-cellular findings occur, especially if the lumbar puncture is performed early or late in regard to when the CNS symptoms developed. [3, 13, 14, 18] In encephalitis caused by EV or HSV a polynuclear dominance can be seen, particularly in an early phase, making differentiation against bacterial infection difficult. [2, 13, 19] As a help, glucose and lactate levels in the CSF are often normal in viral encephalitis, but an increased albumin level may be seen. In HSE, hemorrhagic pleocytosis is sometimes observed [1, 2, 13]. A diagnostic dilemma, when the CSF examination is normal, are cases of other febrile infectious diseases that can also present with encephalopathy without inflammation in CNS. However, the absence of pleocytosis cannot be used to rule out acute encephalitis, but has to be interpreted together with other findings, and repeated examination might be necessary.

1.1.2.1 Identifying the etiology in childhood encephalitis

A detailed history of recent travel, vaccinations, prodromal symptoms or biphasic course are important clues in trying to establish the etiology. A confirmed etiological diagnosis relies on identifying a pathogen in the CNS. Brain biopsies are seldom performed, unless post-mortem, and analysis of the CSF is the standard method of choice. The microbiological testing often includes antigen detection in CSF by PCR for HSV-1, HSV-2, varicella zoster virus (VZV), EV and intrathecal antibodies against Borrelia Burgdorferi in endemic areas. The etiological test battery used will vary between geographical regions and also between seasons. As it is often not possible to rule out a bacterial infection,

Table 1. The frequency of findings and reported symptoms at presentation in acute childhood encephalitis of both known and unknown etiologies

Symptom	Frequency	Reference
Fever	56-100%	Le VT et al, 2010 [12] - children Galanakis E, et al 2009 [8] - children Iff T, et al 1998 [10] - children Granerod J, et al 2010 [11] - adults and children
Headache	45-60%	Iff T, et al 1998 [10] - children Granerod J, et al 2010 [11] - adults and children
Seizures	14-74%	Iff T, et al 1998 [10] – children Wang IJ, et al 2007 [9] - children Le VT et al, 2010 [12] - children Galanakis E, et al 2009 [8] - children Granerod J, et al 2010 [11] - adults and children
Nuchal rigidity	23-29%	Wang IJ, et al 2007 [9] Le VT et al, 2010 [12] - children Granerod J, et al 2010 [11] - adults and children
Altered consciousness	34-76%	Iff T, et al 1998 [10] – children Wang IJ, et al 2007 [9] - children Galanakis E, et al 2009 [8] – children
Focal neurological findings, including ataxia	25-35%	Galanakis E, et al 2009 [8] – children Wang IJ, et al 2007 [9] - children Le VT et al, 2010 [12] - children Granerod J, et al 2010 [11] - adults and children
Symptoms from gastrointestinal tract	21-48%	Iff T, et al 1998 [10] – children Wang IJ, et al 2007 [9] - children Galanakis E, et al 2009 [8] - children Granerod J, et al 2010 [11] - adults and children
Symptoms from respiratory tract	20-50%	Galanakis E, et al 2009 [8] – children Wang IJ, et al 2007 [9] - children Granerod J, et al 2010 [11] - adults and children
Findings	**Frequency**	**Reference**
Pleocytosis in CSF*	39-99%	Iff T, et al 1998 [10] – children Wang IJ, et al 2007 [9] - children Galanakis E, et al 2009 [8] - children Granerod J, et al 2010 [11] - adults and children
Encephalitic changes on CT	18-30%	Galanakis E, et al 2009 [8] – children Wang IJ, et al 2007 [9] - children Granerod J, et al 2010 [11] - adults and children
Encephalitic changes on MRI	41-60%	Galanakis E, et al 2009 [8] – children Wang IJ, et al 2007 [9] - children Granerod J, et al 2010 [11] - adults and children
EEG changes	43-83%	Galanakis E, et al 2009 [8] – children Wang IJ, et al 2007 [9] - children Granerod J, et al 2010 [11] - adults and children

* pleocytosis > 5 x 10^6 WBC/mL

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culture and PCR for bacterial antigen are usually performed simultaneously. Even if the sensitivity of microbiological tests have improved with the PCR technique, antigen testing can be falsely negative early or late in the illness. [13, 14, 18] This is especially important in HSE, where a new analysis of CSF from a repeated lumbar puncture might be needed before acyclovir treatment is suspended. Unfortunately, a pathogen is seldom identified in CSF and etiological analyses are often performed on samples from feces, respiratory tract or serum, depending on presenting symptoms, season, presence of epidemics and vector borne diseases. Identification of a pathogen from another location than CSF has to be interpreted cautiously and together with other findings since it might not be related to the current infection at all. In reality, it is often from these locations that a possible pathogen is identified, if identified at all.

In large parts of central and Eastern Europe, TBE virus (TBEV), an arthropod-borne virus (arbovirus) transmitted by ticks to humans, is a frequent cause of meningoencephalitis in both adults and children. In areas endemic with TBEV, etiological analyses should also include analysis for TBE. [20, 21] Even in studies where extensive etiological testing is performed, the etiology remains unknown in a majority of cases with acute encephalitis. [10, 14, 22, 23] With the development of more sensitive analyses, such as real time PCR, even small amounts of viral antigen can be detected. It is therefore surprising that the number of confirmed etiologies have not increased. The California Encephalitis Project (CEP) was set up in 1998 in order to prospectively describe encephalitis cases in all ages in California, in regard both to etiology and clinical characteristics. Even with a more extensive etiological screening, the etiology could only be established in around 40% of cases. [23] The same figure was seen in a prospective study of childhood encephalitis from the Hospital of sick children in Toronto in 1994-95 [22]. Other studies show similar results with many cases of encephalitis with unidentified etiology [24, 25]. This might be due to the fact that we do not screen for pathogens not yet identified to be neurotropic, or samples are taken at the wrong time point when the pathogen have been cleared and antigen is no longer detectable in CSF. Another explanation is that direct viral entry is less common than previously believed and autoimmune reactions account for a larger number of encephalitis cases than recognized today. Support for the latter theory is that an increasing number of cases of encephalitis with unknown etiology are found to be caused by autoantibodies against receptors or surface antigens in CNS, such as the NMDA-receptor. Indeed, in a study from one cohort in the Californian Encephalitis Project, the frequency of anti-NMDAr encephalitis surpassed that of other identified viral etiologies in cases younger than 30 years old [26]. In some cases, encephalitis is a post-vaccination or post-infectious immunological reaction and identifying a pathogen in CSF is then not possible. In these cases, CNS symptoms typically develops approximately 1-4 weeks after an infection with e.g. VZV, mycoplasma pneumoniae or measles. [2, 27]

1.1.2.2   *The place of EEG examination in encephalitis*

An EEG examination will aid in the diagnosis of encephalitis by confirming cerebral involvement. The EEG picture in acute encephalitis typically shows generalized or focal slowing of the background activity. [13, 27] However, if performed early, the EEG picture can be normal and signs of encephalitis might develop later. If the initial EEG is normal

and clinical suspicion of encephalitis remains, the EEG examination should be repeated. The EEG picture has been shown to be abnormal in 40-80% in studies of children with acute encephalitis and a deteriorating EEG picture has been shown to correlate with a worse prognosis. [8, 9, 28] In HSE, typical EEG changes with temporal abnormalities with spike and wave activity or periodic lateralized epileptiform discharges (PLEDs) are sometimes seen. In these cases the suspicion of herpes virus etiology is strengthened, although these changes can be seen in other conditions.[18] A pathological EEG picture with generalized slowing of the background activity is not specific for encephalitis as other conditions, also causing encephalopathy, might show a similar picture. Apart from confirming CNS involvement, the EEG examination will also reveal epileptic activity and EEG examination is important to rule out non-convulsive status epilepticus in individuals with depressed level of consciousness or severe confusion. [2] How long the EEG changes will persist for in acute encephalitis is not known, but clinical recovery may precede normalization of the EEG. In children with TBE, the EEG-picture was found to still show a significant slowing of background activity one year after the acute illness, but these changes could not be correlated with the outcome [29].

1.1.2.3   *Neuroimaging as a diagnostic tool in encephalitis*
Neuroimaging is often performed at some point in an encephalitic illness. In the early assessment a computer tomography (CT) is sometimes needed to rule out intracranial hemorrhage, stroke or large space occupying lesions such as tumors or abscesses. However, the CT is less useful in detecting inflammatory changes and a magnetic resonance imaging (MRI) should be the method of choice. A MRI is essential both in order to visualize encephalitic changes but also to differentiate against other neuroinflammatory conditions, such as acute disseminated encephalomyelitis (ADEM). In encephalitis, the MRI picture may be normal in the early phase and a serial examination is sometimes needed [1, 2]. Performing MRI in children is complicated by the fact that general anesthesia often is necessary during the examination. Although not specific for HSV-1, the MRI picture may shows inflammatory lesions or necrosis in the fronto-temporal regions in HSE. [1, 18, 30] Other viruses also have a predilection of causing lesions in certain anatomical regions in the brain. For example, in infections with arboviruses such as West Nile virus (WNV) and TBEV, lesions are often visualized in deep grey matter in thalami, basal ganglia and in cerebellum, and EV 71 has been shown to cause lesions in the brainstem [30-32].

1.1.3 Treatment options
The treatment options in acute encephalitis are unfortunately few. Supportive treatment is essential for all, with correction of fluid and electrolyte balance, anticonvulsive drugs and parenteral feeding, when needed. In brainstem involvement close monitoring and support of vital functions are important. Intravenously (iv) administered acyclovir is the drug of choice when HSE cannot be ruled out. Early start of treatment is essential and should not be delayed until microbiological results are complete. Children with suspected CNS infection will often receive both acyclovir and iv antibiotics simultaneously awaiting the microbiological results. Acyclovir is not only used in suspicion of HSE but also used in

severe cases of encephalitis or vasculitis caused by VZV [2, 3, 13, 14]. A clinical dilemma is often when or whether the acyclovir treatment can be discontinued. In HSE, CSF taken in close proximity to the start of neurological symptoms is known to be negative for HSV in approximately 5 % of patients [13-15]. If lumbar puncture was performed early (< 3 days) after onset of neurological symptoms, a repeated lumbar puncture should be performed for new PCR testing for HSV, before terminating acyclovir treatment [2, 18, 19]. Guidelines on when acyclovir treatment can be suspended in adults exists and Thompson et al [13] and Kneen et al [19] have recently suggested a similar algorithm for when acyclovir treatment can be suspended in children. Other antiviral therapies against, for example EV, EBV and CMV are under development, but are not routinely used in encephalitis in immunocompetent children [2, 13]. Indeed, the development of new antiviral or immune-modulatory treatments are much sought after in encephalitis. The immune response in CNS is probably causing neuronal damage and scarring in encephalitis, and using immunotherapy might seem like an appealing treatment option. However, administration of immune-modulatory medication may prove to be detrimental if viral replication is still ongoing. In autoimmune or antibody-mediated encephalitis e.g. ADEM, acute hemorrhagic leukoencephalitis (AHLE) and anti NMDA-receptor encephalitis immunotherapy already has an important place in the medical management. [33]

Prevention with vaccines exists for several of the viruses known to have the potential to cause encephalitis, such as Japanese encephalitis virus (JEV), TBE, VZV, measles and mumps and plays an important preventive role.

1.1.4 Outcome

After introduction of acyclovir in the treatment of HSE, the prognosis has changed dramatically from a mortality of 70% to approximately 20-30%. Even so, a large proportion of children suffer from severe neurological sequelae following HSE. Common residual symptoms are learning difficulties, speech and hearing deficits and epilepsy. [15, 17, 34] In the last decade there have been outbreaks, mainly in Asia, of severe encephalitis in children caused by EV 71, JEV and influenza, with high mortality and sequelae in survivors [12, 35, 36]. Encephalitis of other etiologies or unknown causes are described to have a more favorable outcome with lower mortality and less number with severe neurological sequelae [8, 28, 37]. In areas where TBE is endemic, it is well recognized that approximately one third of adult cases will not recover fully. The main complaints are fatigue, headache, cognitive deficits and persistent paresis. [6] Indeed, in adults with acute encephalitis, neurological and neurocognitive sequelae are frequently observed. [7] The situation in the pediatric population is less well studied and most studies of the long-term outcome after encephalitis in children focus on mortality or severe neurological sequelae. However, there are a few studies indicating that problems after encephalitis in children might be greater than previously recognized. [9, 29, 38] There are difficulties in studying cognitive sequelae after CNS injury in children compared to in adults. The nervous system of a child is under constant development and it is difficult both to detect and assess mild to moderate deficits that might not be apparent until higher demands are

put on the child's cognitive abilities. A child might also have more difficulties in expressing memory problems or other cognitive deficits compared to adults. Moreover, adults usually have a defined level of functioning and a change in the ability to perform or participate in everyday life will often be obvious.

The severity of acute encephalitis varies between individual cases and risk factors for developing severe acute illness have been studied as well as risk factors for death or an incomplete recovery. Yet, outcome after childhood encephalitis seems difficult to predict. Young age, seizures at presentation, deteriorating EEG picture, large lesions on neuroimaging and low Glasgow coma score (GCS) have been suggested to correlate with a poor prognosis, but results differ between studies. [16, 28, 36, 39, 40]

1.1.5 Neuropathogenesis

The route of entry into CNS differ between different viruses and for a large proportion of viruses these mechanisms are still unknown. It is thought that the virus can enter the CNS either via hematogenous, neuronal spread or both. Via the hematogenous route, the virus can enter the CNS either as a free virus, e.g. WNV, or as a virus particle in infected immune cells that passes the blood brain barrier (BBB), e.g. HIV. In the neuronal route, the virus is transported by retrograde axonal transport in the nerve from the periphery (e.g. rabies) or from nerve ganglions (e.g. VZV, HSV) into CNS, thus escaping recognition by the immune system. [41] Once within the CNS, the viral cell tropism will determine both the immune response and localization of the subsequent inflammation and neuronal damage. Some viruses will infect mainly neurons, others have the ability to infect other resident CNS cells such as microglia or astrocytes. In some etiologies, such as TBE, it has been shown that a high level of viremia is a prerequisite for viral entry into CNS. [41-43] Inflammation is an important issue in the neuropathology seen in encephalitis. The inflammatory response is vital for a functional immune defense but is also potentially causing neuronal damage. The level of inflammation or the persistence of inflammation after viral clearance might correlate with outcome after encephalitis. At present, little is known of how the inflammatory reaction is regulated in CNS infections.

1.1.6 Genetic susceptibility

Why a few individuals develop severe complications, such as encephalitis, when infected with some of our most common viruses is unknown. However, there are studies showing genetic differences in our immune responses to viral infection that increase the susceptibility to develop CNS infection. Impaired function of interferon signaling have been shown to be a risk for developing encephalitis caused by HSV-1. Casrouge et al described an autosomal recessive genetic deficiency in the UNC-93B protein in two children with HSE, resulting in impaired cellular interferon responses to viral infection [44]. Furthermore, mutations in the Toll-like receptor 3 (TLR3) have been found in children with HSE in other studies [45, 46]. The TLR3 is an intracellular receptor and stimulation leads to the production of inflammatory cytokines and type 1 interferons, an important function of the innate immune system. In contrast, a functional TLR3 was associated with increased risk of severe infection in adults with TBE [47]. Differences in

the mechanisms behind the development of encephalitis between viruses most likely explain these conflicting results. Moreover, mutations in the Chemokine receptor type 5 (CCR5), a receptor regulating leukocyte trafficking as well as mutations in the 2´-5´oligoadenylaste synthetase (OAS), an antiviral protein induced by type 1 interferons, have been seen in patients with severe TBE and in WNV infection. [48-50] Indeed, genetic differences in the regulation of our immune response to infections seem to play an important role in the susceptibility to develop encephalitis. This is further exemplified by the difference in outcome after encephalitis between children in Japan and Europe. In Japan, there is a 30% mortality in encephalitis caused by influenza virus whereas mortality following encephalitis caused by influenza in children in northern Europe and the US is rare. [35, 51]

1.2   EPIDEMIOLOGY

The true incidence of acute encephalitis is difficult to assess due to variations in definitions and reporting systems. Geographical factors such as climate, presence of epidemics or vectors transmitting neurotropic viruses as well as local immunization programs will also affect the incidence. In the western world, the incidence of childhood encephalitis is estimated to be 2-18/100.000 child-years with the highest incidence in young children. [13, 28, 52, 53] The cause of the encephalitis is usually attributed to be viral since a large proportion occur during, or in close proximity to a viral infection; albeit an etiological agent is only found in half of the cases, at most. A case of encephalitis can be either sporadic, as in the case of encephalitis caused by HSV, occurring during an epidemic such as the outbreaks of enterovirus 71 amongst children in Asia, or occurring in geographically restricted areas due to local arboviruses, e.g. TBE in parts of Europe or Japanese encephalitis in Asia [1, 12, 13, 20, 54]. Before the introduction of immunization programs directed at mumps, measles and rubella (MMR), these viruses caused a large proportion of childhood encephalitis cases yearly. Interestingly, studies in Finland have shown that the incidence of encephalitis has remained the same after the start of the immunization programs, but the etiology has changed. (34) A similar picture is seen in our area, with the same incidence of childhood encephalitis now compared to before the introduction of the national MMR immunization program (M Eriksson, personal communication).

1.2.1 Etiology in sporadic cases of encephalitis
1.2.1.1   *Herpes viruses*
Viruses belonging to the herpes virus family are reported to be the most frequent cause of sporadic encephalitis worldwide [1]. Encephalitis caused by HSV-1 is feared due to its severity and the high risk of mortality and sequelae. Treatment with acyclovir have dramatically changed the prognosis in HSE, but the morbidity is still high. Encephalitis can occur both in primary infection and in reactivation of HSV-1. In a study from France, 42 % of the encephalitis cases with known etiology were caused by HSV, both in adults and children, followed by VZV, a virus also belonging to the herpes virus family [55]. In multi-center studies from England and Taiwan, similar results were seen, with HSV dominating followed by VZV [11, 56]. VZV can cause a variation of neurological

complications such as acute cerebellar ataxia, meningitis, encephalitis and CNS vasculopathies. Acyclovir is used in serious cases. Other viruses from the human herpes viridae family can occasionally also cause encephalitis such as CMV, EBV and HHV-6 [2, 13].

1.2.1.2 Other neurotropic viruses

Non-polio EV are the cause of the majority of cases with aseptic meningitis and is also a known cause of encephalitis in both adults and children [3, 9, 53]. EV comprises of more than 70 serotypes with varying virulence and only a few are important in causing disease in humans. Encephalitis caused by EV is described to cause a self-limiting illness with better prognosis than HSE. However, in Asia, EV 71 has caused several large outbreaks of encephalitis with brainstem involvement and a high mortality and morbidity in children. [9, 54] Another seasonally commonly encountered virus causing encephalitis is influenza, where both type A and B have the potential to cause CNS infection. Studies have shown a high proportion of neurological complications during influenza infection in children, with a large number suffering from persisting sequelae. Serious cases were mostly encountered in the younger age groups. [35, 57] Other viruses with the potential of causing encephalitis are viruses commonly causing respiratory tract infections or gastroenteritis, such as respiratory syncytial virus (RSV), parainfluenzae, rotavirus and calicivirus. In areas without immunization program against childhood infections such as measles, mumps and rubella, these viruses are also important in causing encephalitis in children. [13, 14, 52] Not only viruses cause encephalitis, bacteria such as Mycoplasma pneumoniae, M tuberculosis, Listeria monocytogenes, Borrelia Burgdorferi and Rickettsia species are known causes of encephalitic illness in Europe and Northern America. [1, 13] Indeed, Mycoplasma pneumoniae was described to cause a majority of the cases of childhood encephalitis in Canada in 1994-1995 [22]. Other, rarer, differential diagnoses are parasitic and fungal CNS infections [1, 13].

1.2.2 Arthropod-borne viruses

The geographical variation in the etiology of encephalitis can in part be explained by differences in the presence of neurotropic **ar**thropod-**bo**rne viruses (**arbo**viruses). Arthropod-borne infections are usually transmitted via mosquitos or ticks. One of the most important arthropod-borne infections, worldwide, is Japanese encephalitis. JE is endemic in large parts of Asia and the pacific region. It has been estimated that nearly half of the world's population live in JE endemic areas. The JE virus causes approximately 50.000 cases of encephalitis and 15.000 deaths per year with a majority of cases occurring in children. [58] Most cases of JEV infections are subclinical or asymptomatic, but of those progressing to involving the CNS, mortality is high and survivors have a high risk of neurological sequelae [36, 59]. In southern Vietnam, JE is the cause of nearly 1/3 of childhood cases of encephalitis of known etiology [12]. In Asia and North America, WNV, dengue fever and California encephalitis are other examples of arthropod-borne causes of encephalitis. An arbovirus causing a large proportion of CNS infections in northern Asia and Europe is the TBE virus.

1.2.3 Tick-borne Encephalitis

TBE is endemic in large parts of Europe and northern Asia and 6-12.000 cases are reported annually from endemic areas [20, 60]. The true morbidity might be higher since mild cases will not be diagnosed. The incidence is also difficult to estimate due to variation in case definitions between countries. However, it is clear that the incidence of TBE has increased dramatically over the last two decades, with an estimated 300 - 400% increase. [21, 61, 62] This increase might in part be explained by a larger tick-population, by the spread of ticks to new areas and by increased contact between humans and ticks, as well as a higher awareness of TBE as a cause of CNS infections. Since September 2012 TBE is a notifiable disease in the European Union, making surveillance easier [63]. In Sweden, TBE cases have increased over the last decade both in children and adults despite an increasing immunization frequency against TBE [62, 64]. TBEV is transmitted by the tick species Ixodes ricinus and Ixodes persulcatus. The tick serves both as a vector and, due to its relatively long lifespan over 2 years, it also serves as a reservoir for the virus. Other important hosts and reservoirs for the TBEV are small rodents, from whom the virus can be horizontally transmitted back to the tick when the tick is feeding on a viremic animal. Transfer of virus between ticks has also been observed in ticks co-feeding on a host with no detectable viremia. The prevalence of TBE-infected ticks has been estimated to be 0.1-5% in Europe. Ticks become active in the spring when temperature reaches above 5° C and persist until the temperature falls in the autumn. In Europe, this seasonal activity of the ticks is reflected by the distribution of TBE cases over the year, with a peak in the summer months (Figure 1). [20, 43, 60, 65]
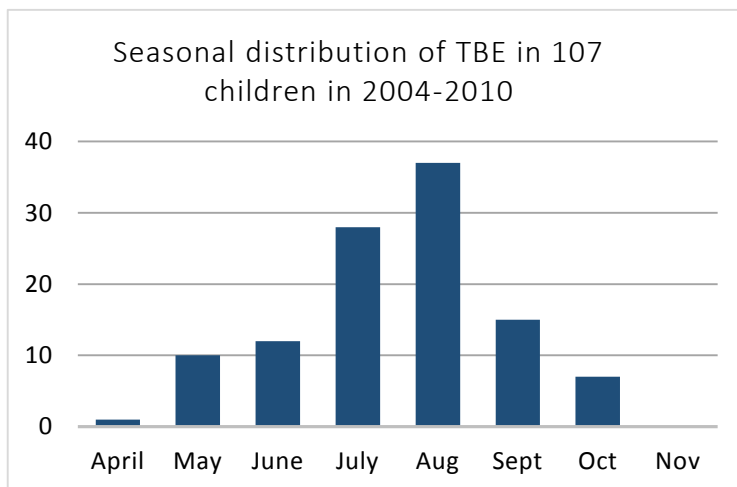

Figure 1. Seasonal distribution of childhood TBE-cases in Stockholm in 2004-2010. All reported cases occurred in April through to October. (Graph created from statistics from the local Swedish Institute for Communicable Disease Control in Stockholm, provided for study III.)

TBE virus is a positive-sense RNA virus of the Flaviviridae family, which also includes WNV, JEV, dengue and yellow fever viruses. There are three subtypes of the TBEV, European, Siberian and Far Eastern TBE strains. The course of the TBE infection seems to vary between the subtypes. An asymptomatic or subclinical illness is seen in a majority of cases with the European subtype, where it is estimated that 5-30% develop clinical symptoms. Mortality is low for the European subtype, approximately 0.5-2%. In contrast, a higher mortality is reported for the Siberian and Far Eastern subtypes, 6-8% and 20-40% respectively. Whether this is due to more virulent strains or reflects differences in reporting systems, is not clear. [20, 43, 60, 65, 66] Infection with the European TBEV usually runs with a biphasic course, after a first phase of viremia causing an influenza-like illness lasting for 4-5 days, a symptom free interval of on average 7 days will follow. In approximately 30%, the infection will thereafter proceed and varying degree of CNS symptoms develops. The severity ranging from mild meningitis to severe encephalomyelitis. It has been described that the severity of TBE-infection increases with age and approximately 30-50 % of adults do not recover fully after TBE, the main complaints are cognitive deficits, headache and fatigue and in some cases persisting paresis. [6, 66-68]

1.2.3.1 TBE in children

TBE in children is often considered a benign illness with a mild clinical course and a favorable outcome, compared to adults. In a large retrospective study from Slovenia it was reported that out of 371 childhood cases of TBE, all survived without sequelae [16]. Even so, the incidence of TBE is increasing in all age groups and there are reports of severe cases and mortality also in children [66, 69, 70]. The incidence of TBE in children has been shown to increase with age, but TBE has also been reported in children only a few months old [71-73]. The symptoms most often described in the acute phase of TBE in children are fever, headache, varying degree of meningism and fatigue. Abdominal pain and nausea is also common and there are cases described to have been mistaken for an acute abdomen which even lead to surgery. Focal neurological symptoms and severe illness causing encephalitis or myelitis seem to be less common in children compared to adults. [16, 43, 70, 74-76] Indeed, the symptoms in the acute phase of TBE in children might be vague and unspecific and the diagnosis can easily be overlooked [77, 78]. In adults, the clinical picture has been shown to correlate with the long-term prognosis [6]. Since the clinical picture generally is less severe in children it is therefore easy to come to the conclusion that TBE is a benign illness in children. However, most studies with larger cohorts examining the outcome after TBE in children have mainly looked at the short-term outcome, or mortality and severe sequelae [21, 37, 70, 75]. We know from follow-up studies on adults that cognitive sequelae, headache and fatigue, leading to a lower quality of life and sometimes inability to return to the same level of work compared to before TBE, are frequently seen [6, 68]. There are studies challenging the notion of TBE being an illness without sequelae in children. Schmolck et al showed that children performed worse on psychometric tests compared to controls and recently, Engman et al also showed significantly more problems after TBE compared to neuroborreliosis and controls [29, 38]. Records from the local Swedish Institute for Communicable Disease

Control in Stockholm, where TBE is endemic, shows that many of the TBE cases reported are identified in children and that the number of cases are increasing [64].

1.2.3.2   *Transmission and route of entry into CNS*
The TBEV is transmitted from the salivary glands of the tick at the time of the bite and removing the tick early does not protect from infection. The virus then infects dendritic cells (DC) in the skin after which they migrate to regional lymph nodes where viral replication takes place. The virus is subsequently spread through the lymphatic system and causes a first phase of viremia and systemic infection. During the viremic phase there is a hematogenic spread to other tissues, especially the reticulo-endothelial system (liver, spleen and bone-marrow) where further virus replication takes place, and the viremia is thus sustained for several days. It is during this phase that the virus causes neuroinvasive disease and it has been shown that a high level of viremia is a prerequisite for CNS entry. [43, 65, 79-81] The exact mechanisms of how the TBEV enters the CNS is not completely understood, but it is thought to be hematogenic, passing the BBB. It has been suggested that cytokines such as IL-6 and TNF released during the infection disrupts the integrity of the BBB and thus enables passage of virus into CNS [79].

1.2.3.3   *Neuropathology in TBE*
In TBE, CNS lesions are most typically seen in thalamus, basal ganglia and brainstem, when visible on MRI. Examination of post-mortem tissues from patients with TBE-infection also show changes in basal ganglia, thalamus, cerebellum, pons, medulla oblongata, brainstem and spinal cord, but all areas of the CNS can be affected. Lesions are mainly localized in the grey matter. [31]The TBEV have the capability to infect most cells in the CNS, but show a predilection for neurons. On microscopic examination, infiltration of lymphocytes are found around perivascular compartments and nerve cell necrosis, glial cell accumulation and phagocytosis of infected neurons are seen. Infected neurons can perish from either apoptosis or necrosis. [43, 65, 79, 81] Moreover, studies on post-mortem tissues have shown the presence of several virus infected neurons with intact morphology, suggesting that lytic infection is not important in neuronal cell death in TBE infection. Rather than the activation of apoptotic cascades, tissue destruction seem to be caused by microglia and cytotoxic CD8+ T-cells. [81] It has been shown that an early response with high titers of neutralizing antibodies in serum during the first viremic phase of TBE seem to be protective of CNS involvement. [82] High titers of intrathecal TBEV-specific antibodies do not correlate to disease severity, suggesting that the cytotoxic T-cell response is more important in viral clearance than humoral activity in TBE encephalitis. [42, 80, 82] The number of free virus particles is probably low in CNS and the spread of virus between cells is believed to be by direct cell-cell contact between neurons, also supporting that the T-cell response is important in reducing the viral load. [43, 79, 81]

1.2.4 New emerging viruses
New viruses causing encephalitis occasionally emerge that together with the spread of viruses to new parts of the world, affects the epidemiology. For example, the introduction

of WNV into Northern America, the spread of JEV from Japan to large areas of Asia and the spread of Toscana virus and TBE to new regions in Europe have changed the epidemiological profile of encephalitis in these regions. [83, 84] An explanation for these changes might be the spread of vectors and hosts to new areas due to climate changes. Changes in travel patterns or outdoor activities will also affect the incidence. New neuroinvasive viruses have also emerged that has not previously been known to cause encephalitic illness, such as Hendra and Nipah viruses reported from parts of Asia [85]. In encephalitis, the etiology is identified in less than 50% of cases and it is likely that there are viruses causing encephalitis that we do not screen for today.

1.2.5 Autoimmune encephalitis

In children, acute encephalopathy might develop several weeks after infection or vaccination and is then most likely an autoimmune reaction. Well known and described autoimmune encephalopathies in children include ADEM and AHLE. Recently, antibody mediated autoimmunity in CNS have been described in an increasing number of children with encephalitis of unknown etiology [26]. In ADEM, inflammatory lesions in the CNS usually develop 1-3 weeks post a viral or bacterial infection or vaccination. The symptoms in ADEM can be diverse but often resembles those of viral encephalitis. MRI is essential for the diagnosis and usually shows typical lesions located in white matter or in the grey matter of basal ganglia and thalamus. ADEM usually responds well to anti-inflammatory treatment and 50-80% of affected children recover without apparent neurological sequelae. [33] In AHLE, the CNS inflammation is more severe and there is often intracranial hemorrhage or infarction, and necrotic areas of the brain parenchyma are also seen. Even with early aggressive treatment with immunotherapy the mortality is high, in the literature described to be as high as 70%, and sequelae are common in surviving individuals. [33] Antibody mediated encephalitis was previously described as a paraneoplastic syndrome predominately seen in adults. Recently, this entity has also been described in a substantial number of children with suspected viral encephalitis. [26] The association with malignancies is less often seen in children compared to in adults. [86] In antibody mediated encephalopathy, antibodies against surface antigens such as ion channels, receptors or associated proteins in the CNS are detected in serum and CSF. Most commonly encountered in children are anti-NMDA-receptor antibodies, where antibodies against the N1-subunit of the NMDA receptor are found. [86] These patients usually present with more pronounced psychiatric symptoms, memory disturbances and movement disorders compared to other causes of acute encephalitis. The symptoms usually progress into more general cerebral involvement with depressed level of consciousness, seizures and hypoventilation, and autonomic instability may develop. [87] The treatment is immunotherapy with high dose of immunoglobulins and/or corticosteroids. Sometimes plasmapharesis is used and in cases who do not respond to first line of treatment, Rituximab or Cyclophosphamide seems to have a beneficial effect. [88] Moreover, in the last couple of years an association of HSE and anti-NMDAr encephalitis have been described in children and adults. Occasionally, individuals present with a relapse after HSE without detectable virus in CSF at the time of the relapse. In some of these cases, anti-NMDAr antibodies have been found in serum and CSF and viral

triggering of anti-NMDA receptor antibody production by the herpes virus infection has been suggested as a probable mechanism. [89]

Table 2. Some of the more commonly known and recognized causes of acute encephalitis.

Sporadic	Arthropod-borne/ Endemic	Epidemic	Bacterial	Autoimmune
Herpes simplex virus	Japanese encephalitis	Enterovirus 71	Mycoplasma pneumoniae	ADEM
Varicella zoster virus	West Nile virus	Respiratory viruses	Borrelia Burgdorferi	AHLE
Enterovirus	Tick-borne encephalitis	Rota virus	Rickettsia	Anti NMDA-r encephalitis
Influenza virus, A and B	Eastern equine virus	Calicivirus	Bartonella Henslae	
CMV	Western equine virus		TBC	
EBV	California encephalitis virus		Listeria	
HHV-6	Toscana virus			
Measles	Dengue fever			
Mumps				
Rubella				

1.3 THE IMMUNE DEFENCE

A functional immune system is crucial for our survival and is needed to fight infections from viruses, bacteria, fungi and other invading pathogens. Upon infection, the immune response consists of two different lines of defense; an early unspecific response – *the innate immune system*, and a late specific response – *the adaptive immune system*.

The innate immune response is triggered immediately upon invasion of a pathogen and leucocytes such as neutrophils, macrophages, DCs and NK-cells becomes activated. Early release of unspecific antibodies, complement factors and interferon type 1 are important to limit the spread of the infection and to aid in the activation of the adaptive immune system. The adaptive immune system is comprised of a cellular defense with the activation of cytotoxic T-cells and helper T-cells, and of a humoral response with the activation of B-cells and the subsequent release of specific antibodies. The adaptive immune response is highly specific and it will take several days from initiation to the presence of functional

effector cells. In addition to providing a specific immune response, the adaptive immune system is also essential for our immunological memory. [90-92]

In viral infections, the immune system will need to respond to both free virus particles and circulating virus infected cells. Some of the virus infected cells will be eliminated by phagocytosis or killed by NK-cells. Antigen presenting cells (APC) of the innate immune system, such as DCs, will take up free virus and present degraded viral particles in the lymphoid tissues to passing naïve CD 4+ T-cells. The naïve CD4+ T-cell will bind to the APC and with the help of co-receptor stimulation, becomes activated. The activated CD4+ T-cell proliferates and differentiates into Th1- and Th2-cells depending on the cytokine stimuli. Some of the APCs will also be infected by the virus. These infected APCs will be recognized by CD8+T-cells that, with the help of co-receptor stimulation, becomes activated cytotoxic CD8+ T-cells capable of killing virus infected cells. [41, 91, 93] Cytokines and chemokines released as a response to infection limits the viral spread and stimulates the migration of immune cells to the site of the infection. Cytokines and chemokines are also essential in the activation and differentiation of the naïve T-cells and B-cells. B-cell activation is stimulated by cytokines released by Th2-cells causing B-cells to proliferate and differentiate into plasma cells that produce a large amount of specific antibodies. [91]

1.3.1 The Immune system in CNS
The immune system in the CNS is separated from the systemic immune system through anatomical barriers such as the BBB and the lack of a lymphatic system. [94] Even if the number of effector cells within CNS are low, there are cells within CNS with immunological properties. The immune cells within CNS comprise mainly of microglia, astrocytes, neurons and oligodendrocytes. [41, 93] Little is known of the regulation of the immune response to viral CNS infection but there is an interaction between cells in CNS and the systemic immune system resulting in an influx of peripheral effector cells into CNS. Depending on e.g. type of virus, viral route of entry into CNS or cell tropism of the virus, the immune responses will differ. In the initial response to infection there is release of cytokines and interferon type 1, leading to influx and activation of immune cells, phagocytosis, apoptosis and release of more inflammatory cytokines. In CNS, it is the microglia who are the main expressors of cytokines together with invading immune cells from the periphery. The effect of this immune activation in CNS will be inflammation. Regulating this inflammatory response is essential as it can be both protective, i.e. reducing the viral load, and harmful causing neuronal death and scarring. [41, 90, 93]

1.4 BIOMARKERS OF CNS INJURY
In acute CNS infections, the extent of the tissue involvement is difficult to assess and neuroimaging in the early phase of the acute illness can often be normal. (8, 9, 11, 22) Biomarkers that reflects the degree of inflammation or tissue destruction in CNS would be very useful and aid in medical decision making. In children, serum is more accessible compared to CSF and having markers reflecting the degree of CNS injury that are measurable in serum would be ideal. However, for a marker to be useful for this purpose

it needs to be specific to CNS and not influenced by inflammation or injury outside the CNS. Indeed, increased serum levels of a CNS marker might rather reflect the degree of disruption of the BBB than correlate directly to the degree of tissue destruction within CNS. In suspected CNS infection, analysis of CSF is standard procedure and identifying biomarkers that correlates with disease severity and outcome in CSF might be more adequate than analyzing biomarkers in serum.

1.4.1 Cytokines and Chemokines

In CNS infections, several cytokines and chemokines are released when the immune system is activated. Some of these will have pro- and others anti-inflammatory properties. How the pro- and anti-inflammatory response is regulated in CNS infections are incompletely understood. However, increased levels of different cytokines and chemokines have been seen in serum and CSF during CNS infections, both in children and adults. For example, pro-inflammatory interferons, cytokines and chemokines such as IL-6, IL-8, and IFN-γ have been seen to be elevated in CNS infection caused by HSE, HHV-6, influenza- and enterovirus in children and adults. [95-98] In children, high levels of IL-6, IL-10 and TNF also correlates with a worse outcome in HHV-6 encephalopathy, Japanese encephalitis and influenza encephalitis [95, 97, 99].

1.4.2 Markers of neuronal damage

In CNS injury, whether due to inflammation, infection, trauma or degenerative processes, there might be a leakage of proteins into CSF or serum that are more or less specific to the CNS. Some of these proteins are used as biomarkers in for example Alzheimer's disease and traumatic brain injury (TBI) and elevated levels have also been seen in studies of CNS infections.

S100B is a calcium-binding protein considered to reflect astrocyte injury. A potential problem is that it is also released from other tissues than the CNS, such as adipocytes, chondrocytes and melanoma cells. High serum levels have also been seen in trauma patients without brain injury. [100-102] However, S100B have been shown to correlate with the severity of brain injury in TBI and also with outcome [100]. Apart from in TBI, S100B has also been shown to increase in CSF and serum in patients with acute CNS infections such as WNV and HSE [103-105]. Recently, S100B was also seen to correlate with mortality in acute encephalitis/encephalopathy in children [106], making it useful as a potential diagnostic marker in severe cases. It is known that the levels of S100B differ with age, and age related reference values in serum of healthy children have recently been published [107]. Tau and pTau are two proteins also used in diagnosing degenerative CNS disorders, such as Alzheimer's disease in which the levels are increased in CSF [101]. Tau is a microtubule-associated protein important for the stability of the microtubules and levels are increased in axonal degeneration [101]. Furthermore, Tau levels have been shown to increase in acute CNS injury and correlates with the outcome after TBI. In CNS infections, Tau is also increased with higher levels in cases with encephalitis compared to meningitis [101, 106, 108]. Glial fibrillary acidic protein (GFAP) is the key building block of the cytoskeleton in astrocytes and it is also involved in the maintenance of the blood-

brain barrier. GFAP is only found in the CNS, and therefore it can be regarded to be a specific biomarker reflecting CNS disease. [100, 101] Elevated levels of GFAP is considered to reflect CNS gliosis and it has been shown to increase in CNS infections such as WNV, HSE and neuroborreliosis as well as in acute encephalopathy of unknown etiology [104-106, 109].

Identifying biomarkers such as cytokines, chemokines or markers of neuronal damage that correlates with the severity and outcome in childhood encephalitis would aid in medical decision making and improve the care of the patient.

2 AIMS

The general aim of these studies was to evaluate the etiology, clinical picture and findings in the acute phase of childhood encephalitis. The aim was also to investigate the long-term outcome in acute encephalitis and in TBE in childhood, in regard to both severe and mild sequelae. We also aimed at trying to identify possible markers in the acute illness that can aid in predicting outcome after childhood encephalitis.

Paper I
The aim of paper one was to study the clinical picture in the acute phase of childhood encephalitis and to evaluate findings of the examinations performed during the acute illness. The aim was also to study the etiological panorama of childhood encephalitis in the Stockholm area in Sweden.

Paper II
The aim of paper two was to examine the long-term outcome in children with acute encephalitis and to examine if there are any prognostic signs or findings in the acute phase that can predict outcome.

Paper III
The aim of paper three was to describe TBE in children and to evaluate the long-term outcome after TBE in childhood, especially in regard to cognitive functions. The hypothesis was that TBE meningitis have a more favorable outcome compared to TBE encephalitis in childhood.

Paper IV
The aim of paper four was to study biomarkers in CSF in children with TBE and to correlate these findings with the clinical picture and with the long-term outcome.

3   METHODOLOGICAL CONSIDERATIONS

The methods used in the individual papers are described in respective paper and will not be discussed in detail in this chapter. Instead, the general outline of the papers and strengths and weaknesses of the methods used will be characterized.

3.1   ETHICAL CONSIDERATIONS

Ethical approval from the local medical ethic committee in Stockholm were received for study II, III and IV before commencing the studies. For study I, the ethical committee considered an ethical approval not to be needed. The study was considered to be quality control of medical patient care. Written informed consent was collected from all parents and from all children above the age of seven who participated in the follow-up studies.

3.2   PATIENTS

Paper I is a population-based cohort study with a retrospective design. All children (1 month-17 years old) treated for acute encephalitis at Astrid Lindgren children's hospital in 2000-2004 were included and medical records were reviewed. Astrid Lindgren children's hospital, was at the time of study I, serving a population of 220.000 children. The hospital also serves as a tertiary referral hospital for children requiring treatment at the pediatric intensive care unit. Out of 148 children with suspected acute encephalitis, 93 children fulfilled the inclusion criteria (see paper I for detailed description) and were included.

Part of paper III is also retrospective in its design. Stockholm is an area endemic for TBE and since 2004 TBE is a notifiable disease with cases reported to the local Swedish Institute for Communicable Disease Control in Stockholm. It has been estimated that 2-20% of individuals infected with TBEV develop clinical symptoms, out of whom 25-30% develop CNS symptoms. [20, 110] In order to only include children with CNS involvement, hospital charts were reviewed for all children (1 month-17 years old) with TBE in the Stockholm area in 2004-2008. Out of 86 children positive for TBE in 2004-2008, 66 children fulfilled the inclusion criteria for TBE with CNS involvement and were included (for detailed description see paper III).

Retrospective studies are always associated with problems that might affect the results, such as recall and selection bias or misclassification. In studies where hospital charts are reviewed retrospectively there is a risk of misclassification due to not all children undergoing the same investigations and assessments, and the documentation cannot be controlled for. In paper I, the incidence of childhood encephalitis is potentially underestimated and more children would perhaps have been included had they undergone more extensive testing. For example, cerebral involvement could have been confirmed if a lumbar puncture or an EEG registration had been performed in some of the children. However, the incidence was similar to what is reported in other studies on childhood encephalitis. The etiology might also have been identified in a larger proportion of children if standardized test batteries had been used. In paper III, the same problems of

possible misclassification exists due to not all children undergoing the same examinations. Furthermore, studying conditions in patients treated in hospitals serving as tertiary referral centers have a risk of overestimating the severity of an illness if milder cases are managed locally. Both study I and III are population based cohort studies, limiting the risk of selection bias. Astrid Lindgren children's hospital is a tertiary referral hospital for children needing treatment at the pediatric intensive care unit (PICU), but it is also the only center providing in-patient care for children in the northern Stockholm area. Three of the children included in paper I were not residents of the serving area but only one of these three children had been referred to the hospital due to the need of intensive care treatment, the other two children were referred from south Stockholm due to lack of in-patient beds at the time of their illness. Although associated with problems that risk biasing the results, an advantage of a retrospective design is the usefulness in studying rare conditions.

Paper II and III are long-term follow-up studies of children with acute encephalitis and children with TBE, respectively. In study II, the follow-up consisted of a structured telephone interview with parent, guardian, or in the case of the child having reached an age of 18 or more, the child was in some cases interviewed. Two questionnaires grading persistent symptoms and every day level of functioning, the Rivermead post-concussion symptoms questionnaire (RPQ) and the Rivermead head injury follow-up questionnaire (RHIFUQ), were used. A subgroup of children with severe persisting symptoms at the time of discharge from hospital and a pathological EEG examination in the acute phase were also followed-up with a new EEG registration and a computerized test of working memory (WM).
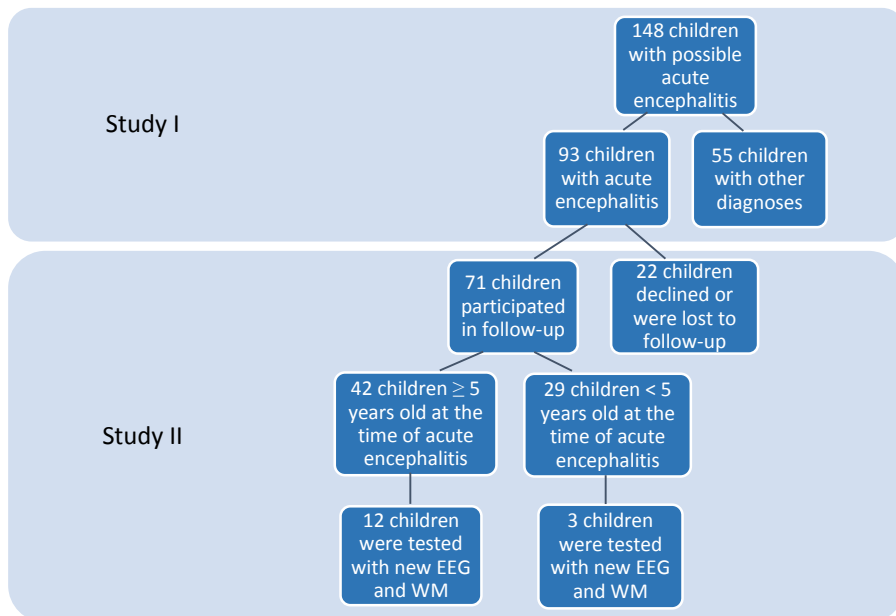

Figure 2. Flow-chart of children included in study I and II. Study II was conducted 3-8 years after the acute encephalitis.

In paper III, 66 children with TBE with CNS involvement were identified and asked to participate in a long-term follow-up 2-7 years after the acute illness. The clinical picture and findings in the acute phase were used to categorize the children with TBE into groups with mild, moderate or severe symptoms. Based on the results from study II, together with previous results from studies of TBE in adults showing a substantial number of cognitive problems following TBE, we aimed at investigating cognitive functions following TBE in children in more detail. The follow-up consisted of questionnaires grading the presence of persisting symptoms (RPQ) and executive functions in everyday life, using BRIEF (Behavior Rating Inventory of Executive Functioning). A subgroup of 10 children with mild TBE (meningitis) and 10 children with severe TBE (encephalitis) also underwent a test of general cognitive functioning, using WISC-IV. Results of the follow-up questionnaires and tests were compared between children with mild and moderate-severe symptoms in the acute phase as well as with standardized norms.
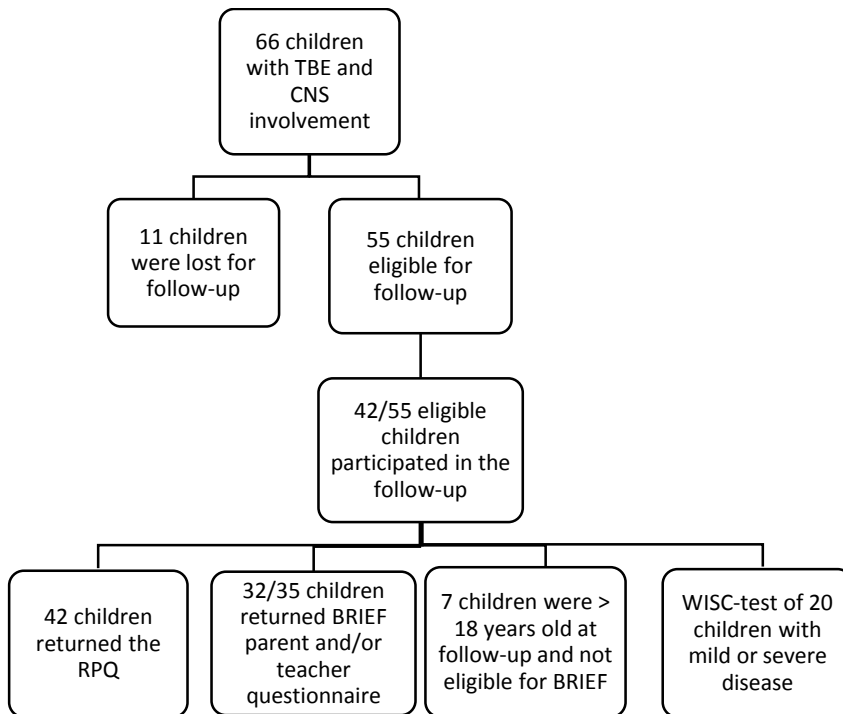

Figure 3. Flow-chart of included children in the long-time follow-up, 2-7 years after TBE, study III.

A limitation of the design of paper II and III is the lack of control groups. However, the questionnaires used in paper II (RPQ and RHIFUQ) and III (RPQ) are designed to detect a change in the presence of symptoms or functions compared to before the acute encephalitis. In a sense, the children serve as their own controls. In study III, the hypothesis was that there would be a difference between children with TBE meningitis and TBE encephalitis and comparisons were made between these groups, but no control group with healthy children was used. In study III, in addition to the RPQ, in which the children are their own controls, two other test batteries were also used, BRIEF and WISC-IV, for which there are standardized age-based normative scores to compare results with.

In study IV, we aimed at finding prognostic markers in CSF in children with TBE. All children with TBE in the Stockholm area in 2004-2008 identified in study III were included. In addition, we also included all children with TBE with CNS involvement during 2009 and 2010 in the Stockholm area, resulting in another nine children. The same questionnaires as in study three were sent out to the children with TBE in 2009-2010.

Routinely, when there is a patient with suspected CNS infection a panel of etiological analyses including HSV-1, HSV-2, EV, VZV, TBE and Borrelia is performed on CSF and/or serum. In cases with clinically mild symptoms and where the suspicion of a tick-borne illness is very strong, the clinician sometimes only perform analysis of TBE and Borrelia. CSF samples from children with TBE in 2004-2010, were retrieved from the microbiological departments at Karolinska University Hospital in Solna and Huddinge. CSF samples are stored long-term at the microbiological department only if analyses of EV and/or herpes viruses have been performed, resulting in 37 available CSF samples.

3.2.1 Definition of encephalitis

The definition used in this thesis for the term acute encephalitis is an acute onset of cerebral dysfunction, defined as encephalopathy lasting for more than 24 hours, or abnormal EEG findings in combination with abnormal neuroimaging results and/or focal neurologic findings and/or seizures. The signs of cerebral dysfunction should be occurring at the same time as signs of infection, such as pleocytosis ($> 5 \times 10^6$ WBC/mL) in the cerebrospinal fluid, fever or elevated infectious parameters (CRP, WBC or SR). In children where the symptoms could be explained by other verified cause such as bacterial meningitis or other neurological or metabolic illness were excluded. Since the diagnosis of encephalitis often relies on the results of examinations such as EEG, neuroimaging or analysis of CSF, children in whom these tests were not performed are at risk of not being included. However, in less stringent definitions children with aseptic meningitis or complex febrile seizures might incorrectly be classified as encephalitis. To compare results from different studies is often difficult due to variations in the definition of encephalitis. In many studies the distinction between infectious encephalitis and cases with septic encephalopathy is not clear and in other studies cases of acute cerebellar ataxia without encephalopathy are included.

3.2.2 Confirmed or possible etiology

The etiology was considered confirmed when a viral agent was detected in CSF. CSF samples were routinely investigated for HSV-1, HSV-2, VZV and EV in most children who underwent a lumbar puncture. In some children, CSF was further analyzed for other pathogens such as Borrelia Burgdorferi, Mycoplasma pneumonia and HHV-6 depending on the clinical picture and season of the year. A pathogen found outside CSF, such as RSV or influenza virus in nasopharyngeal sample, or rotavirus, adenovirus, norovirus or EV in feces were considered as probable etiologies. For TBE, the presence of a significant rise of TBE-specific IgM antibodies in serum was considered as current infection, for all other etiological diagnoses based on serology, a subsequent rise of IgG antibodies was also requested.

3.3 FOLLOW-UP INSTRUMENTS
3.3.1 Questionnaires
3.3.1.1 *Rivermead Post-concussion symptoms Questionnaire (RPQ)*

The RPQ was originally designed by King et al [111] to evaluate symptoms often reported after TBI. The questionnaire consists of 16 items grading the presence of symptoms following head injury compared to before the injury. The severity of each symptom is graded from 0-4 from which a total sum can be calculated. The questionnaire has shown good validity by others studying outcome after TBI [112, 113]. Questions have been raised concerning the internal validity of the questionnaire and it has been suggested that the questions should be separated into different categories rather than summarized into a total sum [114, 115]. In the present study, the total number of persistent symptoms at follow-up and their severity were analyzed and a total sum was not calculated. The RPQ has been used in studies on children after TBI and Lannsjö et al showed that it was not biased depending on age or gender and it can be used from at least the age of 6 years and upwards [115-117]. Several of the symptoms seen after TBI are also frequently reported after CNS infections. However, motor deficits, seizures, speech impairment and personality changes are symptoms often seen after CNS infections but are not covered by the RPQ, why we added four questions addressing these symptoms. Complaints and symptoms covered by the RPQ are also often seen in an otherwise healthy population [118, 119]. According to the ICD-10, three symptoms or more should be present after a TBI in order to be called a post-concussion syndrome. In adults, a post-encephalitic syndrome is described without reference to a specific number of symptoms. In study II and III, the presence of 3 symptoms or more were calculated for all children. The follow-up was performed in some cases as long as 7 years after the acute illness, obviously increasing the risk of recall bias. Nonetheless, a long-term follow up is better at identifying problems persistent over time than studying outcome close to the incident.

3.3.1.2 *Rivermead Head Injury Follow-Up Questionnaire (RHIFUQ)*

The RHIFUQ was designed to evaluate social and functional outcomes following TBI. It has been shown to correlate positively with the presence of symptoms reported on the RPQ. [112, 120] The questionnaire consists of 10 brief questions regarding the ability to participate in social and leisure activities of everyday life. Similar to the RPQ, a change

in these abilities is reported on a five-graded scale (0-4), comparing abilities now to before the acute illness.

3.3.1.3 Behavior Rating Inventory of Executive Functioning (BRIEF)

BRIEF is a questionnaire designed to evaluate a child's executive functioning in everyday activities in home- and school environment, by parents and teachers. It consists of 86 questions that are summarized into two domains and then into a total score. An age-based standard T-score is calculated that can be related to the standardization sample. The standardization sample is divided into separate norms for girls and boys, as well as into four age-groups. The BRIEF questionnaire has shown good reliability and internal consistency, but only moderate correlation between ratings by parents and teachers [121]. It is not diagnose specific but has proven useful in both developmental associated disorders and acquired neurological injuries such as learning difficulties, reading disorders, traumatic brain injury and attention deficits [122-124]. It has been shown to be able to discriminate between ADHD and non-ADHD as well as ADHD-combined type from the ADHD-inattentive type in a clinical setting [124].

3.3.2 EEG

In study II, 15 of the children with the most severe persisting symptoms at the time of discharge as well as a pathological EEG picture in the acute phase were followed up with a new EEG registration. Sixteen electrodes were used and the EEG registrations were analyzed by an experienced neurophysiologist. The previous EEG registrations from the time of the acute illness were also reviewed and compared to the new registrations from the follow-up. The EEG registrations were characterized as being normal or showing mild, moderate or severe slowing of the background activity. Any focal or epileptic activity was also described, if present. EEG changes have been shown to persist over time in children with encephalitis but whether this is prognostic is unclear [29]. During the same session, all children also performed a computerized test of working memory. Results on the EEG registration and the results on the working memory test were compared in order to investigate if a pathological EEG examination would correlate to worse results on the working memory test.

3.3.3 Computerized test of working memory

All children who underwent a new EEG recording also performed a test of reaction time and working memory, using a standardized computerized training program (RoboMemo [Cogmed Cognitive Medical Systems, Stockholm, Sweden]). Simple and complex reaction time as well as the visuo-spatial working memory were tested and compared to controls in the same age span collected from a previous study on epilepsy and working memory training (F. Edin et al, unpublished data). In the test of simple reaction time, the subject is to respond by pressing a button as quickly as possible when a circle appears on the screen. The test is performed both for the left and the right hand, separately. In the test of complex reaction time, the subject is to respond by pressing a button with the left index finger when a circle appears to the left on the screen, and pressing a button with the right index finger when the circle appears to the right on the screen. In the test of visuo-spatial

working memory, the subject is sat in front of a 4x4 grid shown on the screen. Circles will in series randomly be shown for a second one after another in either one of the 16 squares in the grid. The subject is then to mark the correct squares in the correct order as the circles were shown. The test starts with two circles and then increases in difficulty. (Figure 4.) This test has been developed for testing and training of working memory. The test have been used in several studies of working memory, e.g. in children with ADHD, acquired brain injuries and children born prematurely. Training with RoboMemo has also been shown to increase the working memory functions, but whether this actually improves learning abilities have been questioned. [125-128]
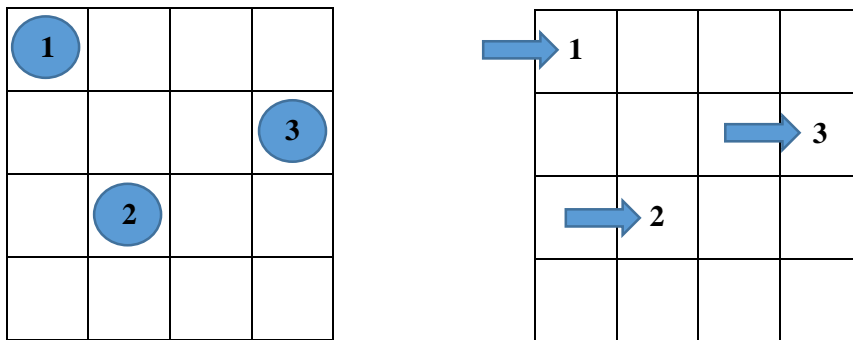

Figure 4. Computerized test of visuospatial working memory. Circles are shown in a 4x4 grid in random order and the subject are asked to mark the squares where the circles appeared, in the appropriate order. The test increases in difficulty with one circle added per trial.

3.3.4 WISC-IV

In study III, we used the Swedish version of the Wechsler Intelligence Scale for Children, 4th edition (WISC-IV), to test the general cognitive ability in 10 children with TBE meningitis and 10 children with TBE encephalitis. The WISC-IV test is an intelligence test developed for children between the ages of 6 and 16 years. The results on the WISC-IV are summarized into four domains; Verbal Comprehension Index, Perceptual Reasoning Index, Processing Speed Index, and Working Memory Index. The results are also summarized into a total IQ-score. The standardization norm is 100 with +/- 15 as one standard deviation [129]. WISC-IV is used as a clinical tool and not only as an intelligence test. In many countries it is used as one part of an assessment battery for diagnosing developmental delay, learning disabilities, ADHD etc. It can be used to show discrepancies between achievements at school and results on intelligence testing, indicating that the child might have learning difficulties. In study III, we did not have a control group of healthy children, but the WISC-IV have normative data to which results

can be compared. Comparisons were also made between children with mild and severe symptoms in the acute phase of TBE. Three of the tested children proved to have difficulties affecting the cognitive ability prior to the TBE and they were therefore excluded from further analysis.

3.4 ANALYSIS OF BIOMARKERS

Two premade multiplex assays were combined and used for detection of 16 cytokines and chemokines in CSF of children with TBE in 2004-2010. In addition, antibodies to detect four markers of neuronal damage, S100B, Tau, p-Tau and GFAP, were added to the assay. In the assay, antibody pairs consisting of a capture antibody linked to a magnetic bead and a biotin-labeled detection antibody were used for each analyte. The assay was validated for no cross-reactions between antibodies and that all pairs detected the intended protein. The multiplex assay was run according to the manufacturer's protocol using Luminex 200 (Luminex Corporation, TX). CSF samples were assessed as undiluted samples of a volume of 50 µL/sample as previous preliminary experiments showed generally low concentrations of several of the measured cytokines in CSF. Due to limitations in volume, only single samples could be run. Standards were added to provide calibration curves. The calibration curve for each analyte was calculated using the Bio-Plex software.

Multiplex assays have several advantages compared to more traditionally used ELISAs. For example, in the multiplex assay, analyses of several cytokines, chemokines and proteins of neuronal damage could be performed simultaneously even with small sample volumes. Obtaining adequate sample volumes from CSF or serum is often an issue, especially in the youngest children, and the ability to perform analysis on small volumes would improve diagnostics in the clinical setting. Several studies have compared levels of cytokines, chemokines and varying proteins in human samples between Luminex assays and ELISA, with varying results. However, many studies have shown high correlation between the two methods. [130-132] In multiplex assays, analysing duplicate samples from each individual is standard in order to detect those with high variations. Due to limitations in CSF volumes we were only able to run single tests for each child. Another factor possibly influencing the results is the sample handling. Several cytokines and chemokines are known to have short half-times, some degrading within minutes while others are more stable. Since samples were retrieved retrospectively, time to initial freezing and the number of freeze-thaw cycles for each sample could not be controlled for, possibly influencing the levels of some of the cytokines. For Luminex assays to be of clinical use, cut off values from healthy individuals need to be established.

4 RESULTS AND DISCUSSION

4.1 SYMPTOMS AND FINDINGS IN CHILDHOOD ENCEPHALITIS

During 2000-2004, 93 children were admitted to Astrid Lindgren Children´s hospital for acute encephalitis (Study I). There was a male predominance with 2/3 of the children being boys. The average age at the time of the acute illness was 7.5 years with a range between 5 weeks to 17.7 years. During the study period, Astrid Lindgren children´s hospital in northern Stockholm served a population of 220.000 children and the incidence of childhood encephalitis was 8.5/100.000, with the highest incidence among children under the age of 10 years (Figure 5). Indeed, the incidence of encephalitis has in several studies been shown to be highest amongst young children, as high as 18/100.000 child years in children under the age of one, compared to 1-2/100.000 in older children and adults [4, 24, 52, 53]. The higher incidence of acute encephalitis amongst young children might be due to their relatively inexperienced and immature immune system. In young children, the number of memory cells are low and compared to older children and adults, their ability to produce cytokines as a response to infection is less well developed. Thus, the younger children are both predisposed to viral infections and also less effective in controlling the infection, compared to adults [92].
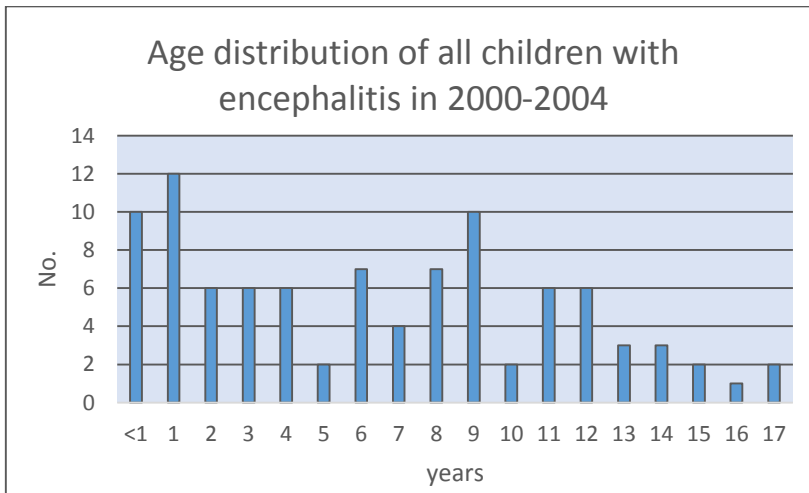

Figure 5. Age distribution of 93 children with acute encephalitis in 2000-2004, study I

In study III, 66 children with TBE and CNS involvement were identified in 2004-2008. The average age was 10.8 years with a range between 3 and 17 years (Figure 6). As in study I, there was a male predominance with 61% of the children being boys. The higher average age in TBE compared to encephalitis of all etiologies most likely reflects the

transmission route of the TBE virus, where contact with an infected tick is a prerequisite for infection [43]. However, TBE has been described to have a less severe clinical course in young children and it is possible that cases amongst the youngest children were missed [21].
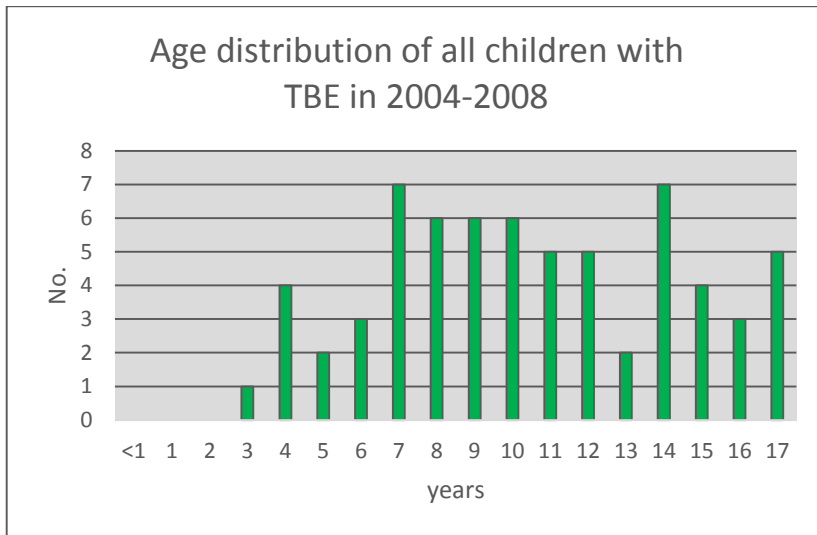

Figure 6. Age distribution of 66 children with TBE with CNS involvement in 2004-2008, study III.

4.1.1 Etiology

As in many previous studies on encephalitis, both in adults and children, the etiology could only be confirmed in a minority of patients. The number of children where the etiology was confirmed by findings in the CSF was only 8/93. However, another 8 cases caused by TBEV were identified by TBE-specific antibodies in serum. In TBE, virus is seldom identified by analysis in CSF and the identification of TBE-specific antibodies in serum is often considered diagnostic. Intrathecal TBE-specific antibodies are not routinely used in clinical practice in our area, unless in cases of suspected vaccination failure. Thus, the number of confirmed etiologies can be considered to be 17% in the present study. A possible etiology was found in 37/93 children from localizations such as nose and/or throat swabs, stool samples or specific IgM and IgG antibodies in serum. A potential problem in determining the etiology in CNS infections is when an agent is found outside CNS. Viral shedding may persist for a prolonged time, e.g. EV in feces, and the causality between encephalitis and the agent found can often be questioned. [133] Furthermore, one must bear in mind that not all cases of encephalitis will present with the symptoms usually associated with the virus. [18, 134, 135] For example, in encephalitis due to HSV-1 and VZV, reactivation is common and it is considered that only 1/3 of HSE cases in adults

occur during primary infection. Thus, symptoms of a current infection with blisters is not present in a large proportion of patients with HSE and a more unspecific prodromal period with upper respiratory tract symptoms is more often noticed. [18, 135, 136] HSV-1 and VZV have often been described to cause a majority of sporadic cases of childhood encephalitis where the etiology is established [8, 11, 55, 56]. Although VZV was one of the dominating etiologies in our study, HSV was only found in 2/93 children with encephalitis, thus contrasting to previous studies. In a study by Koskiniemi et al, the incidence of encephalitis due to measles, mumps and rubella disappeared after the introduction of national immunization programs against these viruses in Finland [52]. Similar results were seen in our study performed in an area where the number of children receiving MMR vaccination is high. However, one child who had returned from vacation in an area of known measles outbreaks developed encephalitis caused by measles after returning home. Due to young age this child had not yet received the first dose of the MMR vaccine. This illustrates the importance of taking a thorough history of vaccination status and recent travels when identifying the etiology in encephalitis. In areas where arboviruses are present, these viruses usually contributes to a large percentage of identified etiologies. Indeed, TBE is endemic in Stockholm and was found to be the leading cause of encephalitis in study I. (Figure 7)

Etiology in childhood encephalitis, 2000-2004

Other (15)*
VZV (5)
Influenza (5)
RSV (6)
EV (6)
TBE (8)
Unknown (48)

Rotavirus(3), HSV-1(2), EBV(2), HHV-6(2), Mycoplasma pneumonia(2), morbillivirus(1), Bartonella Henslae(1), parainfluenzae(1), norovirus(1)
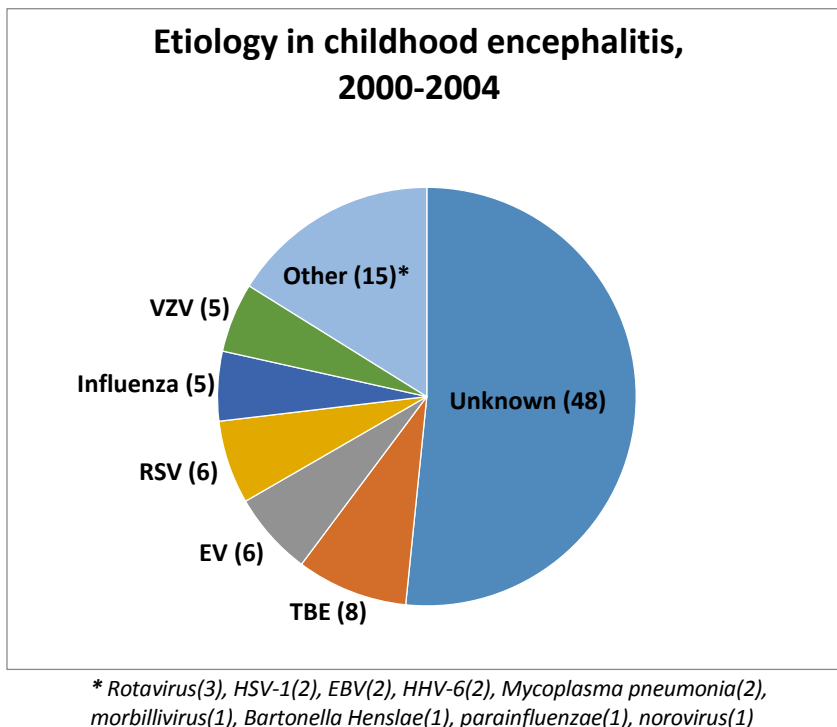
Figure 7. Proportion of the different etiologies causing acute encephalitis in 93 children with encephalitis in 2000-2004, study I. (Number of children)

There is increasing evidence of autoimmune mechanisms explaining some of the cases of encephalitis without proven etiology and in a recent study from the CEP, a large number of children with unidentified etiology was found to have antibodies directed at the NMDA receptor [26]. It has also been shown that a viral CNS infection such as HSV-1 might trigger the development of autoantibodies to CNS antigens and causing a relapse of CNS symptoms after HSE [89]. Whether other viral infections also have this potential has not been investigated. In our study, the presence of autoantibodies was not investigated.

From an epidemiological as well as medical perspective, the large number of unidentified etiologies are a concern. One would have thought that a larger percentage of etiologies would be identified with the development of improved microbiological techniques. This might indicate that autoimmune mechanisms cause a larger percentage of cases of acute encephalitis in both children and adults than previously recognized.

4.1.2 Clinical picture in the acute phase

Clinical symptoms of acute encephalitis need to include signs of recent or ongoing infection as well as neurological symptoms indicating CNS involvement. These signs are not always easily interpreted and CNS symptoms might initially be vague, especially in young children [15-17]. In study I, a majority (80%) of the children with acute encephalitis had fever or a recent history of fever at the time of presentation. Fever was also present in nearly all children with TBE infection (64/66) in study III. In contrast, fever at presentation was only seen in 56% of cases in a study by Iff et al [10].

Altered level of consciousness was seen in 80% of children with acute encephalitis in study I. Altered sensorium might be difficult to assess in a child with high fever, and even more difficult in young children, and repeated evaluations are often necessary. To carefully consider a parents concern of a child behaving differently is also important as this might be a sign of confusion or personality changes. Focal neurological findings and seizures were seen in approximately 40% of the children with acute encephalitis in study I. A similar frequency of these findings have been seen in other studies on children with acute encephalitis [9, 10, 134]. In a study by Misra et al, seizures were present more often in HSE and JEV compared to encephalitis of other etiologies [137]. In our study of children with TBE, seizures were only present in 10% of the children, confirming a difference in symptomatology depending on etiology.

In TBE, symptoms associated with meningism such as nuchal rigidity, sensitivity to light or sound and nausea were present at a higher frequency in children with encephalitis compared to in isolated meningitis. This illustrates that CNS infections often involve more than one anatomic area and that meningoencephalitis is common in TBE in children. Furthermore, children with isolated TBE meningitis often presented with vague and unspecific symptoms such as fever and headache and the CNS involvement could easily have been missed if a lumbar puncture had not been performed. Indeed, only one child with positive TBE serology in whom a lumbar puncture was performed did not have pleocytosis. In this child, CNS involvement could not be confirmed by the clinical picture or findings on other examinations and he was subsequently not included in the study. In

children with TBE, mild symptoms in the acute phase were seen in 45%, moderate in 17% and severe in 38%. (Figure 8) Previous studies have shown similar results with higher proportion of meningitis compared to encephalitis in childhood TBE [16, 66, 74]. In contrast to in adults, myelitis is seldom seen in childhood TBE, and no child had myelitis in the present study. The duration of hospitalization varied between 1-25 days (mean 6.5 days) and care at the PICU was needed for three children, confirming that TBE cause substantial morbidity and potentially even severe acute illness in some children. Similar results were previously also shown in a study by Cizman et al, where 5% of children who were hospitalized due to TBE needed treatment at the ICU [69].
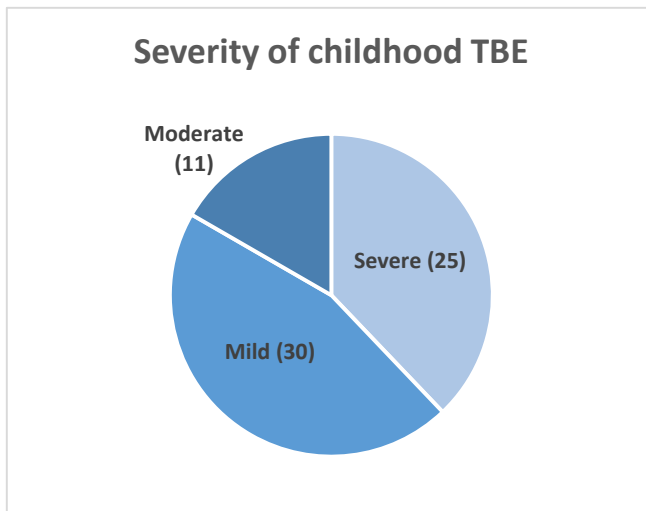

Figure 8. Proportion of children with mild, moderate and severe symptoms in the acute phase of TBE (66 children), study III.

In summary, fever and encephalopathy were present in most children with acute encephalitis and seizures or focal neurological deficits were seen in 40%. In contrast, seizures and focal neurological findings were less frequently seen in TBE, but nearly all children had headache and fever. Approximately half of the children with TBE had meningitis and the remaining had a more severe clinical picture. In children with TBE meningitis, the clinical picture was often unspecific and symptoms of CNS involvement were vague.

4.1.3 Laboratory investigations
When there is a suspicion of a child suffering from acute encephalitis, laboratory work-ups are usually taken to try to differentiate between bacterial and viral infections as well as identifying other differential diagnoses. However, none of the tests available are at all specific in discriminating between bacterial and viral infection. In the present studies serum was analyzed for CRP, WBC and SR in a majority of children, showing normal or

slightly elevated levels. More important in CNS infections is the analysis of the CSF, and lumbar puncture should be performed as early as possible. In study I, CSF was analyzed in 84/93 children and pleocytosis was seen in 55% (46/84), with a monocytic dominance in most. Interestingly, in study III, all children with TBE and CNS involvement in whom CSF was analyzed (65/66) had pleocytosis with a mean WBC count of $80.5x10^6$/mL. Indeed, pleocytosis is seen in a majority if adults and children with TBE by the time CNS symptoms develop [74, 82]. Albumin levels were mildly elevated in 60% of the children with TBE, compared to 28% of the children in study I. In encephalitis, the lack of pleocytosis might raise questions of the correct diagnose, but studies where repeated lumbar puncture have been performed shows that pleocytosis can develop over time [136, 138]. In some cases evidence of viral infection, such as viral DNA in CSF have been found even in absence of elevated WBC count [34, 139]. One child in study I were positive for HHV-6 in CSF but had a normal cell count. Furthermore, viral DNA has also been found in CSF in individuals without signs of ongoing CNS infection [140-142]. Therefore the interpretation of results from CSF analyses must be made together with the clinical picture and results on other tests, such as EEG and neuroimaging.

4.1.4 EEG

In study I, EEG examinations showed a picture compatible with encephalitis in 90% of children in whom a registration was performed. The presence of pathological changes on EEG examinations have in other studies been found in between 40-80% of children [8, 9, 22, 134]. Furthermore, a deteriorating EEG picture has been associated with worse outcome by some authors, while others have not seen a correlation between EEG findings and outcome [9, 39, 136]. Few repeated EEG´s were made in the cohorts included in this thesis. EEG examinations are also important in identifying the presence of seizure activity, especially in cases of suspected non-convulsive seizures or subtle clinical seizures. In children with TBE (study III), an abnormal EEG picture was seen in 27/28 children with moderate to severe symptoms in the acute phase. Only 5 children with mild illness underwent an EEG registration, and it was normal in all. The clinical picture in TBE is sometimes unspecific and an EEG registration might aid in determining the grade of CNS involvement. Even if a majority of the children with encephalitis had an abnormal EEG-picture with changes compatible with encephalitis, seizure activity might be missed in one single recording. Gold et al recently described seizure activity in a substantial number of children without clinical apparent seizures when continuous EEG recordings were used. [143]

In study II, a follow-up study of 71 children with acute encephalitis, 15 children with a pathological EEG in the acute phase underwent a new EEG registration 3-8 years after the acute illness. The EEG picture had improved in all children, but it was still abnormal in 9/15 children with persistent generalized slowing of the background activity. In a previous study by Schmolck et al, follow-up EEG´s in children after TBE also showed a persistent abnormal picture in 58% of children at long-term follow-up. However, the clinical importance of these changes is unclear since a correlation between results of

follow-up EEGs and psychometric tests could not be seen in our study or in the previous study. [29]

4.1.5 Neuroimaging

In order to visualize abnormalities compatible with encephalitis, MRI examination is superior to a CT scan. In study I and III, we found that many children with encephalitis never underwent any neuroimaging. In study I, neuroimaging was performed in only 58% of children with acute encephalitis and a majority of the examinations were CT-scans. Abnormalities compatible with encephalitis, such as diffuse or focal edema or ischemia were seen in 30% of the children who underwent neuroimaging, four children had an MRI picture compatible with ADEM. In children with TBE (study III) only 9/66 children underwent neuroimaging examinations, out of whom a pathological picture with inflammatory changes was seen in one child with severe encephalitis. This child needed care at the PICU for a prolonged time and subsequent inpatient rehabilitation before returning to school. In studies of childhood encephalitis, CT scan shows abnormalities compatible with encephalitis in 20-30% of cases, compared to 40-60% with MRI [8, 9, 11]. Indeed, MRI is significantly better at finding inflammatory changes early in encephalitis compared to CT-scan [144]. Thus, MRI examinations should be performed more frequently in the suspicion of encephalitis and CT examinations only to rule out other life threatening conditions [19, 144]. Unfortunately, MRI examinations are often delayed due to the need of general anesthesia, especially in the young children and some medical centers may lack the ability to perform MRI, making CT the only option.

In summary, the diagnosis of childhood encephalitis is sometimes difficult to make. Many children will present with vague symptoms, but fever and encephalopathy is often present. Other CNS symptoms such as seizures and focal neurological deficits are seen to a less degree, but when present, the diagnosis is easier to make since the CNS involvement is more obvious. An EEG-examination can often be helpful in determining CNS involvement, showing abnormalities in 90% of children with acute encephalitis. MRI was performed in a minority of the children, but is an important examination in order to visualize the extent of inflammation and to differentiate against other conditions. Not all children will have pleocytosis in CSF, but a lumbar puncture is essential also for microbiological examinations. Furthermore, in children with TBE meningitis symptoms were often vague and lumbar puncture revealed pleocytosis in all, proving it to be useful in confirming the CNS involvement. Searching for the etiology outside the CNS is important but results have to be interpreted together with clinical symptoms, other findings and current epidemiological data.

4.2 OUTCOME AFTER CHILDHOOD ENCEPHALITIS

Outcome after childhood encephalitis varies from full recovery to death or severe sequelae, with a worse prognosis in etiologies causing extensive CNS injury with necrosis or hemorrhage, e.g. HSV-1. Studies on outcome after encephalitis varies in definitions of good and poor outcome, length of follow-up as well as in the methodologies used to evaluate outcome, making comparisons difficult.

4.2.1 Short-term outcome

In study I, hospital charts of 93 children with encephalitis revealed an incomplete recovery at the time of discharge in 60% of the children and there were no mortalities. In 32 of the children symptoms were regarded as mild (fatigue, headache, mild irritability) and moderate to severe persisting symptoms such as hemiparesis, dysphasia, seizures, ataxia and moderate cognitive problems were seen in 22 children. Other studies of childhood encephalitis have shown similar results with neurological deficits in around 25% of children at time of discharge [8, 40, 145, 146]. In studies also describing milder persistent symptoms such as headache, fatigue and irritability a higher percentage of incomplete recovery is seen [22, 147]. Furthermore, in etiologies known to cause severe encephalitis, such as HSV and JEV, moderate to severe persistent symptoms at discharge are described in up to 60% of children [17, 148]. In study I, significantly more children who had focal neurological deficits at presentation had persistent symptoms at the time of discharge, compared to children with no focal neurological findings at presentation (84% and 42% respectively). Focal neurological findings at presentation was also seen to correlate with the short-term outcome in a study by Klein et al [146]. Other predictive characteristics that have been suggested are abnormal MRI, young age and etiology, especially HSV-1 [145, 146, 149]. There were no significant correlations between the presence of persisting symptoms at discharge and age, gender, or findings on any of the examinations performed during hospital stay in our study. According to the hospital data, all children with encephalitis due to RSV had made a full recovery at time of discharge. In contrast, persistent symptoms were frequently seen in children with encephalitis due to HSV-1, EBV, rotavirus, EV, TBE and VZV. In VZV, ataxia was the dominating symptom at time of discharge. It is well known that VZV infection can cause cerebellar ataxia in children [150]. In our study, children with isolated cerebellar ataxia without encephalopathy or further neurological deficits, did not fulfill the inclusion criteria and were not included.

The question whether children with persistent symptoms at discharge will make a full recovery over time or not, together with a lack of knowledge of the possible long-term deficits following childhood encephalitis lead us to perform a long-term follow-up of the cohort in study I.

4.2.2 Long-term outcome

In study II, 71/93 children participated in the follow-up that was conducted 3-8 years after the acute encephalitis. Subjective complaints of symptoms not being present before the encephalitis were seen in 54% of the children at follow-up. In children 5 years or older at the time of encephalitis, three persisting symptoms or more were seen in 40%. Problems were mainly seen with concentration, memory and sensitivity to sound. Moreover, a change in personality was seen in nearly 30 % of the children, following encephalitis. Of the children who were under the age of 5 at the time of encephalitis, nearly 30% complained of three symptoms or more at follow-up. In this age group, problems were mainly seen with concentration and a feeling of frustration. These remaining problems lead to difficulties in functioning in everyday life as compared to before the encephalitis in 30% of the children. Difficulties were mainly seen in areas such as "ability to participate

in leisure activities", "finding school more tiring" and "maintaining the same school standards" as compared to before the encephalitis.

The long-term outcome after childhood encephalitis varies greatly in different studies. Part of these variations can be explained by different study designs, definition of sequelae and time to follow-up. However, when studying mortality rates following childhood encephalitis, there seem to be great geographical variations. Higher mortality rates are reported following encephalitis caused by e.g. influenza in Asia compared to western industrialized countries [35, 151]. Furthermore, in areas where JEV is endemic mortality rates as high as 30% are reported in children [12, 36, 59]. There were no mortalities in our cohort, and low or no mortalities are also reported in childhood encephalitis from Finland, England, Greece and Canada [8, 15, 22, 149]. Following encephalitis, 10% of the children developed subsequent epilepsy in our study. Having seizures in the acute phase increased the risk of developing epilepsy 8-fold. Indeed, epilepsy following encephalitis have been described by others and is a known sequelae. Seizures in the acute phase increases the risk of post-encephalitic epilepsy up to 20-fold [9, 40, 152, 153].

No results on examinations or laboratory parameters could be used as prognostic markers for an incomplete recovery in our study. Focal neurological findings in the acute phase that correlated with problems at discharge did not correlate to the long-term outcome. An increased risk of incomplete recovery were seen in children needing treatment at the PICU. Interestingly, in a recent study, significantly more deficits in neuropsychological performance and school functioning were seen in children admitted to the PICU due to meningoencephalitis, compared to age matched controls [154]. In our study, cognitive problems such as impaired concentration and memory functions were commonly described, together with headache, fatigue and personality changes. Neurocognitive problems following encephalitis have been described both in adults and children and in a study by Ooi et al, neuropsychological and behavioral problems were seen in over half of children at follow-up. Moreover, in a study of children with encephalitis caused by EV 71, 20% were found to have problems similar to the difficulties seen in children with ADHD. [148, 155]

The search for predictive markers for the outcome in encephalitis have proven inconclusive with divergent results between different studies. Low GCS, repeated seizures, young age, and abnormalities on MRI and EEG examinations have been seen to correlate to outcome in several studies, but not in others [9, 12, 28, 35, 136, 137, 148]. However, certain etiologies are associated with a high risk of incomplete recovery, such as HSV-1, JEV, EV 71 and influenza [32, 34-36]. In study I, even viruses commonly considered to cause encephalitis with good prognosis in children, such as TBE and RSV showed a potential to cause persistent problems. Sequelae was frequently found in children with TBE, VZV and influenza. In contrast, only one of five children with encephalitis due to EV had persisting symptoms at follow-up. Two children had HSE in the present cohort, and sequelae was seen in both. Unfortunately, in approximately 50% of cases the etiology remains unknown and using etiology as a predictor is often not helpful.

In contrast to study I, where 40% were considered to be fully recovered when leaving hospital (according to medical records), only 9/71 children considered themselves to have made a full recovery at the time of discharge from hospital. This indicates that more subtle problems such as cognitive deficits, headache or fatigue might not be apparent until the child returns to his or her normal environment. In children who recovered completely, symptoms subsided within the first 6-12 months. (Figure 9) Indeed, most children seem to recover within the first 6 months, which have recently been shown in other studies where assessment at 3-6 months were predictive of the long-term outcome [40, 148].
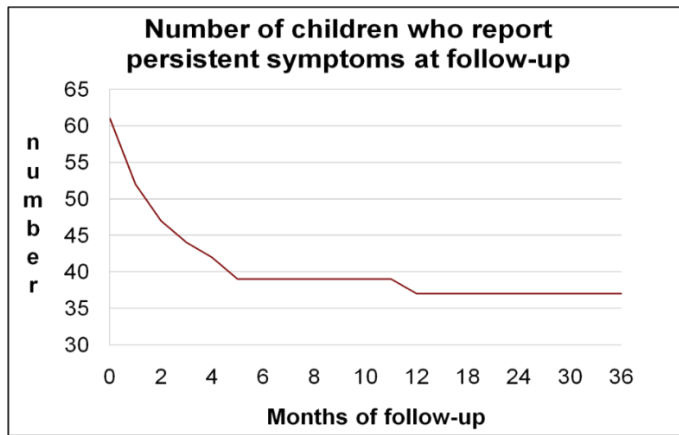

Figure 9. Number of children who report symptoms at follow-up at different time-points out of 71 children included in the follow-up study (study II).

In study III, 42 children with TBE in 2004-2008 participated in a long-term follow-up, 2-7 years after the acute illness. There were no mortalities in this cohort, but incomplete recovery was frequently seen. At follow-up, presence of three symptoms or more were reported by 29/42 children. The most frequently reported persisting symptoms were headache (25/42), memory problems (21/42), fatigue (19/42), irritability (19/42) and concentration problems (18/42). Interestingly, no difference could be seen between children with mild or moderate-severe illness in the acute phase. Furthermore, the executive functioning of children 18 years or younger at the time of follow-up (29 children) were assessed using the BRIEF questionnaire. Significant problems were seen in 30 % of the children on one or more subdomains. Problems were mainly noticed in areas of "ability to initiate problem solving or activity", "organize one's environment and materials", "sustain working memory", and "monitor one's own behavior". Again, there were no differences between children with mild or moderate-severe TBE in the acute phase. The general cognitive functioning was assessed in more detail in 20 children with the WISC-IV test. The test results showed an uneven profile with poor results on working memory. The results on the working memory index were significantly lower compared to

the standardization norm as well as to the results on the other subscales. The results were similar for children with mild and moderate-severe symptoms in the acute phase. (Figure 10)
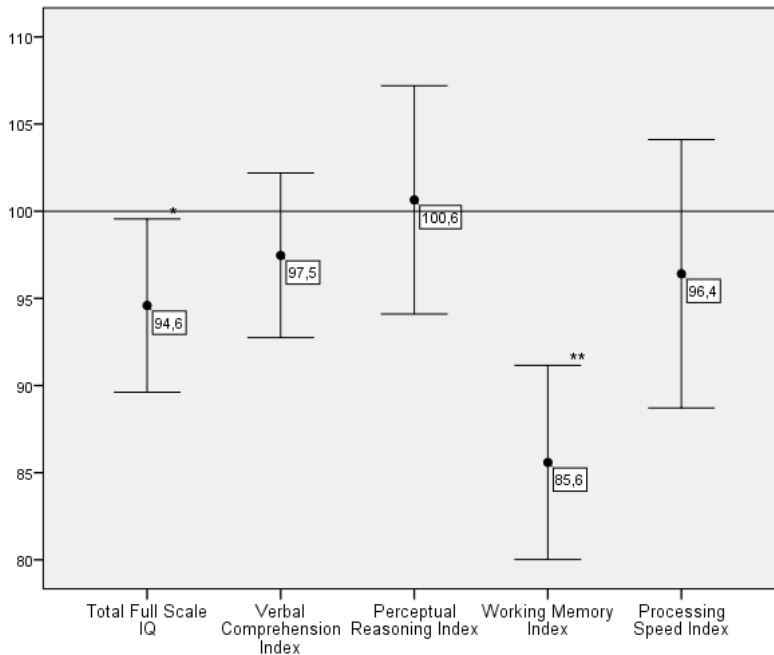

Figure 10. Full total IQ score and mean Index scores on the 4 subscales: Verbal Comprehension Index, Perceptual Reasoning Index, Working memory Index, and Processing Speed Index for 17 children with TBE. Error bars indicating 95% CI. Mean standardization Index = 100. (*P = 0.03, **P <0.001), study III.

Persisting problems are frequently seen in adults following TBE, mainly with cognitive deficits, fatigue and headache but in some cases also persistent paresis [6]. The outcome after TBE in childhood is considered to be more favorable and complete recovery have previously been reported for most children in several larger studies [16, 74, 76], even if severe cases also have been described [69, 70, 75]. Mild to moderate sequelae such as cognitive problems following TBE were not investigated in these studies. Two smaller studies on children with TBE suggests that cognitive problems may be more frequent than previously recognized. [29, 38] The present study confirm these previous findings with cognitive problems after TBE in a large proportion of children.

In TBE, young age have been associated with milder clinical course and favourable outcome. Our results show that many children with TBE have mild symptoms in the acute phase, but incomplete recovery are seen even in these individuals. Hence, the clinical picture should not be used to predict the long-term outcome. Indeed, CNS symptoms in children may be vague and more difficult to assess than in adults. Whether some children with meningitis due to TBE would have been diagnosed as encephalitis if an EEG examination had been performed is unknown. A problem with not all children undergoing the same investigations is risk of misclassification, potentially biasing the results of the present study.

Interestingly, 8/42 of the children with TBE described problems with attention, concentration, reading or learning abilities prior to the TBE. They all had high scores on the symptoms reporting questionnaire (RPQ) with 6 to 15 persisting symptoms at follow-up. Individuals with pre-existing cognitive difficulties are most likely more vulnerable when CNS functions are compromised as in CNS infection or trauma. In contrast to the results in study II, where no gender differences could be seen in regard to results at follow-up, girls had significantly more problems after TBE compared to boys. Such gender differences have also been shown after traumatic brain injury [156], suggesting a vulnerability in girls when the CNS functions are compromised, or a gender difference in symptoms reporting. Furthermore, being identified as being at risk of sequelae might affect the children or parents to report symptoms or problems not related to the TBE infection. However, the results on WISC-IV should not be biased by this and problems with working memory seems to be more frequent in TBE than previously recognized.

In summary, problems following sporadic cases of encephalitis and TBE in childhood are seen in a substantial number of children at long-term follow-up. Cognitive problems, headache, fatigue as well as personality changes in encephalitis are the dominating complaints. On more detailed tests of cognitive functions problems with working memory were seen in children following TBE. The clinical picture in the acute phase is a poor indicator of the long-term outcome, both in sporadic cases of encephalitis and in TBE in childhood.

4.3   BIOMARKERS IN CSF

Since the clinical picture in the acute phase is not useful to predict outcome after childhood TBE, other prognostic markers are needed. In other CNS infections and in traumatic brain injury, levels of biomarkers such as neuronal proteins and cytokines have been shown to correlate with the severity of the acute illness as well as with outcome in some cases [96, 100-102].

In study IV, the levels of IFN-γ, IL-6 and IL-4 correlated with the grade of pleocytosis in CSF in the acute phase of TBE, with higher levels seen with increasing number of WBCs. Pleocytosis in CSF is used clinically to investigate the presence of inflammation in CNS-infections and all children with TBE had pleocytosis. However, in encephalitis due to other etiologies, CSF findings are normal in up to 50% of cases and the CNS involvement might be overlooked. In a study of children with EV meningitis, IL-6 and IL-8 were

elevated in CSF even in the absence of CSF pleocytosis [98]. Thus, IL-6 might potentially be more useful in measuring the presence of CNS inflammation than the WBC count. In infection, young children respond with less pronounced release of cytokines and chemokines compared to older children and adults [92]. This was also seen in our study where the levels of IL-4, IL-6 and IL-8 increased with increasing age. The long-term outcome also correlated to age in study IV, with young children having a more favorable outcome. This indicates that a less pronounced inflammatory reaction in TBE infection is beneficial. Significantly higher levels of IFN-γ, IL-4, IL-6 and IL-8 were seen in children with sequelae compared to children with a good outcome also supporting that the degree of inflammation correlates to outcome after TBE infection. This has also been indicated in encephalitis in children caused by HHV-6, influenza and JEV, where high levels of IL-6, IL-10 and TNF correlated with a poor outcome [95, 97, 99].

Markers of neuronal damage, such as S100B, GFAP and Tau have been shown to be increased in adults with HSE [104] and VZV encephalitis [157] and in childhood encephalitis/encephalopathy with poor outcome [106]. Increased levels of S100B and GFAP were seen in adults with HSE compared to in TBE infection [104], indicating that the direct neuronal damage is more pronounced in HSE compared to in TBE. In our study, the levels of S100B and GFAP were below the detection limit of the assay in all children but one. Levels of Tau were higher in children with sequelae compared to children with a good outcome, but the difference was not statistically significant. However, the presence of Tau in CSF is supporting that there is some degree of neuronal damage even if it is not as pronounced as in encephalitis caused by viruses resulting in lytic infection.

Taken together, it seems that the inflammatory reaction in TBE causes the neuronal damage rather than direct viral induced neuronal injury. Understanding the pathogenesis behind the neuronal injury in acute encephalitis is an important area of research that have the potential of resulting in better medical methods of intervention in the future. In CNS infection, the presence of sustained inflammation in CNS is probably causing neuronal damage. Limiting the inflammatory reaction would be a potential therapeutic target. However, in early stages of the infection, the inflammatory reaction is essential for viral control and clearance. At what time-point the inflammation no longer is beneficial is difficult to establish and needs to be elucidated further.

In summary, markers of neuronal damage might be useful in predicting outcome after CNS infection caused by viruses resulting in direct neuronal destruction, but is not helpful in predicting outcome in childhood TBE. Instead, it seems to be the inflammatory reaction within CNS causing neuronal injury and sequelae after TBE in children, supported by the elevated levels of IFN-γ, IL-4, IL-6 and IL-8 in children with sequelae, in our study.

For these cytokines and chemokines to be of use in clinical practice, cut-off levels for when they are considered to be correlated to a risk of incomplete recovery needs to be established.

5 CONCLUSIONS

From the studies included in this thesis we conclude that the etiology of childhood encephalitis in Stockholm is similar to other areas in Europe where TBE is endemic. The dominating etiologies were TBEV, EV, VZV, influenza virus and RSV. However, the etiology remained unknown in 52% of the cases.

Fever and encephalopathy were seen in a majority of patients with encephalitis. Other symptoms of CNS involvement, such as seizures or focal neurological deficits were less often present. In TBE, nearly all children had fever and headache, but symptoms of CNS involvement were often vague. All children with TBE had pleocytosis, which supports that analysis of CSF is important both for identifying the etiology and also in confirming CNS involvement. The EEG picture was abnormal in 90% of the children with encephalitis and thus useful in confirming CNS involvement.

We conclude that the clinical picture in the acute phase is a poor predictor of the long term outcome in encephalitis as well as in TBE. In children with acute encephalitis, persistent symptoms were seen in more than half of the children, and children who were considered fully recovered at the time of discharge from hospital also had problems at follow-up. In TBE, problems were seen in 70% of the children at follow-up, and both in children with mild and severe symptoms in the acute phase.

Apart from the need of care at the PICU, no other clinical sign, symptom or finding on any examination could be used to predict the outcome. Furthermore, markers of neuronal damage did not discriminate between good or poor outcome in children with TBE. However, children with problems at follow-up had significantly higher levels of IFN-γ, IL-4, IL-6 and IL-8 in CSF than children with a good outcome following TBE. This indicates that the inflammatory response in CNS is important in the development of CNS damage and subsequent sequelae.

Cognitive problems and personality changes were the main complaints after acute encephalitis. In TBE, headache and fatigue as well as cognitive problems dominated. On more detailed tests of cognitive functions, problems with working memory and executive functioning were seen in children following TBE. These symptoms may cause problems in everyday life and especially in learning situations at school. Since all children with encephalitis who made a full recovery did so within 6 to 12 months, we conclude that all children should be monitored for 6-12 months after sporadic encephalitis as well as after TBE with CSN involvement in order to identify long-term sequelae.

6 FUTURE PERSPECTIVES

The studies included in this thesis have addressed the clinical picture, etiology and outcome in childhood encephalitis and TBE. Hopefully, these studies will increase the awareness of the risk of long-term problems following encephalitis and TBE in childhood. However, many areas still need to be elucidated further.

A concern in encephalitis, is the large proportion without an identified etiology. New techniques enabling the identification of even small amounts of viral DNA might increase the number of identified etiologies in the CSF. With the ability of genome sequencing, screening for unknown viral antigen is possible and future studies might result in identification of new viral causes of encephalitis. However, recent research findings also suggest that a larger proportion than previously recognized are caused by antibodies against neuronal antigens, such as the NMDA receptor. [26] Since the etiology often remains unidentified, the frequency of autoimmune causes of encephalitis needs to be addressed further in future studies.

Risk factors for developing encephalitis need to be studied in more detail. The infectious dose, viral load, variations in virulence factors and age have been suggested to be associated with the development of encephalitis. Indeed, why a few individuals develop complications such as encephalitis when encountering some of our more common viruses is unknown. Genetic variations in our immune response that increase the susceptibility to severe infections have been addressed in several studies, but need to be studied in more detail. Identifying the phenotype of these genetic differences will increase our understanding of the pathogenesis in encephalitis and hopefully lead to the development of more individualised treatment options. It will also enable us to identify individuals at risk of developing encephalitis and preventive measures such as vaccination can be recommended.

The outcome in encephalitis is difficult to predict. In the present studies we found that the clinical picture in the acute phase cannot be used to predict outcome in childhood encephalitis and in TBE, and other markers needs to be identified. Identifying markers of neuronal damage in the acute phase that correlates to outcome is a promising area of research. These biomarkers will most likely differ between etiologies due to variations in the pathogenesis. In some etiologies, such as TBE, where lytic infection is less prominent, measuring cytokine and chemokine profiles might be more informative of the ongoing process in the CNS, and might also correlate better with the outcome.

Analysis of cytokine and chemokine profiles in encephalitis is an interesting area of research that will aid in understanding the role of inflammation in causing sequelae. How the inflammatory response is controlled and when it is no longer beneficial for viral clearance needs to be addressed in future studies. Analyses of the temporal profile of cytokines and chemokines involved in the inflammation in encephalitis could be one way of studying this. Whether it is the actual level of the cytokines, different cytokine profiles or sustained inflammation that is important in causing neuronal damage and scarring with subsequent sequelae in encephalitis need to be elucidated. Using multiplex assays are

promising methods to enable these studies even on limited CSF and serum volumes. Identifying normal cut-off levels and levels for when the risk of poor outcome is increased is also important.

Neuroimaging and neurophysiology are other areas where new techniques may prove useful in detecting subtle changes not visible on conventional MRI or EEG recordings, which might aid in diagnosing encephalitis and may also correlate with the outcome. Functional MRI have been shown to correlate with cognitive functions in children and adolescents. [158] Whether results on fMRI can predict long-term outcome and later cognitive functions after encephalitis needs to be studied in more detail. Pathological EEG picture have in some studies shown to correlate with outcome. Whether new methods of registration and interpretation can improve the correlation between the EEG-picture and later sequelae in encephalitis also needs to be studied in more detail.

In order to study the clinical picture, etiology and long-term outcome in childhood encephalitis, prospective studies are needed with standardized test procedures and examinations as well as standardized follow-up. Premorbid problems can be identified in prospective studies with less risk of recall bias compared to in retrospective designs. The evaluation of neurological and cognitive sequelae also needs to be studied in more detail in children following encephalitis with standardized follow-up examinations both in regard to follow-up time and test-batteries used.

Few treatment options are available for encephalitis in childhood today. Undoubtedly, understanding the pathogenesis in encephalitis would enable us to better predict which individuals are at risk of severe illness and poor outcome, and it would also aid in the invention of new treatment strategies.

7 POPULÄRVETENSKAPLIG SAMMANFATTNING

Infektioner i hjärnan, centrala nervsystemet (CNS), är relativt ovanliga men potentiellt mycket allvarliga. Dödsfall förekommer och många drabbade riskerar att få bestående eller långdragna besvär i efterförloppet. CNS infektioner orsakas vanligtvis av bakterier eller virus men i många fall hittar man aldrig orsaken. Infektionen kan engagera olika delar av hjärnan, vid meningit (hjärnhinneinflammation) omfattas hjärnhinnorna och vid encefalit (hjärninflammation) är själva hjärnparenkymet påverkat. Hos barn kan det vara svårt att värdera graden av CNS påverkan och även svårt sjuka barn kan initialt ha vaga och ospecifika symtom. I Stockholm orsakar den fästingburna sjukdomen TBE ett stort antal CNS infektioner varje år, både hos barn och vuxna.

Denna avhandling fokuserar på encefalit hos barn och syftet är att studera etiologin (orsaken), kliniska karakteristika, långtidsprognosen samt förekomst av prognostiska markörer i likvor (ryggmärgsvätskan) hos barn med encefalit och TBE.

Studie I: En journalgranskningsstudie av alla barn (93 st.) som vårdats för akut encefalit vid Astrid Lindgren barnsjukhus i Stockholm, 2000-2004, visade att man kunde identifiera etiologin i endast 48 % av fallen. De etiologier som dominerade var TBE, enterovirus, vattkoppor, influensa och RS-virus. Nästan alla barn hade feber och var medvetandepåverkade, personlighetsförändrade eller förvirrade i det akuta insjuknandet. Neurologiska bortfallssymptom såsom förlamning eller känselbortfall sågs hos 44 % och kramper hos 40 % av barnen. Den undersökning som bäst kunde styrka att det förelåg CNS-påverkan var EEG-registrering och EEG-bilden var patologisk hos 90 % av barnen i det akuta skedet. Endast 55 % hade ökat antal vita blodkroppar i likvor som ett tecken på CNS inflammation/infektion. Drygt hälften av barnen hade kvarstående besvär vid tidpunkten för utskrivning från sjukhus.

Studie II: 71/93 barn från studie I deltog i en långtidsuppföljning 3-8 år efter den akuta sjukdomen. Strukturerade frågeformulär avseende kvarstående symptom samt funktion i vardagen användes vid en telefonintervju med alla familjer. Drygt hälften av barnen var inte återställda vid uppföljningen. De besvär som dominerade var kognitiva problem med koncentrationsförmåga och minnesfunktioner samt personlighetsförändring. Ca 30 % av barnen tyckte att deras funktion i vardagen var påverkad med försämrad förmåga att delta i fritidsaktiviteter och skola. Förutom behov av intensivvård i det akuta skedet så kunde inga andra prognostiska markörer identifieras.

Studie III: Alla barn med TBE i Stockholm 2004-2008 identifierade via register hos Smittskydd Stockholm. De barn med TBE som också hade ett CNS engagemang inkluderades i en långtidsuppföljning 2-7 år efter den akuta sjukdomen. Uppföljningen bestod av 2 frågeformulär avseende kvarstående symptom samt exekutiva funktioner i vardagen. En subgrupp på 20 barn testades av en neuropsykolog för att identifiera kognitiva problem. Kognitiva svårigheter, huvudvärk och trötthet sågs hos två tredjedelar av barnen efter TBE, problem med exekutiva funktion förekom hos en tredjedel av barnen. Vid neuropsykologisk testning sågs signifikanta problem med arbetsminnet jämfört med

övriga kognitiva förmågor. Svårighetsgraden av sjukdomen i det akuta skedet påverkade inte prognosen, lika många barn med milda respektive svåra symptom i det akuta skedet hade problem vid uppföljning.

Studie IV: Av 63 barn med TBE under 2004-2010 kunde sparade likvorprover rekvireras för 37 barn. Dessa prover analyserade avseende hjärnskademarkörer och inflammatoriska markörer. Nivåerna av dessa markörer ställdes i relation till den kliniska bilden och eventuella kvarstående problem vid uppföljning. Barn med kvarstående problem vid uppföljning hade signifikant högre nivåer av inflammationsmarkörerna IFN-γ, IL-4, IL-6 samt IL-8. Nivåerna av hjärnskademarkörerna GFAP, S100B, Tau och pTau skiljde sig inte åt mellan barn som var helt återställda eller barn med kvarstående besvär vid uppföljning. Troligtvis så är den kraftiga inflammation som uppstår i CNS vid TBE infektion inte bara nödvändig för att bekämpa infektionen utan riskerar att även orsaka skada i hjärnparenkymet som ger upphov till problem i efterförloppet.

Slutsats: Etiologin till encefalit hos barn i Stockholm är till stor del lik den i de områden i övriga Europa där TBE är vanligt. De flesta barn med encefalit har feber och medvetandepåverkan i det akuta insjuknandet men vid TBE är symptomen ofta mer vaga och ospecifika. EEG-undersökning och analys av likvor är viktigt för att identifiera CNS-påverkan både vid encefalit och TBE. En stor andel barn med akut encefalit och TBE har långdragna eller kvarstående besvär vid uppföljning. De besvär som dominerar är kognitiva svårigheter med minne och koncentration, personlighetsförändring, trötthet och huvudvärk. Den kliniska bilden i det akuta skedet kan inte användas för att förutsäga vilka barn som får problem i efterförloppet. Därför bör alla barn med encefalit eller TBE erbjudas uppföljning för att kunna identifiera problem i efterförloppet. Vid TBE verkar inflammationen i CNS bidra till uppkomsten av CNS skada med efterföljande problem. Fler studier behövs för att identifiera prognostiska markörer i det akuta skedet som kan användas för att förutsäga vilka barn som riskerar att inte tillfriskna helt.

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