STUDIES ON VIRAL ENCEPHALITIS WITH EMPHASIS ON HERPES SIMPLEX ENCEPHALITIS

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ABSTRACT

In this thesis studies of two types of viral CNS-infections are presented; influenza related encephalitis and herpes simplex encephalitis (HSE).

Influenza related encephalitis/encephalopathy has been reported to increase both in incidence and severity in the Japanese population the last decade. We wanted to assess the incidence of the disease in Sweden and evaluate the clinical picture of the disease. The cases were identified through the Swedish National Inpatient Register, and the patient records were evaluated. Between the years 1987 and 1998 the incidence of influenza related encephalitis was 0.21 cases per million population and year. All 8 cases whose records could be evaluated recovered without sequelae. There were no pandemic influenza outbreaks during the study period and all but one of the evaluated cases occurred during A(H3N2)-dominated seasonal outbreaks. We conclude that, in Sweden during seasonal influenza outbreaks, influenza related encephalitis is a very rare complication, which resolves without sequelae.

HSE is the most common of the sporadically occurring forms of viral encephalitis. We have investigated the incidence, morbidity, case fatality rate, and virological diagnoses of HSE in Sweden during a 12-year period. The incidence was 2.2 cases per million population and year. No diseases predisposing to HSE could be found. The post-HSE morbidity was considerable with elevated risk for epilepsy, neuropsychiatric disability, infections, diabetes and venous thromboembolism. The one-year mortality rate was 14%.

In a material from a clinical trial of acyclovir vs. vidarabine on HSE, we studied if antibody levels and viral load correlates with outcome. Viral load dropped rapidly in the acyclovir treated patient, but there was no difference between outcome groups. In the patients treated with vidarabine, high IgG-antibodies to herpes simplex virus (HSV) in the cerebrospinal fluid (CSF) at admission was protective, indicating that anti-HSV-IgG in CSF has some ability to control the infection.

Acute or subacute neurological deterioration after HSE has been described as relapsing HSE. In a prospective cohort of 32 HSE-patients there were 4 patients with relapse. At relapse none of the patients had positive HSV PCR in the CSF, suggesting that the pathogenesis is not mediated by direct viral cytotoxicity. An immunological imbalance with low anti-inflammatory activity and elevated proinflammatory activity indicates that the pathogenesis in relapsing cases can be immunologically mediated.

This thesis gives baseline epidemiological data on influenza related encephalitis that can be useful when influenza epidemiology changes. A nation wide study establishes that HSE still is a serious disease with high morbidity, despite access to current antiviral therapy. Our studies support the hypothesis that the immunological response to herpes simplex virus can aggravate the brain damage in HSE. Immunomodulating therapy regimens should be studied.
LIST OF PUBLICATIONS


V. **Anders Hjalmarsson**, Elisabeth Aurelius, Martin Glimåker, Anna Tüll Nyman, John Hart, Birgit Sköldenberg. Subacute contralateral relapse in herpes simplex encephalitis. neuropsychological follow-up, magnetic resonance volumetry and markers of inflammation. In manuscript.
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>CMV</td>
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<td>CNS</td>
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<td>CSF</td>
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<td>DNA</td>
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<td>EASIA</td>
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<td>Glial fibrillary acidic protein</td>
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<td>MRI</td>
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<td>NFL</td>
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<td>NSE</td>
<td>Neuron-specific enolase</td>
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<td>PBMC</td>
<td>Peripheral blood mononuclear cell</td>
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<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>RNA</td>
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<td>sCD8</td>
<td>Soluble Cluster of Differentiation 8</td>
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<td>SMR</td>
<td>Standardised mortality ratio</td>
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1 INTRODUCTION

The main scope of this thesis is different aspects of herpes simplex encephalitis in non-neonatal patients. As a comparison we also studied influenza related encephalitis. The documented ability of both viruses to cause neuroinflammation is one of the few things these viruses have in common. Influenza virus is a RNA-virus, spread by droplets, causing outbreaks, and the virus is not specific for humans. Herpes simplex virus is a DNA-virus specific for man, and has been our companion for millions of years (McGeoch, Cook et al. 1995). The route of infection is principally through direct mucosal contact or mucosal secretions. The virus develops latency in sensory ganglia neurons and can be reactivated later in life causing disease without reinfection (Koelle and Corey 2008).

1.1 INFLUENZA ENCEPHALITIS

There are three types of influenza viruses, A, B and C. The classification is based on antigenic differences. They belong to the family Orthomyxoviridae. Their genome consists of segmented single strand RNA and they have a host cell derived envelope (Treanor 2000).

Influenza A virus is the cause of pandemics. It is antigenically unstable and new combinations of surface glycoproteins, haemagglutinines and neuraminidases occur. This leads to outbreaks in the population who lacks immunity to the new strain (Studahl 2003).

Influenza virus epidemics have been causing considerable morbidity during history. There have been approximately three serious pandemics every century in the last 300
years (Kilbourne 1997). During the last 120 years there have been five pandemics. The first pandemic that was documented in detail was the Russian flu 1889-1890 (Skog, Hauska et al. 2008).

The Spanish flu (1918-1919) is the pandemic that has caused most deaths, approximately 50 million world-wide (Johnson and Mueller 2002). That an influenza virus infection can lead to encephalitis or encephalopathy has been suspected since the Spanish flu. There has been much debate if encephalitis lethargica was caused by the influenza virus, but the majority of evidence at hand today does not support this theory (McCall, Henry et al. 2001; McCall, Vilensky et al. 2008).

There were cases of encephalitis during the Asian flu 1958-9 (Flewett and Hoult 1958). The influenza strains which have caused the seasonal flu the last decades cannot cause a productive infection in the CNS. One reason for this is that the virus demands presence of extracellular trypsin type proteases present in the airways for splicing hemagglutinin HA0 to HA1 and HA2 (Kido, Yokogoshi et al. 1992; Murakami, Towatari et al. 2001). Without this splicing, the new virions cannot penetrate the cell membranes and are thus not infectious. Virus strains with HA-variants which can be cleaved with plasmin or other proteases that also occur in the CNS can theoretically cause a productive infection of the brain (Kido, Yokogoshi et al. 1992; Davis, Kornfeld et al. 2000).

The pathogenesis of influenza encephalitis is largely unknown but has been suggested to be post infectious immune-mediated or due to a non-permissive infection maintained from replication in the airways (Flewett and Hoult 1958; Davis, Kornfeld et al. 2000). The clinical picture ranges from a few days with disorientation and full recovery to
fulminant deadly necrotizing encephalopathy, the latter predominantly reported in Japanese children (Kasai, Togashi et al. 2000; Okabe, Yamashita et al. 2000; Yoshikawa, Yamazaki et al. 2001). In Japan (population 127 million in the year 2000, Statistic Yearbook of Japan), there were 472 cases of influenza related encephalopathy in children up to 15 years of age during the years 1998-2002 (Wada, Morishima et al. 2009). Other neurological complications of influenza are febrile convulsions, Reye’s syndrome, myelitis and Guillain-Barre’s syndrome (Hayase and Tobita 1997; Studahl 2003).

The H5N1 bird flu is a virus which has caused human disease recently (Thanh, van Doorn et al. 2008). On July 1st 2009 there were 436 confirmed cases worldwide, of those 262 died from the disease (World Health Organization). The bird flu has very limited spread between humans. Bird contact is mainly required. But the disease is more severe than the H1N1-flu, and encephalitis cases have been described (Thanh, van Doorn et al. 2008). As highly pathogenic influenza virus can be cleaved by the intracellular protease furin, there is also a possibility that humans can contract a productive influenza infection in the CNS (De Jong, Bach et al. 2005; Kido, Okumura et al. 2009).

Figure 1. Influenza virus A (H5N1), Electron microscopy photo by Kjell-Olof Hedlund, SMI.
We are now in the beginning of the first pandemic of the 21\textsuperscript{st} century by the novel H1N1 flu. The complication rate and mortality in the current flu seems to be low, comparable with average seasonal flu. According to ECDC data from 18\textsuperscript{th} of August 2009, there are 39,427 confirmed cases in the EU/EFTA countries and of those 63 fatal cases (European Centre for Disease Prevention and Control). Nevertheless, the risk that the flu changes character is not negligible. There are cases of encephalitis / encephalopathy described associated with the novel influenza A(H1N1) (Centers for Disease control and Prevention 2009).

1.2 HERPES SIMPLEX ENCEPHALITIS

1.2.1 Background

Herpes simplex encephalitis is the most common of the sporadically occurring encephalitides (Sköldenberg 1991). The connection between herpes simplex virus and infection of the central nervous system was suspected in the 1920s, when a vaccination trial was conducted. The hypothesis was that encephalitis lethargica was caused by herpes simplex virus (Mathewson-Commission 1929). Goodpasture and Teague showed that encephalitis could be produced in rabbits by corneal inoculation with HSV from human labial lesions (Goodpasture and Teague 1923). The first cases with convincing evidence of herpes simplex encephalitis were a child in 1941 and an adult in 1944 (Smith, Lenette et al. 1941; Zarafonetis and Smadel 1944). The diagnosis was based on the presence of inclusion bodies in brain tissue in combination with propagation of the virus in different strains of animals, and finally neutralisation with antiserum from individuals infected by known herpes strains. In the 1940s, serology tests were also performed by neutralisation tests in animals or eggs. Other diagnostic methods were developed which made in vitro serology possible, the most important
was the complement fixation test. This was applied for HSV-infections (Hayward 1949).

1.2.2 Herpes simplex virus type 1 and 2

HSV belongs to the subfamily *Alphaherpesvirinae* and the genus *Simplexvirus* (Davison 2007). The main clinical manifestations of HSV type 1 and 2, herpes febrilis (cold sores) and herpes genitalis have been known for long. Cold sores were described in Roman time and genital herpes was described by the French physician Jean Astruc in 1736 (Hutfield 1966). Convincing evidence that there were two types of HSV was established in the 1960s (Schneweis 1962; Dowdle, Nahmias et al. 1967). Herpes simplex encephalitis has been considered to be caused almost exclusively by herpes simplex type 1. The typical CNS-manifestation of herpes simplex type 2 is meningitis. Nevertheless, in 4-7% of the patients with non-neonatal focal herpetic encephalitis, HSV type 2 has been the evident aetiology (Nahmias, Whitley et al. 1982; Aurelius, Johansson et al. 1993).

1.2.3 Viral diagnosis of HSE

Viral diagnosis of HSE developed in the 1960s, when Maccallum 1964 published the method of virus isolation from brain biopsy (Maccallum, Potter et al. 1964). This enabled virological confirmation of the diagnosis in only a few days. Treatable differential diagnoses could also be diagnosed by the method. It is an invasive procedure with a reported complication rate of about 3%, which is acceptable considering the severity of the disease (Whitley 1997).

In the 1970s and 1980s, demonstration of intrathecal antibody production to HSV was used, first with complement fixation methods (Maccallum, Chinn et al. 1974;
McKendrick 1979), later with radioimmunoassay (Sköldenberg, Kalimo et al. 1981) and enzyme immunoassay (Aurelius, Forsgren et al. 1989; Forsgren, Sköldenberg et al. 1989). The serum-CSF antibody analysis is comparable to the modern PCR-method regarding sensitivity. For a negative result to be reliable it requires that the patient has been ill at least ten days before samples are taken.

Brain biopsy remained the gold standard for the diagnosis of HSE until the 1990s when it was replaced by detection of HSV-DNA in CSF by polymerase chain reaction (Puchhammer-Stockl, Popow-Kraupp et al. 1990; Rowley, Whitley et al. 1990; Aurelius, Johansson et al. 1991; Lakeman and Whitley 1995). PCR is the best available diagnostic method for HSE, but in CSF-samples taken short after onset, and if antiviral therapy is rapidly initiated, there are cases that are HSV-PCR negative (Aurelius, Johansson et al. 1991; Studahl, Bergström et al. 1998; Tyler 2004). It has been suggested that both PCR and intrathecal antibody production should be used to have the most reliable diagnostic strategy (Cinque, Cleator et al. 1996).

The HSV DNA PCR methods have been developed since they were introduced. The conventional PCR is a two-step procedure with amplification and then detection by gel electrophoresis or EIA (Kimura, Shibata et al. 1990; Puchhammer-Stoeckl, Heinz et al. 1993). To increase sensitivity and specificity, nested PCR was developed (Aurelius, Johansson et al. 1991). Nested PCR has two amplification steps, the second round with primer pair for hybridization sites within the first amplicon. This procedure is more time consuming than conventional PCR. In real-time PCR, the amplification and detection, by analysis fluorescence produced by labels reacting to the formation of double-stranded DNA, take place in the same step (Mackay, Arden et al. 2002). This method is used for quantification of the HSV-DNA copies in the sample.
1.2.4 Pathological-anatomical findings in HSE

HSE is an acute, focal, necrotizing encephalitis in which hemorrhages can develop. The characteristic localisation is in the temporal lobe. Both temporal lobes can be affected but not symmetrically. In addition to the temporal lobe, the insula, hippocampus, amygdala and the lobes adjacent to the temporal lobe can be affected (Boos and Esiri 1986).

1.2.5 Therapy in HSE

There was no antiviral treatment available until mid 1960s when pyrimidine analogues developed for chemotherapy in malignant diseases were shown to have antiviral effect (Breeden, Hall et al. 1966). Idoxuridine and cytarabine had unsatisfactory effect, but vidarabine reduced the mortality in HSE from 70% in historic controls to 28-50% (Whitley, Soong et al. 1977; Sköldenberg, Forsgren et al. 1984). The breakthrough came in the 1980s with acyclovir, a purine nucleoside analogue, which is phosphorylated by herpes specific thymidine kinase in infected cells, and thereby trapped in the cell. The phosphorylated acyclovir is a substrate for the viral DNA-polymerase and terminates the viral replication. The specific accumulation in infected cells results in low systemic toxicity (Kleymann 2006). Today, with acyclovir therapy, the mortality is about 15-20 %. However, the morbidity in the survivors is still considerable. Acyclovir is an effective antiviral substance, but the penetration to the CNS is slow and incomplete. New highly lipophilic antiviral agents are needed to get a better penetration to infected brain tissue (James, Kimberlin et al. 2009).

The current recommended therapy for HSE is Acyclovir IV 10-15 mg/kg TID for 21 days (James, Kimberlin et al. 2009). In the clinical trials, which established acyclovir therapy for HSE, the duration of therapy was 10 days (Sköldenberg, Forsgren et al.
1984; Whitley, Alford et al. 1986) The duration of the treatment has been increased since the drug was introduced. The reason for this is that even during therapy, HSV PCR has been positive in some cases up to about three weeks, and this has correlated with more severe outcome (James, Kimberlin et al. 2009).

Immunomodulating approaches have been tested. Due to a high intracranial pressure corticosteroids have been administered to HSE-patients. In a recent retrospective study adjuvant therapy with corticosteroids was beneficial (Kamei, Sekizawa et al. 2005). As this was a retrospective study, there was no standardised regimen, prednisolone or dexamethasone was used and the duration of treatment spans between two days to six weeks. There are results from a mouse model indicating that late administration of glucocorticoids is neuroprotective (Sergerie, Boivin et al. 2007). There have been studies on adjuvant therapy with interferons, but these have not shown better results than placebo (Wintergerst, Kugler et al. 2005).

1.2.6 Seroprevalence of HSE and incidence of HSE

The Herpes simplex virus is a very common virus. There are considerable differences due to geography and socio-economic status when in life the infection is acquired. The seroprevalence to HSV type 1 in age groups older than 70 years is approximately 90% in most studies (Smith and Robinson 2002). Even though the causing virus is very common, HSE is a rare disease. The incidence of HSE varies between 2-4 cases per million population and year (Sköldenberg, Forsgren et al. 1984; Whitley 1988; Sköldenberg 1991). There is no seasonal variation (Whitley and Gnann 2002). The disease affects both sexes to the same extent (Whitley, Soong et al. 1982; Whitley and Gnann 2002). People of all ages can be affected, but the majority (50-60%) of the
patients are older than 50 years (Whitley and Gnann 2002). No risk factors for developing the disease have been identified (Whitley, Soong et al. 1982).

1.2.7 Clinical picture of HSE

There are no specific clinical symptoms in HSE that differ from other encephalitides (Whitley, Soong et al. 1982; Kohl 1988). About half of the patients experience a prodromal illness less than a week before the onset of encephalitic symptoms. The prodromal illness can be fever, headache, and general malaise. Gastrointestinal or respiratory symptoms occur, especially in children. During the acute onset, the symptoms include fever to almost 100%, headache, personality and behavioural changes, and focal neurological signs. Seizures during the acute stage occur in 33-67%, more common than in other encephalitides. About 25% of the patients have recurring epileptic seizures long time after the acute stage. About the same proportion develop neuro-psychiatric sequelae. Unfavourable clinical indicators are long time from onset to treatment, coma at presentation and high age (Sköldenberg, Forsgren et al. 1984; Whitley, Alford et al. 1986; Raschilas, Wolff et al. 2002).

1.2.8 HSE and neurosurgery / cerebral irradiation

There are reports on HSE occurring a few weeks after neurosurgery (Bourgeois, Vinikoff et al. 1999; Spuler, Blaszyk et al. 1999; Aldea, Joly et al. 2003; Spacca, Mallucci et al. 2007). HSE as postoperative complication occurs both in children and adults with malignant and non-malignant diseases. There are about ten reported cases in the literature, but the diagnosis should be considered in these patient groups, if deteriorating during the postoperative stage.
1.2.9 HSE in the immunologically compromised host

HSE does not occur in transplant recipients more often than in normal hosts (Crippa and Cinque 2006; Whitley 2006). In HIV patients, the typical clinical picture of HSE is rare and is seen only in patients with a preserved immune response. In the HIV patients with severely impaired immunity the symptoms are similar to subacute focal encephalitis or ventriculo-encephalitis seen in the encephalitis caused by CMV. In the great majority of cases were HSV is demonstrated, there is also CMV-infected cells in the same prevalence or higher (Vago, Nebuloni et al. 1996; Cinque, Vago et al. 1998). This leads to difficulties in deciding which virus contributes most to the disease.

1.2.10 Clinical, neurophysiological and radiological diagnosis of HSE

In clinical diagnosis of a suspected case of HSE, the results of lumbar puncture, neuroradiological and neurophysiological investigations should be evaluated since the results of the PCR usually take about 24 hours, and can be false negative during the first three days after onset (Studahl, Bergström et al. 1998; Tyler 2004). Negative early PCR seems to be more common in children (17%) than in adults (3-5%) (Elbers, Bitnun et al. 2007). If there is a strong suspicion that the patient has HSE despite negative PCR, a second lumbar puncture should be performed to repeat the HSV PCR. Pleocytosis occurs in approximately 90 percent of the cases (Sköldenberg and Forsgren 1985; McGrath, Anderson et al. 1997; Whitley 2006). The EEG is characterized by spike and slow-wave activity and periodic lateralized epileptiform discharges in the area of the temporal lobe. The sensitivity of the EEG has been estimated to 84 percent, but the changes are unspecific (Adams and Jennett 1967; Ch’ien, Boehm et al. 1977). CT-scan is the radiologic method most frequently available when the patient is admitted, and temporal lobe changes on CT scan indicate the diagnosis in a majority of the patients within five days after onset of neurological symptoms (Hindmarsh,
Lindqvist et al. 1986). MRI is superior to CT-scan in showing the early oedematous changes, T2-weighed images are considered the method of choice (Tien, Feldberg et al. 1993). In infants and neonates, diffusion weighed images are more sensitive than T2-weighed or fluid-attenuated inversion recovery images (Maschke, Kastrup et al. 2004).

![Figure 2. HSE 12 days after onset, T2 weighed MRI.](image)

### 1.2.11 HSE and epilepsy

HSE is often complicated by seizures (Annegers, Hauser et al. 1988). The incidence of post-HSE epilepsy has been estimated to approximately 24%. Simple or complex partial seizures and secondary generalised seizures occurs (McGrath, Anderson et al. 1997). In children, the incidence is higher (44%) (Elbers, Bitnun et al. 2007). Unilateral and particularly bilateral periodic lateralised epileptiform discharges on the EEG have been related to unfavourable outcome in HSE in adult patients (McGrath, Anderson et al. 1997).
1.2.12 Relapse of HSE

There are patients who after their initial acute HSE-disease develop new neurological symptoms. The clinical picture in these cases is not uniform. There are at least three pathophysiologic mechanisms that may account for these complications: delayed symptoms induced by the initial infection, immunological mediated inflammatory disorders as in post infectious encephalitis, or recurrence of intracerebral viral replication (Koenig, Rabinowitz et al. 1979; Kimura, Aso et al. 1992; De Tiège, Rozenberg et al. 2003). The incidence is reported to be between 4% and 26% and occurs more commonly in children (Sköldenberg, Forsgren et al. 1984; Kimura, Aso et al. 1992). The clinical picture differs somewhat between non-neonatal children and adults. In children, extrapyramidal movements occur in some cases the first month after HSE-onset. This type of relapse does not respond to acyclovir therapy and often recurrence of virus cannot be demonstrated (De Tiège, Rozenberg et al. 2003). Another type of relapse is associated with new neurological symptoms of various types, fever, new neuro-radiological lesions and recurrence of viral DNA in the CSF. This is more common in children but also occurs in adults (Kimura, Aso et al. 1992; Landau, Miller et al. 2005). This type of relapse is most common a short time after withdrawal of acyclovir treatment, indicating insufficient length of therapy. In adults the HSV-PCR of the CSF is negative in the majority of cases, based on a number of cases or case series (Dennett, Klapper et al. 1996). The clinical presentation varies from similar symptoms as at the initial onset, to subacute, afebrile cognitive deterioration over months to years (Sköldenberg, Forsgren et al. 1984; Dennett, Klapper et al. 1996; Yamada, Kameyama et al. 2003; Landau, Miller et al. 2005).

A further sub classification of HSE-cases has been suggested, to enable identification of patients with a more severe prognosis. This “prolonged HSE group” consists of
those comatose at admission, those who do not improve after two weeks on intravenous acyclovir therapy, and relapsing cases (Shoji 2009; Taira, Kamei et al. 2009). The authors suggest this classification based on 23 patients and of those 8 were classified to the prolonged group. More evidence for the validity of a sub classification is needed. However, it could be useful if alternate therapy regimens will show to be beneficial for this group, e.g. prolonged antiviral therapy or addition of corticosteroids or other immunomodulating treatment.

1.2.13 Genetic susceptibility to HSE

A frequency of 13% of consanguineous families has been reported in a French pediatric HSE-study. This suggests that a genetic predisposition to HSE exists. In this material, consisting of 86 confirmed cases, the PBMC-response to HSV-infection was tested, and two patients were found who had low INF-α and β-response. Further specific stimulation tests led to the conclusion that the patients had defects in their TLR-7, -8 and -9 stimulation. These toll-like receptors mediate virus detection and interferon response. The two patients had different mutations of the UNC-93B gene. UNC-93B is a protein in the endoplasmatic reticulum involved in the activation of TLR 3, 7 and 9. The authors convincingly show that this is the cause of the encephalitis in these two patients (Casrouge, Zhang et al. 2006). The same group has also demonstrated TLR3 deficiency in two unrelated children with HSE (Zhang, Jouanguy et al. 2007). These mutations may occur in other HSE patients, but the majority of HSE patients do not have them (Casrouge, Zhang et al. 2006). These findings encourage further research on other genetic deficiencies that could increase the susceptibility to HSE.
2 AIMS OF THE THESIS

The aims of this thesis are:

• To assess the incidence of influenza related encephalitis in Sweden, and evaluate the clinical picture of the disease.

• To assess the incidence of herpes simplex encephalitis in Sweden, and estimate mortality, outcome and evaluate possible risk factors for the disease.

• To investigate if laboratory parameters - intrathecal antibody levels, and quantitative HSV DNA PCR - are useful for predicting outcome in herpes simplex encephalitis.

• To assess the incidence of relapsing HSE, and describe the pathogenesis, clinical picture and outcome.
3 PATIENTS AND CONTROLS

In paper I and II we have used Swedish national computer registers to find the patients. Sweden (population 8.9 million 2000) has a national healthcare system based on administratively independent county councils. The private hospital sector is small and provides mostly elective care. Since 1964, the Swedish National Board of Health and Welfare has compiled data on individual hospital discharges in the National Inpatient Register, and since 1987 the register covers all Swedish hospitals. About 1.4 million admissions are recorded annually. In addition to a national registration number uniquely identifying every resident of Sweden, information of sex, age, place of residence, hospital and department, date of admission and discharge are collected. Each record also contains medical data, including diagnoses at discharge (coded according to the Swedish version of the International Classification of Diseases, 9th and 10th revision, ICD9 and ICD10).

The national cause of death register covers all Swedish residents who die, with data on the causes of death in 99.5% of the cases.

The cases were identified retrospectively in the computer registers by ICD-codes. In paper I we describe the 21 detected cases of influenza related encephalitis during a 12-year period. In paper II the cases found in the inpatient register were virologically confirmed. Data from the registers of the virological laboratories in the country was added, resulting in 236 cases during a 12-year period.

In paper III, the patients originate from the Swedish trial of acyclovir versus vidarabine. This was a nationwide clinical trial covering a period of 2 ¾ years in the 1980s. Among
127 consecutive patients with suspected HSE, 53 patients had a virologically confirmed HSE diagnosis.

In paper IV, the 4 relapsing patients originate from 32 consecutive patients who were treated for acute HSE and survived the acute phase at the Department of Infectious Diseases, Danderyd Hospital, Stockholm, during a 22-year period, 1977–95. Of the 32 patients, 26 had been treated with acyclovir for 10–21 days and 6 with vidarabine. The patients were followed for 6.1 years (range 24–228 months) after HSE. Their median age was 51.7 years (range 17–89) and 21 (57%) were females. Non-relapsing cases from the cohort were used as controls for comparison with the relapsing patients.

Paper V is a case report on a relapse case, with presentation of a follow up protocol using neuropsychiatric testing and repeated volumetric MRI, complement to paper IV.
4 METHODS

In paper I and II, the National Inpatient Register was used to identify the patients. In paper I on influenza related encephalitis, a problem was that there is no specific ICD-code for this diagnosis. In this disease there is also a possibility that the influenza disease precedes the onset of encephalitis by 1-2 weeks. Three approaches were used to find the patients in the register. All hospitalizations with influenza as 1st or 2nd diagnosis and encephalitis as 1st or 2nd diagnosis. All hospitalizations with encephalitis as 1st or 2nd diagnosis and influenza as 1st or 2nd diagnosis. And last, all hospitalizations with influenza as 1st diagnosis, and another admission within a month with encephalitis. The patients’ records were evaluated resulting in a considerable proportion of drop outs.

In paper II the data retrieval was less complicated since there is a specific ICD-code for HSE. For all patients, data on hospital admissions that occurred before and after the first HSE-related admission were also collected. The data set was linked to the National Register of Death and Causes of Death.

Since we had access to the national identification number of each patient we could validate each clinical diagnosis of HSE, because this registration number is also used by the virological laboratories in their local registries for patient identification.

Verified HSE was defined as a positive finding of HSV-1 by DNA PCR of CSF samples or as detection of intrathecal HSV-1 antibody production. From available data it was not possible to decide whether a verified HSV-2 infection in the CNS actually was HSE and not aseptic meningitis, which is the most common CNS manifestation of
infection due to HSV-2. For this reason, we only investigated HSV type 1 encephalitis, as we have no method to find the approximately 7% of HSE-cases caused by HSV type 2 without access to the patient records.

The verified cases were assessed with regard to hospital care before and after the HSE-onset, and mortality data from the National cause of death register was added. Because of the low incidence of hospital admissions in each diagnostic category, it was not feasible to analyze pre-HSE and post-HSE admissions formally by use of standardized incidence ratios. Instead, we compared the patients’ previous and subsequent hospital admissions by year at risk with the mean national annual rates of hospital admission during the period 1998–2001, which were provided by the Centre for Epidemiology at the National Board of Health and Welfare (Stockholm, Sweden). To estimate the accuracy of this data, we also compared the data with the national incidence of hospital admissions in the age group 65–69 years, because this was the age group closest to the median age of the patients in our series.

Mortality was analyzed by comparing the number of observed deaths with the number of expected deaths in an age-, sex-, and calendar year–matched group (the standardized mortality ratio, SMR).

In paper III, archive data from the clinical trial on acyclovir vs. vidarabine in Sweden was used (Sköldenberg, Forsgren et al. 1984). The diagnostic method used at the time of the study was intrathecal serology, and in some cases brain biopsy was performed. The antibody analyses were made by indirect EIA (Forsgren, Sköldenberg et al. 1989). The patients’ serum and CSF levels of anti-HSV-IgG were analysed statistically by means of logistic regression, to investigate if there was any correlation with antibody
levels at admission to hospital and outcome. A survival analysis was also performed with the same focus on evaluating the potential predictive value of the IgG levels. In a subset of the patients we were able to analyse intrathecal viral load by quantitative HSV-PCR (Bai, Hosler et al. 1997). Because of the small numbers these results are presented as case profiles.

In paper IV, the 4 relapsing cases were compared with matched non-relapsing cases from the same cohort. The definition of relapse was based on clinical signs; suddenly appearing new or aggravated neurological symptoms or signs that were not connected with any other complicating disease. Transient seizures were not considered as a relapse. Soluble markers of inflammation, markers of cell damage and viral load were analysed in CSF-samples from the patients.

IFN-γ and IL-10 were determined by sandwich enzyme immunoassays (EASIA) developed by Medgenix Diagnostics SA (Fleur, Belgium).

Soluble CD8 was determined using an enzyme immunoassay from T Cell Diagnostics (Cambridge, MA)

Cell damage markers:
GFAP (Glial fibrillary acidic protein), measured with a previously described ELISA (Rosengren LE et al. 1994).

NFL (the light subunit of the neurofilament core), analysed with ELISA (Rosengren LE et al. 1996)
S-100 (soluble dimeric proteins with not fully known functions found in astrocyte cytoplasm), and NSE (Neuron-specific enolase) were analysed with commercially available luminescence immunoassays (Sangtec Medical, Bromma, Sweden).

Viral load was analysed with quantitative HSV-PCR (Schloss, van Loon et al. 2003).

In paper V we used volumetric MRI to assess the extent of brain damage. This has been used previously in studies of various neurological diseases including HSE (Yoneda, Mori et al. 1994; Caparros-Lefebvre, Girard-Buttaz et al. 1996). It is performed by manually defining the borders of cerebral lesions visualised by MRI. Computer software is used to calculate the volumes of the lesions.
5 RESULTS

5.1 PAPER I

The aim of the study was to investigate the incidence and severity of influenza related encephalitis in Sweden by using national computer registers.

Between the years 1987 and 1998 there were 14,250 admissions due to influenza virus infection, corresponding to 13.7 admissions per 100,000 persons and year. Of those, 10 patients had an accompanying diagnosis of encephalitis. With further register analysis in order to detect those patients with post-infectious encephalitis, a total of 21 patients with influenza related encephalitis were found, corresponding to an incidence of 0.21 cases per million individuals and year. There was a male predominance (76%) and none of the patients died during admission.

We were able to validate 8 of the patients. All but one occurred during H3N2-dominated seasonal outbreaks. The main cause for the high drop out rate was register related factors. Data search to find the identity of the patients failed or, in one case, lack of data on treating department in the register. The second most common cause was patients not responding to our request to access their hospital records. All of the eight validated cases were mild and resolved completely within six weeks.
Figure 3. Influenza in Sweden 1987-1998. Admitted patients and laboratory confirmed cases by month.

### 5.2 PAPER II

The aim of the study was to investigate incidence, morbidity, case fatality rate, and virological diagnoses of HSE in patients admitted to hospital with HSE in Sweden during the period 1990–2001. National registers were used to identify the patients and the registers of the virological laboratories were used to validate the cases.

During the study period, 638 patients had a hospital admission with HSE as primary diagnosis. Of these, 236 had convincing virological confirmation of acute HSV type 1 infection of the CNS. There were 11 cases that had untyped HSV-infection and 75 patients had HSV type 2 infection, some of which possibly had HSE. However, with
our data it is not possible to conclude which patients had HSE or aseptic meningitis. The incidence of HSE in this study is 2.2 cases per million individuals and year. If virologically unconfirmed, but possible cases are included, the incidence rises to 3.2 per million and year.

During the five years preceding the onset of HSE, the frequency of hospitalisation does not differ from the general population. After HSE-onset (median follow-up time: 4.3 years) 87% of the patients were readmitted, with a median 4 admissions per patient and a 6 days median length of stay. The most common cause of readmission was epilepsy, which was 60-90 times more common in the post-HSE group than in the general population. Infections were the second commonest cause, followed by neuropsychiatric causes. Diabetes and thrombo-embolic disorders were also frequent.

The 1-year mortality rate was 14.0%, and the overall mortality rate during the median 4.3 years of follow-up was 25.4%. The mortality rate was significantly increased in the first year after diagnosis but not later. There was no difference in mortality between the sexes, and there was no significant increase in mortality in age groups <70 years old. The most common cause of death was HSE, followed by cardiovascular diseases.
Figure 4. Cases of herpes simplex encephalitis due to herpes simplex virus type 1 in Sweden, 1990–2001, by patient age at hospital admission.

5.3 PAPER III

The aim of the study was to investigate if levels of serum- and CSF-antibodies correlate with outcome in HSE. The material came from a clinical trial comparing the current acyclovir therapy with the previous vidarabine therapy. In a subset of patients, viral load was analysed.

The results of the statistical analysis of serum- and CSF-anti-HSV-IgG were that in patients treated with vidarabine, the CSF-levels were significantly higher in the mildest outcome group. The risk of dying was 75% lower for each log unit titre increase. In the acyclovir groups this was not seen. There was also a significant association between serum levels of anti-HSV IgG and death. The risk of dying was 3.87 times increased
per log-unit titre increase. However, when comparing outcome in the groups mild/moderate to severe/death, this association disappears, which calls for caution when interpreting the results on serum levels.

The anti-HSV-IgM results were negative in serum in 25/50 patients and in CSF in 38/50 patients. A non-significant trend was found, which suggests that high serum-IgM at admission might be unfavourable. The anti-HSV-IgA levels in serum were tested in late samples, due to the design of the initial trial. No difference was detected in IgA levels with respect to the outcome groups.

CSF response of IgM class can be very late, especially in elderly patients, and was demonstrated in 28/42 patients followed for more than 10 days. Intrathecal IgA response was detected in 27/31 patients. The CSF IgM activity was similar irrespective of treatment and outcome. The levels and duration did not parallel that of HSV-DNA.

We analysed the viral load by Taqman-PCR in those patients for whom we were able to retrieve samples taken within the first 3 days after the neurological onset. There is a considerable overlap between the groups, with lower concentrations of virus-DNA in the acyclovir group. The results clearly show the superior effect of acyclovir compared with vidarabine in reducing the viral load in the CSF. There is no distinct difference between outcome groups. Further statistical analysis was not performed because of the small numbers in each group.

5.4 PAPERS IV AND V

The aim was to study the occurrence of relapse in a consecutive material of 32 surviving HSE-patients, and analyse potential activity markers in CSF samples.
A total of 3 out of 26 acyclovir treated patients had a relapse, and 1 out of 6 vidarabine treated patients. All but one of the relapses occurred within the first 4 months after HSE-onset. After the relapse episode, the 4 patients recovered to the pre-relapse level within 2-8 weeks. Two of the patients had new or aggravated lesions on CT-scan at relapse.

The CSF findings at relapse were subtle compared to the pronounced pleocytosis and protein elevation at HSE-onset. None of the 13 CSF samples taken the first 32 days after relapse were HSV-PCR positive in the 4 relapsing patients. There was no correlation with viral load or duration of positive PCR at onset between relapsing and non-relapsing patients. INF-γ levels were elevated in 50% of the relapse patients, but 50% of the controls also had elevated levels. Soluble CD8 levels peaked at one month after HSE-onset and peaks were also detected during the first week of relapse. IL-10 was elevated in all patients during the first week after acute HSE, but not at relapse. The sCD8/IL-10 ratio was higher at relapse than at the onset of acute HSE. The glial and neuron destruction markers (GFAP, NSE and NFL) were high at onset, but showed no new peaks at relapse. The markers were higher in the acute stage in the relapse cases than in the non-relapsing.

The patient in the case report differs from the patients in paper IV in several ways. His relapse occurred seven months after the initial onset, with new contra lateral lesions on MRI. After having recovered and resumed work after the acute episode, he is post-relapse permanently dependent of assistance in his daily activities. The kinetics of the CSF-parameters did not differ from that of the other relapse cases. HSV PCR was negative at relapse as in the cases in paper IV. The relapse is objectively documented
by decreased performance in neuropsychological tests and increased volumes of brain lesions are demonstrated by volumetric MRI.
6 DISCUSSION

In paper I, we evaluate the incidence and severity of influenza related encephalitis in Sweden during a period with relatively low influenza activity. No pandemic occurred during the period. Encephalitic complications were rare and resolved quickly.

There are several kinds of influenza related encephalitis reported in the literature. The acute type is seen mainly in children, with the acute necrotizing encephalitis in Japanese children. The postinfectious encephalitis is more common in adults. The different clinical entities of the disease need further characterisation.

The present study provides baseline data for future studies, since the morbidity associated with influenza infections is expected to rise in the next pandemic, whether caused by bird flu, the novel H1N1-flu or a hitherto unknown type.

Paper II is a nation wide epidemiological study of herpes simplex encephalitis during a twelve year period and 236 verified cases are evaluated. The register data makes it possible to study pre- and post-morbidity as well as mortality. The strength of the study is the large number of cases, virological confirmation and the nationwide coverage. The weakness is that we had no access to the patients’ records, which would have given additional data. The main findings confirm previous smaller studies, with regard to incidence of post HSE – epilepsy and neuropsychiatric disability. The quality of the computer registers also enables us to state that there are no hospital care treated diseases that predispose for HSE. HSE, despite acyclovir treatment, is still a very serious disease, with considerable morbidity and mortality.
In paper III, we study anti-HSV antibody response and viral load in HSE. The material is unique since it consists of two treatment arms, acyclovir and vidarabine. The results show that high IgG response in CSF is prognostically favourable in the vidarabine group, which suggests that these antibodies are capable of limiting the infection. The analysis of quantitative PCR showed that acyclovir is much more effective than vidarabine in reducing viral load in the CSF. No difference in viral load between outcome groups could be demonstrated. It is difficult to draw conclusions from our quantitative PCR data since the number of patients is small and unevenly distributed between outcome groups. This is a consequence of the study design, as the material originates from a clinical trial in which one of the treatments tested was superior to the other. The main conclusion is that neither quantitative PCR nor antibody levels can be used as a prognostic marker for the individual patient.

In papers IV and V, we study relapsing HSE cases. The incidence in adult patients is approximately 1/8. The definition of relapse is based entirely on clinical signs and symptoms. None of the patients had recurrence of positive HSV-PCR or any other laboratory data to confirm the relapse. These are the most common findings in adult patients with relapses. Analysing cytokines and cell damage markers resulted in the conclusion that, at relapse, there is no anti-inflammatory response (represented by IL-10) that balance the pro-inflammatory response (represented by sCD8). I.e. the pathogenesis in relapsing HSE could be insufficient anti-inflammatory response rather than pro-inflammatory activation. This supports the idea of immuno modulating therapy in relapsing HSE. The cell damage markers had the same kinetics in both relapsing and non-relapsing cases, which suggests a pathogenetic mechanism that is not based on recurrence of the lytic viral infection. There could be a viral activation in relapses even though the HSV-PCR remains negative. A local reactivation in the brain
tissue has been suggested, and HSV has been demonstrated by in situ hybridisation in brain tissue from a relapsing patient (Yamada, Kameyama et al. 2003).

In paper V, we use a follow up protocol, including standardised neuropsychological testing and repeated MRI with volumetry. The described relapse case differs from the cases included in paper IV in time to relapse, in the contra-lateral new MRI-changes and in that he did not recover to the pre-relapse level. As with the cases in paper IV, there are no presently used diagnostic laboratory parameters to define the relapse diagnosis. If repeated MRI and neuropsychological follow-up was used as a routine in HSE patients, this might improve the chance to detect and objectify relapses which otherwise is difficult to diagnose, due to of the lack of diagnostic methods.

Summing up the thesis, the first two studies describe the epidemiology and clinical courses of one mild type of encephalitis (influenza related encephalitis) and one severe type (HSE). As influenza related encephalitis is extremely rare, about 1/10 as common as HSE, which in turn also is very rare, we had no possibilities to further study this disease. The original plan was to study influenza pathogenicity, but the low prevalence of seasonal influenza during the last decade led to change of scope for my PhD-studies. The remaining studies focused on prognostic aspects of HSE. The acute management of the disease is well studied and today’s diagnostic methods are highly sensitive and specific and easily accessible with a low level of discomfort for the patient. Despite adequate diagnostic methods and effective anti-viral therapy, the long time prognosis after the disease is still serious in many patients and 13% of the patients have later neurological deterioration diagnosed as a relapse. There are clinical prognostic parameters, such as consciousness at admission, age, time from onset to therapy. But there are no diagnostic methods that could indicate which patient is at risk for a relapse.
or high level of neurological handicap. Such methods would shed light on the pathogenesis and possibly lead to new therapeutic strategies. In paper III, we show that antibody response (IgG) in the CSF has a role in controlling the infection, but only if treatment is less effective than acyclovir. It is also shown that acyclovir is effective in rapidly reducing the viral load in CSF, but no difference in viral load is seen between outcome groups. In paper IV the incidence of relapse of HSE is 1 of 8 cases and the efforts to find objective diagnostic criteria is as yet unsuccessful. The cytokine analyses suggest that the inflammatory activity at relapse is caused by the absence of anti-inflammatory activity, rather than proinflammatory activity.

Papers III-V support the hypothesis that outcome after HSE to a significant extent is decided by the immunological response to the infection and to a lesser extent by viral load. The immuno-modulating therapeutic strategies studied until today are limited to the addition of corticosteroids to the antiviral therapy, with beneficial effect. The current state of evidence for corticosteroid treatment or other immunomodulating therapy is not sufficient for inclusion into therapeutic recommendations. Nevertheless, there are many indications that much of the damage caused by HSE, both in the acute stage and at relapse, is mediated by the host immune defence rather by direct cytotoxicity of the virus, which suggests that immunomodulating therapy may be beneficial in HSE.
7 CONCLUSIONS

In Sweden, during seasonal H3N2-dominated outbreaks, influenza related encephalitis is a very rare complication, which resolves without sequelae.

The incidence of HSE in Sweden was 2.2 cases per million population per year. There are no diseases that are predisposing for HSE. The post HSE-morbidity is considerable, with elevated risk for recurrent seizures, infections, diabetes and venous thromboembolism.

Quantitative HSV-PCR and antibody levels in serum and CSF are diagnostic in HSE, but have no prognostic value in the individual patient.

High levels of anti-HSV-IgG in CSF are protective in patients treated with vidarabine.

1/8 patients with HSE has a relapse episode and 3 of 4 relapses occur during the first 4 months after the acute HSE onset. Relapse of HSE is a clinical diagnosis, which yet cannot be confirmed by laboratory tests. The clinical picture of the cases is not uniform, suggesting different pathogenetic mechanisms.

At relapse there is, contrary to acute HSE, no anti-inflammatory IL-10 response to modulate the rise in pro-inflammatory activity (sCD 8).
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9 REFERENCES


