Cytomegalovirus and herpes simplex virus infections in the fetus and newborn infant, with regard to neurodevelopmental disabilities

Mona-Lisa Engman
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Mona-Lisa Engman
To my beloved son Andreas
ABSTRACT

The congenital cytomegalovirus infection (CMV) is the most common congenital infection causing childhood morbidity. The majority (85%) of infected infants have no signs of infection in the newborn period, and when sequelae such as a hearing deficit or neurological impairment manifest themselves, the possibility of a CMV infection is easily overlooked.

Neonatal herpes simplex virus (HSV) infection is a rare but devastating infection. Improvements with regard to outcome have been achieved by antiviral treatment, but the morbidity remains unacceptably high in children following neonatal HSV encephalitis.

The general aim of the thesis is to increase the knowledge of childhood morbidity related to CMV and HSV infections.

In the first study the prevalence of congenital CMV infection in a Swedish cohort and the increased risk of hearing deficit in infants with congenital CMV infection compared to uninfected infants were investigated. The results revealed that two infants of 1000 were born with congenital CMV infection and that the risk of congenital hearing deficit in infected infants was 21 times higher than in uninfected ones in this cohort.

In study II neurological morbidity associated with a congenital CMV infection is studied by retrospectively performed diagnosis in children with neurological morbidity on the basis of cerebral cortical malformations. The results revealed that four out of 26 children had a congenital CMV infection but only one had any symptoms suggesting a congenital infection in the neonatal period. Two of the children developed severe disabilities with mental retardation, autism, spastic cerebral palsy and deafness. A third child had epilepsy and unilateral cerebral palsy, while the fourth had a coordination dysfunction and hearing deficit.

Parental reactions in conjunction with screening was evaluated and the results showed that parents of infants with a false positive screening test remained worried about the child’s current and future health after receiving a normal confirmatory test. No difference was found for anxiety and traumatic stress comparing parents to true positive infants, false positive infants and controls.

In study IV, the neuropsychological outcome after neonatal HSV encephalitis was evaluated. The findings revealed that a considerable proportion of the children had cognitive impairments in combination with attention deficits and difficulties with expressive speech. In addition one child with a low average intellectual function suffered a relapse and was subsequently found to have severe mental retardation.

We conclude that congenital CMV infection should be considered in children with hearing deficit or cerebral cortical malformations of unknown origin. If treatment options improve and hearing can be preserved by antiviral treatment, screening could be an option to identify infants at risk. The parents of infants with a false positive screening have been identified as a vulnerable group that requires follow-up as well as parents of true positive infants.

Neuropsychological assessment is essential in the habilitation of the child with neonatal HSV encephalitis and is a tool to monitor deterioration of cerebral function related to relapses.
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<tr>
<td>ABR</td>
<td>Auditory brainstem response</td>
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<tr>
<td>CASG</td>
<td>Collaborative Antiviral Study Group</td>
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<td>CI</td>
<td>Cochlear implantations</td>
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<td>CMV</td>
<td>Cytomegalovirus</td>
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<td>cCMV</td>
<td>Congenital CMV</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<td>CT</td>
<td>Computed tomography</td>
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<td>dBHL</td>
<td>Decibel hearing level</td>
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<td>DBS</td>
<td>Dried blood spot</td>
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<td>DIC</td>
<td>Disseminated intravascular coagulation</td>
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<td>DISS</td>
<td>Disseminated disease</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>DSM IV</td>
<td>Diagnostic and Statistical Manual of Mental Disorders IV</td>
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<td>hCMV</td>
<td>Human cytomegalovirus</td>
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<td>HHV</td>
<td>Human herpes virus</td>
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<tr>
<td>IES-R</td>
<td>Impact of Event Scale-Revised</td>
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<tr>
<td>EEG</td>
<td>Electroencephalography</td>
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<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
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<td>HSV</td>
<td>Herpes simplex virus</td>
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<td>HSV-1</td>
<td>Herpes simplex virus type 1</td>
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<td>IgM</td>
<td>Immunoglobulin M antibodies</td>
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<td>IgG</td>
<td>Immunoglobulin G antibodies</td>
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<td>IQ</td>
<td>Intelligence quotient</td>
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<tr>
<td>MMR</td>
<td>Measles, mumps and rubella</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>PIQ</td>
<td>Performance intelligence quotient</td>
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<tr>
<td>PTSD</td>
<td>Post Traumatisk Stress Disorder</td>
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<tr>
<td>SEM</td>
<td>Skin-eye-mouth disease</td>
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<tr>
<td>SNHL</td>
<td>Sensory neural hearing loss</td>
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<tr>
<td>STAI-S</td>
<td>Spielberger State-Trait Anxiety Inventory</td>
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<tr>
<td>TEOAE</td>
<td>Transient-evoked otoacoustic emissions</td>
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<tr>
<td>TORCH</td>
<td>Toxoplasmosis, Rubella, Cytomegalovirus, Herpes simplex virus</td>
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<tr>
<td>VIQ</td>
<td>Verbal intelligence quotient</td>
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<tr>
<td>WISC III</td>
<td>Wechsler Intelligence Scale for Children III</td>
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<td>S.O.N.-R</td>
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1 INTRODUCTION

This thesis focuses on childhood morbidity caused by herpes viruses transmitted vertically from mother to infant during pregnancy or delivery. The herpes viruses, cytomegalovirus (CMV) and herpes simplex virus (HSV) are grouped together with other congenital infections that can impact upon child health under the classification of TORCH infections.

1.1 TORCH INFECTIONS

The infections comprising the group of so-called TORCH infections are congenital TOxoplasmosis, congenital Rubella, congenital Cytomegalovirus infection and neonatal Herpes simplex virus infection.

1.1.1 Congenital toxoplasmosis

Congenital toxoplasmosis is caused by the parasite Toxoplasma Gondii. This pathogen is a single celled obligate intracellular parasite with a wide range of hosts (1). Humans get infected through ingestion of infected undercooked meat or contact with contaminated soil (1-3).

If a pregnant woman acquires a primary infection of congenital toxoplasmosis during gestation transmission to the fetus may occur. The risk of transmission increases during late gestation but the risk of sequelae occurring is greatest if the fetus is infected early on in the gestation period. In Sweden, toxoplasmosis has a low birth prevalence (4), with about 8-10 infants being infected every year. The majority (85-90%) of infants are asymptomatic at birth, but at risk of developing sequelae later (1, 3), especially in the form of visual impairment, or occasionally, neurological impairments that only become apparent as the child develops. Strain specific differences in the pathogenesis have been suggested to play a role in the variability identified in the clinical outcome (5).

Symptomatic infants characteristically present with hydrocephalus, chorioretinitis and intracranial calcifications (6, 7). If the diagnosis of congenital toxoplasmosis is confirmed in a neonate a combination of antibiotics is recommended for various time periods depending on the country concerned, with the aim being to prevent visual and neurological sequelae (3, 8-10). In Sweden, one year of therapy is currently recommended, however, randomised controlled trials are needed in order to evaluate the outcome in children that have been treated in this manner.

1.1.2 Congenital rubella

The rubella virus can cause miscarriages, stillbirths and fetal anomalies when acquired during early pregnancy (11). Congenital rubella syndrome is characterised by intrauterine growth retardation, microcephaly, purpuric lesions, deafness, cataracts, chorioretinitis and cardiac anomalies (12). Fetopathy arising from congenital rubella has effectively been eliminated by vaccination in countries with high quality vaccination programs (11-13). Vaccination against measles, mumps and rubella (MMR) was included in the childhood vaccination programme in Sweden in1982. In addition, antenatal screening for rubella and the vaccination of rubella seronegative women post partum was introduced, with the result that the last case of congenital
rubella in Sweden was noted in 1986 (11). In countries with less well developed vaccination programmes however, congenital rubella still occurs.

1.1.3 Congenital cytomegalovirus infection

Nowadays, since disabilities arising from congenital rubella have been prevented by vaccination, congenital CMV (cCMV) infection is the most common intrauterine infection causing childhood morbidity (13, 14). Congenital CMV infection like congenital toxoplasmosis, is asymptomatic in the neonate in the majority (85-90%) of infected infants (15-19). Symptomatic infants have a high risk of developing sequelae, but as many as 10-15% of neonatally asymptomatic infants are at risk of developing permanent disabilities with a delayed onset as a result of CMV infection (15-18, 20-23). The sequelae include hearing deficits, visual impairment and neurological disabilities (15-25). Thus, congenital CMV infection poses a significant child health problem, but the morbidity is easily overlooked because of the absence of symptoms in the neonate (18).

1.1.4 Neonatal herpes simplex virus infection

In contrast to congenital CMV infection and congenital toxoplasmosis HSV infections are mostly symptomatic in the neonate (26) and are frequently lethal or associated with a high risk of morbidity (26-28). The infection may be acquired in utero, during delivery or postnatally. The infection is manifested either as localised disease involving skin, and/or the eyes or mouth, as a disseminated disease with sepsis like symptoms or as encephalitis (26, 29).

Early institution of antiviral therapy with acyclovir is essential for the prognosis but, neurological morbidity remains high even in treated infants if the central nervous system is affected (26, 28). Thus, preventive measures are urgently needed to reduce neurological sequelae.

1.2 HERPES VIRUSES

Viruses are small pathogens without organelles and devoid of a metabolism of their own. They are necessarily dependent on a living host cell for protein synthesis and replication. Viruses are classified according to the nature of the nucleic acid (DNA or RNA) in the virion, the symmetry of the capsid, the presence or absence of an envelope and the dimensions of the virion and capsid.

CMV and HSV are double-stranded DNA-containing enveloped viruses (figure 1, page 3) belonging to the herpes virus family. Other members of the family are the Epstein-Barr virus, varicella zoster virus, and the human herpesvirus (HHV) 5-8 (30-32). Herpes viruses are very common in populations and they are mostly acquired early in life. CMV and HSV are distributed worldwide without seasonal variation and are transmitted between humans during close interpersonal contact (15, 16, 19, 29, 33).
In a significant proportion of immunocompetent healthy individuals, the primary infection proceeds without any clinical signs. The herpes viruses cannot be cleared from the body, but persist for the lifetime of the host in a latent state, controlled by the adaptive immune response. Reactivations can occur spontaneously or be triggered by factors such as pregnancy, sun-light, steroids, tissue damage, fever and stress (19, 29, 30, 32, 34). During reactivation, symptoms are usually absent as well, and therefore the individuals remain unaware of their infection (35, 36). The fact that a significant proportion of infections are subclinical have impact on their spreading in the community.

CMV is the largest member of the family (37) and is highly species specific. There are many different strains of human CMV (hCMV). In addition to primary infection and reactivation, CMV infection with a different strain of virus is possible, i.e., reinfection (16, 19, 33, 38). When virus replication occurs, characteristic cell enlargement develops known as “cytomegalic inclusion disease” (16). After primary infection, the CMV virus remains latent in myeloid and dendritic cell progenitors. The antigen presenting cells may allow transport of the virus to endothelial cells, the salivary glands, the urinary tract epithelium and the uterine endothelium upon reactivation of the infection (16). During reactivation, the virus is subsequently shed intermittently from the urine, saliva, breast milk and genital secretions (19).

Herpes simplex virus type 1 (HSV-1) is commonly associated with nongenital infection, while herpes simplex virus type 2 (HSV-2) infection is associated with genital disease (28, 29). HSV-1 and 2 are characterised by neurovirulence (29, 32, 37) and the ability to establish latency in sensory ganglia. The viruses have a short reproductive cycle and rapidly destroy the host cell (37).

The virus first infects epithelial cells of the skin, oral cavity or genital tract. Thereafter virions enter cutaneous sensory neurons and are transported by retrograde flow along axons that connect the point of entry to the nuclei of sensory neurons (39). Following primary infection, the virus resides in the neurons of the sensory ganglia (trigeminal ganglia, sacral ganglia, and cervical ganglia) in a latent state (26, 29, 32). The virus reactivates periodically, with subsequent antegrade translocation of the virus back to skin and mucosal surfaces, causing viral excretion, often in the absence of any other symptoms (26, 39).
1.3 IMMUNE DEFENCE

1.3.1 Innate immune defence

The innate immune response is the first line of defence in the combat against intruding pathogens (33). It is unspecific and has no memory. This defence includes innate recognition and microbial killing by immune cells (macrophages, dendritic cells and neutrophils, NK cells and mast cells) exposed to antigens as well as defence by interferons. After antigen uptake and processing dendritic cells and macrophages migrate to the draining lymphnode where activation of adaptive immune response takes place by antigen presentation to T-cells.

1.3.2 Adaptive immune defence

1.3.2.1 Cellular immune defence

Cellular immunity is an important host defence against viruses and other intracellular pathogens (40). The defence against CMV infection is mediated by the combination of virus-specific CD4 and CD8 T-cell responses (30, 33). T-cells are important in the defence against viral infections because they produce cytokines and kill infected target cells (41).

In the fetus immune cells develop as early as 3 to 4 weeks of gestational age (42) and it has been shown that cellular immune responses can be detected in fetuses with a gestational age of 22 weeks (40), but T-lymphocytes are not believed to be fully functional in fetuses. As the immune system develops, T-cells are selected or regulated to generate immunological tolerance of self and to ensure that they will be reactive against foreign antigens (43). It has been shown that the increased vulnerability to viral infections such as CMV and HSV in fetuses and neonates compared to adults, is associated with decreased reactivity to antigens (42). Reduced cytokine production rather than a lower frequency or absolute number of CMV-specific T-cells is responsible for the difficulties in controlling CMV infection (30). The difficulty that congenitally infected infants have in gaining control of the immunity to the infection is reflected by prolonged viral load in the urine (15, 16, 30). The risk of serious disease and of developing sequelae in congenitally infected infants with CMV is believed to be related to the immaturity of the immune system and failure to gain control over the infection through viral clearance (40).

1.3.2.2 Humoral immune defence

In contrast to congenital rubella and congenital toxoplasmosis maternal antibodies do not protect against intrauterine or perinatal transmission of CMV or HSV, but risk of viral transmission to the fetus is reduced by pre-existing maternal immunity (44). As antibodies protect against viral spreading in the bloodstream (33, 42), it is believed that IgG antibodies transferred via the placenta protect against the spread of the infection by the blood to the fetus. The risk of transmission of CMV declines from about 30-50% without maternal immunity to about 0.2-3.4% if maternal immunity existed before the pregnancy (13, 15, 16, 44). The risk of transmission of HSV to the infant during delivery diminishes from about 30-50% if the mother has a primary infection to less than 1-3% if the mother has a non-primary infection (45, 46).
1.3.3  Immune defence against viral brain infections

HSV and CMV have the capacity to invade and replicate in the CNS. Innate and adaptive immune responses are important for the defence against viral brain infections (42). The first line of immune defence to viral brain infection is mediated through macrophages, microglia (the-end differentiated resident brain macrophages), NK cells and cytokines (42). Thereafter, antigen-specific defence with infiltrating immune cells takes place (42).

The cell types in the CNS that are infected in a congenital CMV infection are incompletely characterised as yet (42). All cell types (brain microvascular endothelial cells, astrocytes, neuronal cells, oligodendroglial cells, microglia/macrophages, neural progenitor cells and neural stem cells) have been shown to be susceptible to CMV but differentiated neurons are refractory to CMV replication (42).

Neural stem cells are located in the ventricular and subventricular region (42). These cells have the ability to proliferate, differentiate (into neurons, astrocytes and oligodendrocytes) and migrate. CMV replication in neural precursor cells has been shown to inhibit proliferation of the cells as well as differentiation into neurons and astrocytes (47, 48). Thus, infection of neural stem cells with CMV may affect brain development in the fetus (42), with a wide spectrum of neurological sequelae being possible, dependent on the phase of the brain development during infection.

1.4  BRAIN DEVELOPMENT

The main phases of brain development are organized into primary neurolation, prosencephalic development, neural proliferation, neural migration, organisation and myelination (49-51) (figure 2, page 6).

The first phase, primary neurolation, begins at four weeks of gestation when a thin sheet of neuroepithelial cells proliferates rapidly to form the neural tube (42, 49). The center of the tube forms the ventricular system and neuroepithelial cells form the different brain regions (42). Neural stem cells reside in the subventricular and ventricular zones. Disturbance of primary neurolation ends up in defects associated with neural tube closure, such as anencephaly, encephalocele and myelomeningocele (49).

Prosencephalic development includes prosencephalic formation, procencephalic cleavage and midline prosencephalic formation, all of which take place at a gestational age of 2-3 months (49, 51). Prosencephalon will give rise to the cerebral hemispheres and thalamus in the mature brain. Abnormalities occurring during prosencephalic development may for instance, end up in causing holoprosencephaly.

Neurons and glia are derived from the ventricular and subventricular zones (50) and cortical development begins with cell proliferation in the ventricular area (52). Neuronal proliferation peaks at a gestational age of 3-4 months. Disturbances in proliferation result in a small brain size and, in most cases, are associated with severe mental retardation (50). The rubella virus has the capacity to disturb proliferation and, subsequently, to give rise to microcephaly. Even CMV may disturb proliferation, but destructive lesions and cortical malformations also contribute to a small brain size (50).

Through the migration of neural cells from the proliferative zones in the ventricular area, the cerebral cortex and deep nuclear structures are formed. Extensive cell migration along specialised radial glia to their final position in the cerebral cortex occurs at a gestational age of 3-5 months (50-54). Incomplete neuronal migration
causes aberrations in the gyral development (50-52, 54, 55). In addition to aberrations in the gyral development agenesis of the corpus callosum and the absence of the septum pellucidum are common findings as they develop coincidentally with the cerebral cortex (50).

Organization begins from the fifth month of gestation and continues postnatally for several years (50, 51). The first step in the organization is the alignment, orientation and layering of cortical neurons, which is followed by an outgrowth of dendrites and axons, synaptogenesis and synapse elimination, apoptosis and glial proliferation and differentiation to astrocytes and oligodendroglia (50). Proper organization of the brain is of the utmost importance for cognitive function. Disturbances affecting organizational events, resulting in, for example, anomalies such as decreased dendritic branching, have been linked to mental retardation (50). Whether neurological sequelae following infections and other perinatal insults are related to disturbances in cortical organization is not currently well understood but have to be further elucidated (50).
The last event in brain development is myelination. Firstly, oligodendroglial proliferation and differentiation take place, thereafter deposition of myelin membrane around the axons follows (50). This phase begins during the second trimester, but peaks after birth (51) and continues into adulthood (50, 51). Myelination within the cerebral hemispheres, in particular, is involved in the higher level associative functions that mature late in adolescence (50). Hypothyroidism, inborn errors of metabolism, periventricular leucomalacia and malnutrition in early infancy may give rise to impaired myelination (50).

1.5 CEREBRAL CORTICAL MALFORMATION

Cerebral cortical malformations are relatively rare, in fact the prevalence in different populations is not even known. In a population-based European multicenter study of 351 children with cerebral palsy assessed by magnetic resonance imaging (MRI) 9% had cerebral cortical malformations (56).

Characteristic cerebral cortical abnormalities attributable to disturbance in cell migration include schizencephaly, lissencephaly, pachygyria, polymicrogyria and cortical heterotopia (50-52, 55, 57). Aberration of gyral development in schizencephaly results in deep grey matter-lined clefts (Figure 3).

The cerebral cortex within the clefts may be thickened and look like pachygyria or polymicrogyria (50). Lissencephaly is characterised by the brain having few or no gyri. The gyri associated with pachygyria are unusually broad and relatively few in number. The brain of a person with polymicrogyria has a cortical surface with a great number of plications. Neuronal heterotopia are clusters of nerve cells that have been arrested in the cerebral white matter during migration. The heterotopias may be focal or organized in a diffuse laminar band in the subcortical white matter, the latter being named “band heterotopia” or “double cortex”. A thick cortical plate with heterotopic grey matter is characteristic of band heterotopia (50).

Migration can be disturbed by inborn genetic errors, such as chromosomal abnormalities or single gene defects, inborn errors of metabolism or can arise from exposure to teratogenic factors during the third to fifth months of gestation (50, 51, 55, 57-61).

Along with other exogenous factors, congenital CMV infection has been clinically linked to malformations of the cerebral cortex (17, 50, 60, 62-65).
1.6 NEUROLOGICAL DYSFUNCTION

The extent, location and timing of damage related to brain development have an impact on the neurological sequelae. The pattern of neurological dysfunction that can be manifested as a result of congenital/neonatal infections is wide. CMV and HSV infection can cause cerebral palsy, epilepsy, cognitive impairments, speech difficulties, hearing deficits and visual impairment. However, it must be emphasised that the neurological symptoms will not be apparent before the age at which a skill is supposed to be acquired. Motor disabilities are most obvious and are easy to recognise early on. For instance cerebral palsy is often suspected when the infant is about 6 -10 months owing to abnormalities in the child’s motor abilities while sequelae such as cognitive impairment and impairment of executive functions will not be apparent until late in adolescence. Executive functions, important for purposeful behaviour such as planning and the ability to shifts one’s attention (66) cannot be expected to be fully developed before 12 – 16 years of age. Behavioural problems resulting from impairment of executive functions include impulsivity, attention deficits, lack of planning and perseveration of action (66). In adults, encephalitis has been shown to result in poor recent and remote memory (66), making neuropsychological assessment crucial in the evaluation of higher cerebral function after encephalitis. Furthermore lesions to the limbic system and amygdalo-frontal pathways have been reported to result in anxiety and mood disorders in adult patients (66).

As yet, the neuropsychological outcome following childhood HSV-induced encephalitis has only been covered briefly, and even then, mostly as single case reports (67, 68).

1.7 HEARING DEFICIT

The prevalence of hearing loss in newborns has been estimated to be 1-4 infants per 1000 (20, 23, 69). The causes of hearing deficits vary widely and include many inherited conditions, such as mutation in the connexin 26 gene, chromosomal aberrations and syndromic deafness, but include many other causes such as asphyxia, prematurity, fetal alcohol syndrome, severe hyperbilirubinemia and congenital infections (69). Hearing loss can be divided into mild hearing loss (21-30dBHL), moderate hearing loss (31-60 dBHL), severe hearing loss (61-90 dBHL) and profound hearing loss (>91 dBHL) (70). Unrecognized bilateral hearing loss will have an adverse effect on speech development. The early identification of hearing loss is vital as hearing has been shown to be important for both language acquisition and social development, and therefore as soon as hearing deficiencies are identified, appropriate interventions need to be undertaken (69, 71, 72). It has for instance, been shown that interventions, before 6 month of age can diminish the negative effects on speech and language acquisition (73). Cochlear implantation before 12 months of age is a tool for improving speech recognition in congenitally deaf infants (72). In order to ensure the advantages of early detection, newborn hearing screening has been implemented in several countries (69, 73, 74).
1.8 CYTOMEGALOVIRUS INFECTIONS

1.8.1 Epidemiology

CMV infection is acquired by contact with infected bodily fluids such as urine, saliva, breast milk and genital secretions (15, 16, 19). The seroprevalence in a population will depend on socioeconomic status, society’s attitude to breast feeding and the consequent method of feeding infants, attendance at day-care centres and sexual habits (15, 16, 19, 23, 75). In the developing countries, seroconversion occurs early in life (15, 16, 23) and the seroprevalence is high (90-100%). In developed countries about 40-70% of women of child-bearing age have CMV IgG antibodies as a sign of passed infection (15, 16, 22, 23).

About 1-4% of seronegative pregnant women will acquire a primary infection during gestation (15, 33, 44). One of the most important risk factors for pregnant women to acquire a primary CMV infection is exposure to young children with a subclinical infection, as they will shed the CMV virus in urine or saliva (16, 18, 76, 77). Horizontal transmission among young children in day-care centres is common, and susceptible parents subsequently get infected when exposed to urine or saliva from their own infected children (15, 16, 18, 33, 76). Hence, this reservoir of CMV virus poses a significant risk for primary infection in pregnant women (15). Another possible means of acquiring the CMV infection during pregnancy is between sexual partners (16, 23, 33, 44, 77).

Primary maternal infection during pregnancy poses a considerable risk to the fetus of acquiring a congenital CMV infection. The risk of transmission of the infection to the fetus via the placenta is reported to be about 30-50% (15, 19, 44). CMV can, however, also be transmitted to the fetus after the reactivation of a latent infection or as a result of reinfection with another CMV strain in IgG seropositive pregnant women (15, 16). If the mother has a recurrent infection, the risk of transmission to the fetus is significantly lower than if the mother has a primary infection, about 0.2-3.4% rather than 30-50% (15, 16, 44). Infants experience sequelae after recurrent as well as primary maternal infection during pregnancy (22, 23, 78, 79). There is no difference in the risk of transmission between the trimesters of a pregnancy (16), however, as mentioned in the previous section, severe sequelae are considered to be more common if the fetus has become infected early in the gestation.

The prevalence of congenital CMV infection varies widely around the world, with reported infection rates ranging from 0.2% to 2.5% of newborn infants (16, 18, 22, 33, 44). Two recent systematic reviews have reported an estimated average birth prevalence of 0.65-0.7% (18, 19). As one might anticipate infection rates for congenital CMV infection tend to be higher in populations with high seroprevalence (15, 16, 23). The positive correlation between higher maternal seroprevalence and high birth prevalence of CMV can be explained by the fact that there are an increased number of pregnant women at risk of reactivations and an increased risk of acquiring reinfections in high seroprevalence populations. Furthermore, seronegative pregnant women have an increased risk of exposure and, subsequently, of experiencing a primary infection (19). According to a study in Sweden conducted in 1970s and 1980s, the prevalence in newborns was 0.5% (22). Since then, it is reasonable to imaging that the prevalence of congenital CMV infection might have changed as a result of increased immigration and changes in life style.
An even larger number of infants acquire CMV infection during birth, or postnatally through consumption of CMV laden breast milk. In contrast to cCMV infection, there seems to be no risk of neurological sequelae in postnatally acquired infection (23, 33, 44) in children born at or near term. However, in preterm infants born before 30 weeks of gestational age and with a birth weight below 1000 gram, a risk of severe CMV disease as a result of transmission in CMV laden breast-milk has been noted (80).

1.8.2 Clinical manifestations in pregnant mothers

In the majority (90%) of pregnant women, a CMV primary infection will pass without any apparent clinical signs. In individuals with symptoms, the infection presents as an infectious mononucleosis syndrome accompanied by lymphadenopathy, fever, muscle pain, headache and tiredness (16, 23, 33).

1.8.3 Clinical manifestations in the neonate

Only about 10-15% of infected infants present with overt signs of disease in the neonatal period. Infants that exhibit clinical abnormalities are classified as having symptomatic congenital infection (15, 16, 18, 23).

The most commonly observed symptoms are petechiae, jaundice and hepatosplenomegaly. About 6-35% of infected infants are born prematurely (33). CMV infection has the capacity to cause placental insufficiency that may cause fetal growth retardation (33, 42), and even influences neuropathogenesis, as mentioned previously (42). Neurological signs in the newborn include microcephaly, seizures, hypotonia and lethargy with poor suck (16, 23, 33). In symptomatic neonates, hearing loss is common and chorioretinitis occurs in about 15-30% of them (23, 25, 33). Interstitial pneumonitis, defects in dentition, with yellow discoloration and inguinal hernia, may also be present in the newborn as a sign of congenital CMV infection.

Congenital CMV infection causes a serious disease in about 5% of infants and is a potentially life threatening disease in neonates, mainly owing to their respiratory insufficiency, but also because of their potential to develop severe hepatic disease, bleeding disorders or to pick up secondary bacterial infections. The mortality rate during the first 6 weeks of life has been reported to be 4-12% (16, 23, 33).

1.8.4 Permanent disabilities

The long-term sequelae attributable to cCMV infection include hearing deficits, visual impairment and neurological disabilities (15, 17-22, 24, 25, 81). A wide range of neurological morbidity, including microcephaly, mental retardation, autism, motor disturbances, cerebral palsy and epilepsy may be the result of cCMV infection. In a review of studies reporting sequelae in neonatally symptomatic children, as well as asymptomatic ones, identified by screening, 17-20% of the children were estimated to manifest permanent disabilities (18). In this estimate, 40-58% of neonatally symptomatic infants and 13.5% of asymptomatic infants developed sequelae. Neonatally symptomatic infants are more likely to experience multiple and more disabling sequelae (16, 18, 23, 82), but the magnitude of sequelae following symptomatic infection varies considerably (27-95%) between studies because of the different criteria used to define symptomatic disease. Several studies have reported that
about 10-15% of neonatally asymptomatic infants subsequently develop long-term sequelae, particularly hearing loss (15-18, 22, 33).

CMV is the leading cause of non-genetic sensorineural hearing loss (SNHL) in children (15, 21, 22, 24, 81, 83). A considerable proportion of infants with symptomatic disease (40-60%) will manifest hearing deficits (23, 81, 82, 84), and hearing loss has been found to evolve in 7-25% of neonatally asymptomatic infants (20, 23, 33, 79, 84). The hearing loss can be either unilateral or bilateral and varies in degree from mild to profound (81). Delayed onset, progression and fluctuation in hearing deficits are important characteristics of cCMV related hearing deficits (16, 20, 21, 24, 72, 79, 81, 84-86). As many as 50% of hearing losses attributable to cCMV have been found to have delayed onset and will consequently not be picked up by a newborn hearing screening programme (21). Such delayed onset hearing loss might not be identified until at least 6 years of age (16, 21, 81, 86). Progressive hearing loss is rather common (85) and in 30-80% of children with unilateral hearing loss, subsequent development of hearing deficits in the normal ear has been found, as has progressive hearing loss in the affected ear (85).

Choriorethinitis has been observed in 15-30% of symptomatic infants (16, 18, 25). In neonatally asymptomatic children subsequent visual impairment and neurological sequelae have been described as well (15, 18, 22), but their occurrence has been less thoroughly studied than hearing deficits. Chorioretinitis in infants who are asymptomatic at birth is considered unusual (23, 25). As far as neurological disabilities are concerned cognitive or other neurological sequelae have been reported to occur in about 6.5% of asymptomatic newborns (18) according to a recent review. However, the results concerning neurological sequelae are inconsistent (23, 33). Some studies report no risk of late-onset neurological or cognitive impairment (87) attributable to a neonatal asymptomatic infection, while others do (18, 22, 33). One study involving a long term follow-up of cCMV infected children, revealed no evidence of cognitive impairment in the children without neurological signs at 12 months of age, when they were assessed at seven years of age (88).

1.8.5 Diagnosis

1.8.5.1 Diagnosis in the mother

Maternal primary CMV infection is proved by IgG seroconversion. The interpretation of IgM gives rise to more difficulties. IgM may persist for several months after primary infection and can reappear at reactivations and reinfection (89). IgM may also be false positive owing to other viral infections and autoimmune diseases (89).

The CMV-IgG avidity test is useful as a tool for dating the time of infection. The avidity measures the binding capacity between antigen and IgG antibodies and increases with time. Thus, low avidity is related to recent infection (89).

1.8.5.2 Prenatal diagnoses

A prenatal diagnosis can be attained through amniocentesis and cytomegalovirus DNA PCR analysis of amniotic fluid. The analysis has a high sensitivity when performed after a gestational age of 21 weeks (23, 33, 89). There may be a delay between the maternal infection and fetal infection, and in line with this observation, an interval of seven weeks between the diagnosis of a maternal primary infection and the
performance of amniocentesis have been proposed (33). Amniocentesis carries a risk of fetal loss of about 1% (44).

Structural abnormalities can be detected by ultrasound, but the possibilities of predicting fetal infection and future morbidity are poor (89).

1.8.5.3 Diagnosis in the child

1.8.5.3.1 Viral detection

Viral culture and/or CMV DNA detection by PCR on samples of urine or saliva are the preferred diagnostic methods. Virus isolation in urine or saliva is highly sensitive for the detection of cCMV infection and is considered a gold standard (23, 44, 89). In order to prove a congenital infection, however, the virus has to be detected before a time-lapse of greater than 2 weeks after birth (16, 23, 33, 89). A viral load detected thereafter may be related to infection acquired during birth, by exposure to infected genital secretions, or postnatally from breast milk if the mother is CMV IgG seropositive (16, 23, 33).

1.8.5.3.2 Serology

The detection of antibodies has limitations in the diagnosis of congenital CMV infection (33). However, serologic methods can be used in combination with other diagnostic tools in CMV diagnosis. The absence of IgG antibodies rules out the possibility of congenital CMV infection, but as IgG antibodies are passively transferred across the placenta, their presence cannot be used to prove a congenital CMV infection in the newborn. If the titre of CMV IgG antibodies declines over the first 6-8 months of life and then disappear, congenital CMV infection can also be ruled out. In contrast, the presence of IgM antibodies is related to fetal infection because they cannot pass the placenta. Thus, IgM antibodies are considered to be diagnostic of a congenital infection if they are found to be present during the first 2-3 weeks of life (15, 44). Unfortunately, the diagnostic sensitivity to IgM antibodies is only 70% (16, 44, 89) and, false positive reactions occur.

1.8.5.3.3 Retrospective diagnosis

The dried blood spot sample (DBS) collected 3-5 days after birth that is obtained in order to screen for inborn errors of metabolism can be used for a retrospective diagnosis by analysing for CMV DNA. (17, 44, 60, 62, 90-92). The DBS are stored in the event that they might be required in conjunction with inborn errors of metabolism or other diseases. Therefore, upon obtaining parental consent, the DBS taken from a child can be retrieved and analysed after consent from parents.

1.8.5.3.4 Other diagnostic assessment

Laboratory-identified abnormalities in infants with signs of congenital CMV disease often include conjugated hyperbilirubinemia, low platelet count and elevations of hepatic transaminases (16, 23).

Neuroimaging may reveal brain anomalies associated with congenital CMV infection, including ventricular enlargement, cortical atrophy, cerebral cortical malformations, white matter abnormalities, parenchymal cysts, ependymal cysts, cerebellar lesions and calcifications (16, 42). The calcifications associated with a cCMV infection tend to
occur in the periventricular area, in contrast to the generally scattered calcifications observed in conjunction with congenital toxoplasmosis. In infants with a congenital CMV infection, apoptotic neuronal damage can also be observed, particularly around the periventricular zone of the brain (42).

An ophthalmological assessment and long-term audiological follow-up is needed in all children with a known cCMV infection.

1.8.6 Treatment

1.8.6.1 Prenatal treatment

Ganciclovir cannot be used during pregnancy because of teratogenicity (93). In a pilot study (93), antiviral treatment with valaciclovir (8g/day) was given to pregnant women with a primary CMV infection who wished to continue their pregnancy after proven symptomatic fetal infection. In providing this treatment, the aim is to try to alter the postnatal outcome in a beneficial way because of the decreased fetal viral load. The results, inevitably measured in terms of an enhanced postnatal outcome, are hard to evaluate owing to the absence of controls, but no adverse effects were noted from using this treatment.

Hyperimmunoglobulin has also been tried in the hope of altering the outcome of intrauterine infection and in an attempt to prevent transmission to the fetus (94). Administration of CMV hyperimmunoglobulin to pregnant women with a primary infection has, however, been shown to be associated with a reduction in the placental thickness (42). In a non-randomised trial (94), the findings indicate that CMV hyperimmunoglobulin seems to enhance the prognosis in fetuses that have already been infected, as well as aiding in the prevention of infection in uninfected fetuses when given to pregnant women with primary CMV infection. However, the therapeutic and prophylactic benefits of the use of CMV hyperimmunoglobulins remain to be determined in a controlled fashion.

1.8.6.2 Treatment of the neonate

Ganciclovir is the only drug available for the treatment of congenital CMV infection, but because of its known side effects, treatment based on this drug requires careful consideration (37, 95). The currently recommended dosage is 6 weeks of intravenous ganciclovir at a dosage of 6 mg/kg twice a day (95) in selected neonates at a high risk of morbidity (figure 4).

The antiviral effect of ganciclovir arises from inhibition of CMV DNA synthesis and, therefore, of viral replication (37, 96). Ganciclovir is virostatic and suppresses active viral replication during treatment, but upon cessation of the therapy, the excretion of the virus recurs (15, 97).

The safety and efficacy of ganciclovir for the treatment of congenital CMV infection have been evaluated in a phase II study (97) and a phase III randomised trial (83). The
side effects of ganciclovir include bone marrow toxicity, particularly neutropenia, but also thrombocytopenia and anemia (83, 95, 97).

Elevated levels of serum creatinine, liver transaminases and bilirubin may also be a consequence of ganciclovir treatment (95). Furthermore, animal studies have revealed it to be carcinogenic and give rise to gonadal toxicity (16, 37, 83). Thus, the long-term safety associated with the administration of ganciclovir to young children has not been established (83).

The phase III study indicated that the frequency and magnitude of hearing deficits may be lessened by antiviral therapy if it is instituted in the neonatal period (83). A recent study also indicated possible beneficial effects on neurodevelopmental outcome at 12 months in treated infants compared with untreated ones (98).

Ganciclovir therapy has been approved in conjunction with rapidly progressive hepatitis, and life or sight-threatening infections (16, 95). Given the high risk of severe neurological sequelae, symptomatic cCMV infection with involvement of the CNS in infants younger than 4 weeks has been considered an indication for ganciclovir therapy by many experts (15, 95, 99), although others consider that treatment should only be given to selected infants within randomised trials until the drug’s toxicity and long-term safety have been evaluated (100). Because of potential bone marrow suppression, weekly monitoring of the blood cell count is required throughout the course of ganciclovir therapy (83, 95).

1.8.7 Prevention

The primary goal from the point of view of prevention is the development of a safe and efficient vaccine, however any vaccine must be able to both induce neutralizing antibodies and introduce cell-mediated immunity (33). Several CMV candidate vaccines including live attenuated vaccines and subunit vaccines have been evaluated in clinical trials (42) but, despite these evaluations, no vaccine has yet been found to protect from cCMV infection. For example, a live attenuated vaccine produced with the Towne strain has been shown to elicit a CMV-specific cell-mediated immune response as well as neutralizing antibodies in immunocompetent adults. However, the vaccine did not protect from CMV infection in seronegative fertile women (42). A subunit vaccine that targets the envelope gB glycoprotein has been evaluated in a phase II study (101) and is currently being evaluated in a phase III efficacy study. This investigation involved the post partum vaccination of seronegative women with the goal of reducing the risk of primary infection leading to congenital infection in subsequent pregnancies (42).

As long as an effective vaccine is not available other methods of preventing CMV acquisition by pregnant women have to be considered. Another approach that has been proposed is that pregnant women could be counselled about the potential risk of acquiring primary CMV infection and given advice on how to prevent CMV infection by adopting hygienic measures (13, 15, 19) such as frequent hand washing with soap, especially after contact with CMV-laden secretions. On the other hand, providing information to pregnant women about potential causes of harm to their unborn child could have an adverse affect on the psychological wellbeing of certain women.

As mentioned before, another intervention that has been proposed is that of administering CMV hyperimmunoglobulin to pregnant women with a known primary CMV infection in order to prevent its transmission to the fetus. A clinical study has
indicated a favourable effect (94), however as this was an uncontrolled investigation, the potential benefits of CMV hyperimmunoglobulin need to be evaluated in randomised controlled studies before this approach could be adopted.

1.8.8 Screening

Screening as a tool for the early detection of potentially preventable morbidity was introduced for phenylketonuria in the 1960s (102). Currently, screening is conducted for phenylketonuria, hypothyroidism, congenital adrenal cortical hypoplasia, galactosemia, and biotinidase deficiency in the newborn screening programme in Sweden. Decisions concerning whether to include other diseases, for example fatty acid oxidation disorders and organic acidurias, are under consideration.

Delayed-onset or progressive hearing loss would be missed by newborn hearing screening programmes (21). Neonatal CMV screening, conducted in combination with newborn hearing screening has been proposed in order to detect late onset hearing loss in this high risk group through the implementation of appropriate audiological follow-up of cCMV infected children (23, 24, 103, 104). As mentioned previously, earlier recognition of hearing deficits and the introduction of interventions to compensate for hearing loss impact positively on language development and social skills (71-73).

Neonatal CMV screening raises three major concerns. The first is that the ability to predict which infant will continue to be asymptomatic and which infant will manifest sequelae later is poor (33, 44). Another is that a positive result to the test may cause parental anxiety that could affect the parent-child relationship in a sensitive period of life (102, 105). The third major concern is that a false-positive screening result could cause parental anxiety even after a normal confirmatory test (102, 106).

Prenatal screening cannot be recommended. Besides parental anxiety and difficult ethical considerations, prenatal screening would lead to unnecessary abortions of asymptomatic fetuses without risk of future morbidity (44, 107).

Because screening could cause anxiety, parental psychological reactions to the screening process would need to be evaluated carefully before a screening programme could be implemented (102).

1.9 HERPES SIMPLEX VIRUS INFECTIONS

1.9.1 Epidemiology

Acquisition of HSV-1 infection often occurs during childhood (26, 108), while HSV-2 infections are acquired in adolescence or later through sexual contact (26). Up to 75-90% of children in the lower socioeconomic populations are seropositive to HSV-1 before 10 years of age (26). In higher socioeconomic populations, the infection is acquired later and the seroprevalence is lower (about 30-40%). In the majority of cases, primary HSV infection in children is asymptomatic (26).

About 20-30% of fertile women are seropositive to HSV-2 (26, 35, 109). Lately an increasing prevalence of HSV-1 genital infection has been noted (26, 28). Maternal genital infection can be classified as primary, non-primary first episode, or recurrent. Primary genital infection with HSV is defined as a new infection with either HSV-1 or HSV-2 infection in HSV seronegative women. A non-primary first episode is defined as HSV-genital infection in a person with a prior infection with HSV-1 (26, 34).
Genital HSV infection in pregnant women poses a risk for neonatal HSV infection (36, 46, 110). About 2% of women in USA acquire a primary genital HSV infection during pregnancy (45, 111). As mentioned previously, the risk of transmission of the infection to the newborn during delivery is about 30-50% if the mother has a primary HSV infection, compared to about 1-3% if the mother has a recurrent infection (28, 45, 46, 110).

The lower seroprevalence of HSV-1 has been suggested to have an impact on the risk of transmission of HSV-2 genital infection by the lack of partial protection from immunity against HSV-1 (26). However, results have been inconsistent. Brown et al did not find any difference in the risk of acquiring neonatal HSV infection between infants born to seronegative compared to HSV-1 seropositive mothers when investigating pooled analysis of sera in 3 cohorts from USA and Sweden (46).

HSV infection in the fetus/newborn can be acquired in utero, intrapartum and postnatally (26, 28, 37, 110). The majority (85%) of infants with a neonatal herpes virus infection are infected during passage through the birth canal. The risk of transmission to the fetus during delivery is increased in the case of prolonged rupture of membranes (> 6 hours), use of fetal scalp monitors or if the mother has a primary infection resulting in a high viral load (34, 36, 110).

Intrauterine viral transmission to the fetus is rare. Only 5% get infected in utero and the risk of congenital HSV infection is highest during the first 20 weeks of pregnancy. Fetal HSV infection may cause abortion, stillbirth and congenital anomalies with a high morbidity rate in surviving infants (45, 110). About 10% are infected post partum. The source of infection postnatally may be contact with relatives or hospital personnel with active herpetic lesions of the mouth or skin (29, 110).

The estimated incidence of neonatal HSV infection varies between 1/1500 and 1/30000 (35, 37, 45, 112). In the Stockholm area, the incidence of neonatal HSV infection has been estimated to be one case in 12,000-13,000 births (112) and in the United States about 1 infant in 3200 deliveries (113). About 70-85% of neonatal HSV infections are caused by HSV-2 (35, 45), and about 25-30% of neonatal infections are caused by HSV-1 (26).

1.9.2 Clinical manifestations in pregnant mothers

In the majority of women (80-90%), genital herpes infections are asymptomatic and remain unrecognized (26, 35, 109). Individuals with symptoms manifest painful vesiculocerative genital lesions. In some individuals complications such as urinary retention or meningitis occur (39) and in rare cases, the viruses give rise to meningoencephalitis (39), mostly caused by HSV-1.

1.9.3 Clinical manifestations in the neonate

Neonatal HSV infection is classified into three categories in accordance with the extent of the disease: (1) skin, eyes, and/or mouth (SEM) disease; (2) Disseminated (DISS) disease involving multiple visceral organs such as the lung, liver, adrenal glands, skin, eye and/or the brain; and (3) central nervous system (CNS) disease with or without skin, eye, or mouth involvement (26, 110, 114).

In SEM disease, skin vesicles of 1-2 mm in diameter on an erythematous base are typically observed and occur in 90% of cases. Signs of eye involvement include
1.9.4 Permanent disabilities

Long-term morbidity after a neonatal HSV disease is associated with the extent of the disease (110, 113) and the type of virus (27, 36, 110, 116, 117). Disease localised to the skin, eyes and mouth is associated with a good prognosis in the majority (about 89%) of cases (117). However frequent skin recurrences may be related to poor immunological control of the HSV infection (118). It has been suggested that combined with cutaneous lesions, viremia occur and there is a subsequent risk of spreading to the CNS, even in infants with SEM (110, 117). Three episodes or more of cutaneous HSV recurrences during the first 6 months of life predict a risk of a poorer than average neurodevelopmental prognosis (117, 119, 120). Furthermore, SEM disease may develop into DISS disease or CNS disease if it is not properly treated (26).
In DISS disease, 35-40% of infants and in CNS disease 64-70% of infants are at risk of having sequelae (110, 113, 117). For those infants with central nervous system involvement, the risk of neurological sequelae has been shown to be increased if seizures are present when antiviral treatment is initiated (110, 113, 117). Persistence of HSV DNA in the cerebrospinal fluid at the end of antiviral treatment has been shown to be linked to a poor neurodevelopmental outcome (116).

Infection with HSV-2 has been linked to a higher risk of morbidity than HSV-1 (27, 28, 36, 116, 117). The mechanisms behind the increased risk has been suggested to be that HSV-2 is less susceptible to acyclovir than HSV-1. (117).

1.9.5 Diagnosis

1.9.5.1 Diagnosis in the mother

Viral detection from vesicles in the genital tract provides the diagnosis. However, serological diagnosis could be used to evaluate the risk of transmission to the neonate. If the mother has vesicles in the genital tract and is IgG seronegative, primary HSV infection should be suspected and a high risk of transmission during delivery should be anticipated. Antibodies to HSV-1 cannot distinguish between antibodies due to infection of the oropharynx and those arising from genital infection. However the presence of HSV-2 antibodies in pregnant women is suggestive of recurrent infection if vesicles are found in the genital tract (26).

1.9.5.2 Diagnosis in the neonate

1.9.5.2.1 Viral detection

The presence of a replicating virus from any location is an important tool for establishing neonatal HSV disease (114). The likelihood of obtaining a positive HSV culture in infected infants is highest when the culture is taken from skin lesions or conjunctivae, regardless of the subgroup of the disease (113). In addition, viruses can be isolated from the mouth, nasopharynx, urine, cerebrospinal fluid (CSF), blood and stools (29, 113). Detection of viral DNA by PCR from any specimen, including cerebrospinal fluid, has improved the possibility of obtaining a rapid diagnosis (114, 116, 121, 122). Herpes simplex virus DNA detection in CSF by PCR has been proven to have good specificity and sensitivity in adult patients with HSV encephalitis (121). The sensitivity in neonatal HSV CNS disease has been reported to be lower, about 74% (28, 110, 114, 116, 118, 119). The diagnostic sensitivity can be increased to about 90% by combining herpes simplex virus PCR in CSF and herpes simplex virus PCR in blood (28, 110, 116). Thus, negative herpes simplex virus DNA PCR in cerebrospinal fluid does not exclude HSV with central nervous system involvement (28, 110, 114, 116, 118, 119, 122, 123), especially when the samples are taken early in the course of the disease. Consequently, serial lumbar puncture has to be performed to diagnose infants whose clinical course reveals a likelihood of HSV infection with CNS involvement (116, 118).

1.9.5.2.2 Other diagnostic tools

Diagnostic evaluations should also include, liver transaminases and a cerebrospinal fluid examination conducted to determine the CSF indices (26, 29, 124). Liver transaminases are commonly elevated in DISS disease (125). CSF examination often
reveals pleocytosis and proteinosis (28), but some infants with a CNS infection have normal CSF, making a certain diagnosis difficult to obtain. In HSV encephalitis an increasing albumin content is usually seen in conjunction with HSV encephalitis during the course of the disease.

Abnormalities are seen from electroencephalography (EEG) in the majority (about 80%) of infants with CNS involvement at some stage during the progression of the disease, but abnormalities mostly appear after the first signs of CNS involvement have been presented (116). Thus serial investigations may be needed to detect abnormalities on EEG that are suggestive of encephalitis.

HSV infections give rise to widespread hemorrhagic necrosis, with devastating effects on brain parenchyma (34). About 65% of patients have been shown to have abnormalities on neuroimaging (113). These lesions can be detected by MRI after 1-3 days after the onset of clinical symptoms (116). Abnormalities in computed tomography (CT) will appear later (116). Abnormalities that are visible through neuroimaging include localized or multifocal areas of abnormal parenchymal attenuation, atrophy, edema, and hemorrhaging involving the temporal, frontal, parietal, and subcortical regions of the brain (26, 116).

1.9.6 Treatment

Effective antiviral agents have been available for the treatment of neonatal HSV disease since the late 1970s (113). Early institution of therapy is essential to prevent widespread viral dissemination and, especially, replication of the virus within the CNS (113). The duration of the treatment depends on the extent of the disease. Currently, the recommended dose is acyclovir 20 mg/kg every 8 hours given intravenously for 2 weeks for skin, eye and mouth disease and for 3 weeks in the case of CNS or DISS disease (37, 110, 114) (figure 5).

Figure 5. Treatment of neonatal herpes simplex virus infection

Treatment with acyclovir is associated with few side effects, however neutropenia have been reported (29, 37, 108, 110, 124). Thus, the absolute neutrophile count must be monitored at least twice weekly during the course of the acyclovir therapy (114, 124). If the absolute neutrophile count falls below 500/m³ it might be necessary to decrease the dosage or to administer granulocyte colony-stimulating factor (110, 124). Nephrotoxicity is another well known side effect (110, 124).
Infants with CNS involvement should be monitored with a repeat lumbar puncture to ensure that the CSF is PCR negative before the intravenous acyclovir therapy is discontinued (110, 114, 116).

1.9.7 Prevention

Prevention of neonatal HSV infections is a challenge because the majority of latently infected fertile women are unaware of that they have an HSV infection and because asymptomatic shedding occurs during delivery (35). Even primary infection during pregnancy tends to occur without symptoms (26). Only about 25% of the women who give birth to an infant with a neonatal HSV infection have some relevant symptoms at the time of delivery (26).

Efforts to prevent the spread of genital herpes include the advocacy of safer sex habits involving the use of condoms but as genital herpes is commonly spread as a consequence of asymptomatic shedding, such prophylactic strategies are unlikely to be met with great success (39). In order to prevent genital HSV infection in fertile women, the use of condoms during pregnancy could be promoted (29, 111), especially in serodiscordant couples.

The highest risk for transmission to the newborn infant is when a woman with a primary genital infection during the third trimester, gives birth vaginally (110). If a primary HSV genital infection occurs later than gestational week 35, or if active HSV lesions are present in the birth canal at the onset of delivery, caesarean section is recommended (29, 35, 45). Infants born vaginally to mother’s who have had a recent primary HSV infection should receive phrophylactic treatment with acyclovir (60 mg/kg/day) intravenously for 10 days (110).

Another preventive measure is prophylactic administration of acyclovir to pregnant women with a genital herpes outbreak close to delivery (45, 109, 110), but as subclinical shedding can occur during therapy transmission to the neonate may be possible in spite of treatment (110).

If there is a risk of perinatal exposure to HSV during vaginal delivery, culture should be obtained from the infants’s conjunctivae, oropharynx and nasopharynx at 24 hours of age in order to achieve an early diagnosis (45, 110). The infant should be monitored thoroughly until neonatal infection can be excluded. If there is any presenting sign of infection, treatment should be instituted immediately.
2 AIM

2.1 GENERAL AIM

The general aim of this thesis is to increase the knowledge of childhood morbidity related to CMV and HSV infections, to provide a basis for rational decision-making concerning preventive measures, treatment programmes and future research.

2.2 SPECIFIC AIMS

The specific aims of this research were to:

**Paper I:** Study the prevalence of congenital CMV infection in a Swedish population of newborns and to investigate the relative risk of hearing deficit in newborns with congenital CMV infection.

**Paper II:** Investigate the impact of congenital CMV infection as a causative agent in cerebral cortical malformations.

**Paper III:** Study parental psychological reactions in conjunction with neonatal screening for congenital cytomegalovirus infection.

**Paper IV:** Investigate the neuropsychological morbidity after neonatal herpes virus encephalitis and to determine the relationship between neonatal neuroimaging and the neuropsychological outcome.
3 METHODS

3.1 DESIGN AND SUBJECTS

3.1.1 Studies I and III

Study 1 is a prospective cohort study where the birth prevalence of cCMV infection is evaluated by screening. In contrast, study 3 is a case-control study intended to evaluate parental psychological reactions associated with neonatal CMV screening. A breakdown of the subjects included is presented in figure 6 and the procedures adopted are illustrated in figure 7, page 23.

In Study 1, infants born at two participating hospitals, Karolinska University Hospital, Huddinge, and Södertälje Hospital were consecutively screened for congenital CMV infection, through the identification and subsequent analysis of CMV DNA on the DBS. The study was performed over a 12 months-long period (October 2003-June 2004; August 2004-October 2004).

Parents were informed of the screening at the maternity ward by their midwives and written information on the study was provided in order to obtain informed consent. During the period in which the research was conducted 6199 infants were born and the parents of 6060 (98%) infants gave their consent to their infant’s participation. At the participating sites, the parents of all infants were offered an assessment of their infant’s hearing within a newborn hearing screening programme. The increased risk of a hearing deficit in cCMV infected infants was evaluated by comparing the prevalence of
hearing deficits in cCMV infected infants and in uninfected infants identified in the newborn hearing screening programme.

The parents of infants with a positive screening test were contacted by a pediatrician and offered an appointment the following day. At this meeting, the parents were informed about congenital CMV infection, their infant was clinically assessed and a confirmatory test was conducted on a urine sample for rapid culture of CMV. At the meeting, the parents were also informed about the psychological follow-up study (study III), which included an assessment based on questionnaires (table 1, page 29). Parents were provided with pre-paid self-addressed envelopes to return the completed questionnaire forms directly to the research psychologist. Thus, the pediatrician was blind to how individual parents had responded. Matched control parents were selected by contacting the parents of the next infant on the list with a negative CMV screening test. The control parents received information about the investigation and the questionnaires by letter.

**Figure 7**

The parents were informed of the result of the urine sample in a telephone call from the pediatrician made after about one week. Infants with a true positive CMV screening test were followed-up until four years of age to monitor their growth, developmental
milestones, and any visual impairment and hearing deficits. Infants with a negative urine sample were considered to have had a false positive screening test and were not followed-up.

3.1.2 Study II

Study II is a descriptive study with a mainly retrospective design. The group under investigation consisted of patients with neurological disabilities, such as cerebral palsy, epilepsy, developmental delay and mental retardation, in whom cerebral cortical malformations had been detected by neuroimaging. The children concerned had been diagnosed at the neuropediatric clinic of the Karolinska University Hospital, Huddinge over a period of two decades (1988-2008). Children born before 2000 were included retrospectively while, children born thereafter were included prospectively at the time when the diagnosis was made.

During the period in which the research was conducted, 30 children who met the inclusion criteria were identified. The parents of one child refused to allow the child to participate and one child had died. In addition, two of the children had been born abroad and were excluded because it was not possible to get retrospective diagnoses, through an analysis of a DBS. Thus 26 children were included.

A retrospective diagnosis of cCMV infection was established through the detection of cytomegalovirus DNA on a DBS. If the child was CMV IgG negative, a cCMV infection could be excluded, and consequently the DBS was not analysed. Neuroimaging was retrospectively reviewed by a child neuroradiologist. Although the neuroradiologist had been informed about the purpose of the study, at no point did he know the results of the virological analysis. The records were examined by a pediatrician.

3.1.3 Study IV

Study IV consists of a cohort study conducted by a retrospectively undertaken neuropsychological follow-up. In the cohort of 267,690 infants born in the Stockholm area over a period of 12 years (1989-2000), 18 infants were diagnosed with neonatal HSV infection and one with a congenital HSV infection (figure 8, page 25). Surveillance of HSV infections was performed at the virological laboratories serving the area and the infants concerned were subsequently followed up clinically. Of the infants with a neonatal HSV infection, 5 had SEM disease and 12 had CNS disease. One had a postnatally acquired infection classified as DISS disease with CNS involvement. The infant who contracted the infection in utero had a widely disseminated disease also involving the CNS. Thus 14 infants fulfilled the inclusion criteria of the study, ie a neonatal HSV infection/congenital HSV infection with involvement of the central nervous system. The parents of these children were contacted by letter and the parents of four children refused to participate and one child was lost to follow-up. Thus nine children were included. Each of these children was clinically assessed, and the child’s records were reviewed; neuroimaging was reviewed in a blinded fashion by a pediatric neuroradiologist and a neuropsychological assessment was performed by an experienced neuropsychologist.
3.2 ASSESSMENTS

3.2.1 Studies I and II

3.2.1.1 CMV DNA analysis on DBS

The DBS obtained at birth were used for the extraction of DNA and, subsequently the polymerase chain reaction (PCR) assay was used to detect any eventual CMV DNA in the sample. PCR is a technique used to amplify a specific region of a DNA strand (32) and thereby generate a huge number of copies of the sequence concerned. It has been used in diagnostics and science since the 1980s. In diagnostics, a target sequence of DNA, for example a sequence of CMV viral DNA is selected for amplification, then the PCR reaction is performed in cycles of heating and cooling. Upon heating, the two strands in the double stranded DNA molecule are separated into single stranded DNA (a process known as denaturation). Subsequently, each strand is used as a template in DNA synthesis at a lower temperature. The selectivity to the selected region is achieved by the use of primers (short DNA fragments) containing sequences which are complementary to the starting point of the target DNA region. The primer binds to the DNA template when the temperature decreases (annealing). The DNA polymerase then builds a new DNA strain from nucleotides (the DNA building blocks) by using the DNA sequence concerned as a template, with the primer as the starting point (the extension). Each cycle doubles the number of DNA molecules.

Real-Time PCR can be used to determine the numbers of DNA copies in the sample. The quantification is achieved through analysis of the product while the reaction is in progress by using fluorescent dyes which react with the amplified product.
In study I, DBS collected at the age of 3-5 days for screening of inborn errors of metabolism were used to screen for CMV DNA using a TaqMan-based quantitative real-time PCR. The method was chosen because it had been proven to have a high sensitivity and specificity earlier (90, 91, 126), as well as being feasible for use in the available samples.

In study II, DBS were used to establish a retrospective diagnosis of cCMV infection by use of the qualitative nested PCR technique.

The extraction of DNA was performed as follows: two discs from each DBS card were placed in a tube with 50 µl of cell culture medium, Minimal Essential Medium (GibroBRL/Life Technologies, UK). The samples were then incubated at 56°C for 1 h and at 95°C for 10 min. After rapid cooling, the samples were centrifuged for 5 min at 14,000 r.p.m. and the supernatant from each sample was moved to new tubes and stored at -70°C for a minimum of one hour before they were analysed (90).

The quantitative TaqMan-based cytomegalovirus DNA PCR assay has previously been described by Yun et al. (127). In brief, the primers and probe were selected from the pol region and the PCR reaction was performed in a 96-well optical reaction plate. The amplification was performed under the following conditions: 50°C for 2 min; 95°C for 10 min; 50 cycles of 95°C for 10 s and 58°C for 15 s. The number of threshold copies/unit volume was set to be at least 1 after at most 50 cycles. The final amplified product was a 66 base pair fragment. The human albumin gene was used as a control for the DNA extraction to detect inhibition causing false negative results. Positive cases were analysed in triplicate. In order to reduce the risk of false negative results, every hundredth negative sample (n=62) was reanalysed.

The method of qualitative nested PCR has been described by Fischler et al (126). In brief, a nested PCR was used consisting of 30 + 30 cycles. The outer and inner primers were derived from the immediate early region. The amplification started with an initial cycle of denaturation at 95°C for 5 minutes, primer annealing at 55°C for 30 seconds, and primer extension at 72°C for 1 minute, followed by 28 similar cycles with a denaturation time of just 30 seconds. The last cycle differed only with regard to the primer extension time, which was 5 minutes. After the first amplification, 5 µl of the amplified product was transferred to a new reaction mixture and another 30 cycles were carried out.

A set of HLA DQ primers, GH 26/27, was used as a control of DNA extraction to detect inhibition causing false negative results.

In order to rule out the possibility of cross-contamination from neighbouring cards, the stored cards before and after the card of the infant concerned were also analysed.

### 3.2.2 Study I

#### 3.2.2.1 Serological analysis of pregnant women

In the first trimester, pregnant women in Sweden are screened for rubella, HIV, hepatitis B and syphilis (11). This blood sample, taken in early pregnancy was used to analyse CMV IgG antibodies and make a comparison with the findings in post-delivery sera.

Primary maternal infection was defined by seroconversion between analysis in the first trimester and postpartum sera or the presence of CMV-specific immunoglobulin M (IgM) antibodies during pregnancy. Maternal infection was defined to be recurrent if CMV immunoglobulin G (IgG) specific antibodies without IgM were detected in the...
serum from the first trimester and if an elevation in the levels of IgG antibodies could be identified when a comparison was made between prepartum and postpartum sera.

In study I, serum samples from 1000 pregnant women were investigated for CMV IgG antibodies by an enzyme linked immunosorbent assay (ELISA) (128) to elucidate the seroprevalence among pregnant women in a Swedish population. Purified nuclear CMV antigen (AD169) was used and the cut-off level for seropositivity was an absorbance of ≥0.2 at a dilution of 1/100.

3.2.2.2 CMV analysis of stillborn infant

At the participating hospitals, the possibility of occurrence of intrauterine CMV infection is routinely assessed in all cases of stillbirth (from 22 weeks of gestation) by CMV IgG analysis of maternal sera and CMV DNA analysis of the placenta.

3.2.2.3 Hearing assessment

In paper I a hearing assessment was performed by screening newborns within a bedside universal newborn hearing screening programme. Neonatal hearing screening, based on transient-evoked otoacoustic emissions (TEOAE) was implemented in southern Stockholm in 1998. TEOAE are physiological activity within the cochlea that occur in response to acoustic stimuli such as clicks and tone pips. TEOAE were recorded in the non-linear quickscreen mode with ILO 288 (Otodynamics Ltd.) according to the following pass criteria: whole wave reproducibility ≥ 70%, signal-to-noise ratio (S/N) ≥ 3 dB in at least 3 of the upper 4 wide frequency bands provided by ILO instrument (centre frequencies from about 1500 to 4000 Hz +/- 400 Hz b.w.) and at least 50 sweeps.

A follow-up of the children’s hearing was performed by behavioural audiometry at 2 years of age (± 3 months) and play audiometry at 4 years of age (± 6 months). The majority of assessments were performed at the audiological clinic by an audiologist, but one of the children was only assessed by a trained nurse at the well-baby clinic at 4 years of age.

3.2.2.4 Ophthalmological assessment

The ocular examinations in study I were conducted by a paediatric ophthalmologist. The assessment was adapted to the age and cooperativeness of each child. Visual acuity was assessed with Teller acuity cards (Vistech Consultants, Inc.) or by testing the child’s ability to fixate and follow small objects. In older children, the best corrected decimal visual acuity with optotypes was assessed. Other evaluations included tests of ocular motility, binocularity (Lang, Forch, Switzerland) and a cover test performed near to the eye to detect strabismus. Retinoscopy and indirect ophthalmoscopy were performed after administration of cycloplegic eyedrops.

3.2.3 Study III

3.2.3.1 Investigation of parental attitudes and psychological reactions

The assessment instruments used in study III to evaluate parental attitudes and psychological reactions related to neonatal CMV screening are outlined in table 1, page 29.
Parental attitudes to screening in general and the parents’ experiences of their participation in the current study were elucidated by a study specific questionnaire. In this questionnaire, questions related to the parents’ worry about their infant’s current and future health were also included.

Situational anxiety was assessed by the state anxiety module of the Spielberger State-Trait Anxiety Inventory (STAI-S) (129). The inventory measures the state of people’s current anxiety levels and is a widely used self-reporting instrument with well-documented psychometric properties.

Stress outcomes were assessed using the Impact of Event Scale-Revised (IES-R) (130, 131). The IES-R is designed to address stress responses in the dimensions of intrusion, avoidance, and hyper-arousal. The three symptom categories correspond with the B-, C-, D-criteria of Post Traumatic Stress Disease (PTSD), according to the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) (132).
<table>
<thead>
<tr>
<th>Instrument</th>
<th>Aim</th>
<th>Items</th>
<th>Scale steps</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Study specific questionnaire</em></td>
<td>To investigate attitudes and experiences</td>
<td>What was your experience of being asked to participate in CMV screening?</td>
<td>5 scale steps a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>What is your attitude in general to screening infants for rare diseases?</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>What is your general opinion of the screening of rare, but untreatable conditions in infants?</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>How would you evaluate the pre-screening information provided?</td>
<td></td>
</tr>
<tr>
<td><em>Study specific questionnaire</em></td>
<td>To investigate worry</td>
<td>Are you worried about your infant’s current physical health?</td>
<td>4 scale steps b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Are you worried about your infant’s future health?</td>
<td></td>
</tr>
<tr>
<td><em>STAI-S</em></td>
<td>To investigate anxiety</td>
<td>20 items are included in the scale. Representative items include statements such as “I feel nervous” and “I feel worried”</td>
<td>4 scale steps c)</td>
</tr>
<tr>
<td><em>IES-R</em></td>
<td>To investigate post traumatic stress</td>
<td>22 items have been included divided into: Intrusion 8 items</td>
<td>5 scale steps d)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Avoidance 8 items</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hyperarousal 6 items</td>
<td></td>
</tr>
</tbody>
</table>

a) 1=very negative; 2=negative; 3=neither negative/nor positive; 4=positive; 5=very positive  
b) 1=not at all; 2=to some extent; 3=rather; 4=considerably  
c) 1=not at all; 2=to some extent; 3=rather; 4=very much  
d) 0=not at all; 1=to some extent; 2=rather; 3 considerably; 4=very much
3.2.4 Study IV

3.2.4.1 Neuropsychological assessment

The nine children included were between 2.5 and 13 years of age at the time of their assessment. One boy had a repeat assessment three years after the original assessment was conducted because of clinical deterioration.

Cognitive functions were assessed using standardised test material including the Griffiths Mental Development Scales I and II (133), the Wechsler Intelligence Scale for Children (WISC) (134), and the Snijders-Oomen Non-Verbal Intelligence Test Revised (S.O.N.-R) (135). The psychologist chose the test that best corresponded with each child’s developmental level and ability to cooperate.

An overview of the tests used in the assessments of cognitive functions is provided in table 2.

<table>
<thead>
<tr>
<th>Type of test</th>
<th>Age in years</th>
<th>Verbal function (scale)</th>
<th>Performance function (scale)</th>
<th>Global cognitive function (scale)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Griffiths I scale</td>
<td>0 – 2</td>
<td>C (Hearing and Speech)</td>
<td>D (Eye and Hand Coordination)</td>
<td>C + D + E</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>E (Performance)</td>
<td></td>
</tr>
<tr>
<td>Griffiths II scale</td>
<td>2 – 8</td>
<td>C (Hearing and Speech)</td>
<td>D (Eye and Hand Coordination)</td>
<td>C + D + E + F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F (Practical reasoning)</td>
<td>E (Performance)</td>
<td></td>
</tr>
<tr>
<td>WISC III</td>
<td>6 – 16</td>
<td>Verbal Intelligence Quotient (VIQ)</td>
<td>Performance Intelligence Quotient (PIQ)</td>
<td>Full-Scale Intelligence Quotient (VIQ + PIQ)</td>
</tr>
<tr>
<td>S.O.N.-R</td>
<td>2.5 – 7</td>
<td>5.5 – 17</td>
<td>Generalized Intelligence Quotient</td>
<td></td>
</tr>
</tbody>
</table>

Griffiths I is composed of 5 scales (A,B,C,D,E) and Griffiths II of 6 scales (A,B,C,D,E,F). The scales measure the following skills; the A scale (locomotion), the B scale (personal and social skills), the C scale (Hearing and Speech), the D scale (Eye and Hand Coordination), the E scale (Performance), and the F scale (Practical Reasoning). For the purpose of this study, the A scale was excluded to avoid the assessment of cognitive functions that might be affected by any reduced motor ability arising from neurological impairment. The B scale was also excluded because the mixed tasks in this scale have little relevance for the evaluation of cognitive functions.

The WISC measures the Verbal Intelligence Quotient (IQ), Performance IQ and Full-Scale IQ. Corresponding to verbal IQ and performance IQ in WISC, the Griffiths C and F scales were used to assess verbal function and the D and E ones the assessment of performance function.
The Snijder-Oomen Non-Verbal Test-Revised was used to measure cognitive functions without being influenced by the ability to communicate using spoken language.

3.3 STATISTICS

In paper I, the risk of hearing deficit in cCMV-infected infants compared to uninfected infants was calculated by determining the odds ratio using logistic regression and its corresponding 95% confidence interval. An odds ratio equal to 1.0 was tested using the Wald statistic. An odds ratio greater than 1 is interpreted as implying an increased risk of a hearing deficit in the CMV group. An odds ratio equal to 1.0 indicates that there is no difference between the groups comprised of children with and without CMV infection. P-values of < 0.05 were considered to be statistically significant.

In paper III, the differences between groups in terms of worry, anxiety and stress were analysed using one-way ANOVAs. Post hoc comparisons were made using the Tukey test. All tests were two-sided and p< 0.05 was considered to be statistically significant.

3.4 ETHICAL CONSIDERATIONS

All papers have been approved by the regional ethics committee. Ethical implications in research are related to the principle of autonomy and the receipt of informed consent. With regard to autonomy, there is a duty to protect children because of their diminished autonomy. The children who participated in the studies reported here have all been assessed in accordance with the guidelines for clinical practise.

The most concerning ethical issue in this research project was performing screening in asymptomatic infants for a potentially harmful infection with delayed onset of morbidity and without adequate treatment options. It cannot be excluded that parents could feel profound anxiety and stress associated with a positive screening test. In order to further evaluate this issue, paper III was designed to disclose parental reactions in conjunction with screening from a psychological and ethical point of view.
4 RESULTS

4.1 RESULTS OF STUDY I

4.1.1 Congenital CMV infection

The screening of 6060 newborns resulted in 12 infants with confirmed cCMV infection (true positive screening test) and 9 infants with positive CMV screening test but negative confirmatory virus isolation in urine (false positive screening test). Furthermore a possible positive screening test occurred in 6 infants (only a few CMV DNA copies were identified in one out of 3 PCR runs). The latter would not have been considered a positive test in clinical practise, but for scientific reasons a confirmatory urine sample was taken and found to be negative in all cases. No stillborn infant were found to have congenital CMV infection.

Requiring both of the criteria specifying congenital infection to be fulfilled (positive screening and positive confirmatory urine sample) we estimated the CMV birth prevalence to be 0.2% (95% CI 0.1%-0.3%).

None of the infants had any overt signs of infection at birth. Their birth weight and head circumference were appropriate for their gestational age. Three of the infants were born preterm (table 3). In one case, the prematurity could have been caused by a twin pregnancy. The twin boys were born in gestational week 34 and only one of them was congenitally infected, however the other acquired a postnatal CMV infection.

One boy born to a mother with a recurrent CMV infection had a unilateral hearing deficit detected by the newborn hearing screening and confirmed by auditory brain stem response at one month of age. A follow-up conducted with play audiometry at four years of age revealed severe unilateral hearing deficit (70-85 dBHL). At the follow-up conducted at four years of age, another child (child K) determined to have normal hearing in the newborn screening was found to have a mild unilateral hearing deficit (20-35 dB HL). While this mild hearing deficit was intermittent and the child also had signs of otosalpingitis, the otosalpingitis was the most probable explanation of the hearing deficit, but fluctuations in hearing, related to the CMV infection cannot be excluded.

No signs of chorioretinitis were found in any child in the ophthalmological assessment up to 3 years of age.
Table 3. Epidemiological and clinical data of children with CMV infection identified by screening.

<table>
<thead>
<tr>
<th>Infant</th>
<th>Gender</th>
<th>Gestational age (weeks)</th>
<th>Maternal infection</th>
<th>CMV DNA Copies on DBS sample (M)</th>
<th>Virus isolation in urine (age in days)</th>
<th>Newborn hearing screening</th>
<th>Follow-up of hearing at four years of age</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Female</td>
<td>40</td>
<td>Primary</td>
<td>9</td>
<td>Pos (21)</td>
<td>N</td>
<td>–</td>
</tr>
<tr>
<td>B</td>
<td>Female</td>
<td>41</td>
<td>Primary</td>
<td>≤5</td>
<td>Pos (21)</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>C</td>
<td>Female</td>
<td>40</td>
<td>Primary</td>
<td>≤5</td>
<td>Pos (29)</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>D</td>
<td>Male</td>
<td>34</td>
<td>Secondary</td>
<td>58</td>
<td>Pos (35)</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>E</td>
<td>Male</td>
<td>38</td>
<td>Probable primary</td>
<td>18</td>
<td>Pos (18)</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>F</td>
<td>Male</td>
<td>40</td>
<td>Secondary</td>
<td>140</td>
<td>Pos (24)</td>
<td>UHL</td>
<td>Severe UHL</td>
</tr>
<tr>
<td>G</td>
<td>Male</td>
<td>36</td>
<td>Primary</td>
<td>≤5</td>
<td>Pos (17)</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>H</td>
<td>Male</td>
<td>35</td>
<td>Primary</td>
<td>13</td>
<td>Pos (26)</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>I</td>
<td>Female</td>
<td>39</td>
<td>Secondary</td>
<td>39</td>
<td>Pos (33)</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>J</td>
<td>Male</td>
<td>40</td>
<td>Secondary</td>
<td>276</td>
<td>Pos (42)</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>K</td>
<td>Female</td>
<td>40</td>
<td>Primary</td>
<td>25</td>
<td>Pos (35)</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>M</td>
<td>Female</td>
<td>37</td>
<td>Suspect secondary</td>
<td>≤5</td>
<td>Pos (49)</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

M = mean value of three PCR runs
N = normal
UHL = unilateral hearing loss

4.1.2 Maternal seroprevalence

Analysis of IgG antibodies in the prepartum sera, taken during the first trimester revealed that 72% of the pregnant women were CMV IgG positive and thus 28% were susceptible to acquiring a primary CMV infection during pregnancy.

4.1.3 Relative risk of hearing deficit

Of the 6119 infants screened within the newborn hearing screening programme, 27 (0.4%) had either a unilateral or a bilateral hearing deficit. One of the infants had cCMV infection. He had no family history of hearing deficits and there was no evidence for any other causative agent of the hearing deficit. The odds ratio for hearing
deficit in CMV-infected infants compared with uninfected infants was 21.3 (95% CI 2.6-170.8; p=0.4).

4.2 RESULTS OF STUDY II

Four out of 26 children with cerebral cortical malformations had CMV DNA detected on the DBS, indicating congenital CMV infection. Neither of the DBS for neighbouring cards were positive for CMV DNA, hence contamination could be excluded. Six children were CMV IgG negative and 16 had no detectable CMV DNA on DBS. The clinical data for the CMV positive children are presented in table 4.

Table 4. Clinical characteristics of the children with congenital CMV infection.

<table>
<thead>
<tr>
<th>Child</th>
<th>Gender</th>
<th>Neonatal symptoms</th>
<th>Result of Neuroimaging</th>
<th>Neurological Sequelae</th>
<th>Hearing deficit</th>
<th>Cochlear implantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Female</td>
<td>Petechiae</td>
<td>Band heterotopia</td>
<td>ULCP, EP, MR, autism</td>
<td>Bilateral</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>No</td>
<td>Unilateral pachygyria</td>
<td>Coordination dysfunction</td>
<td>Bilateral</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>Female</td>
<td>No</td>
<td>Unilateral Sylvian pachygyria</td>
<td>ULCP, EP, MR, autism</td>
<td>Bilateral</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>No</td>
<td>Right perisylvian pachygyria</td>
<td>ULCP, EP</td>
<td>No</td>
<td>–</td>
</tr>
</tbody>
</table>

ULCP = unilateral cerebral palsy; EP = epilepsy; MR = mental retardation

Two of the children with a congenital CMV infection had severe disabilities, including mental retardation, autism, cerebral palsy and bilateral deafness (table 4). Only one had symptoms in the neonatal period suggestive of a congenital CMV infection. In fact, the diagnosis in the symptomatic girl was established during pregnancy because of growth retardation identified in the sixth gestational month. Fetal head ultrasound revealed ventricular dilation. Serology of the mother showed CMV IgG antibodies without IgM antibodies, pointing at a recurrent maternal CMV infection. The amniocentesis revealed CMV DNA in the amniotic fluid. The mother was treated with antiviral therapy (valaciclovir) during the last 6 weeks of gestation in order to decrease the viral load in the hope of altering the postnatal outcome in a beneficial way. At birth, in gestational week 38, the girl had petechiae. The trombocyte count was within the normal range. The child’s head circumference was appropriate for the gestational age at birth, but the head growth thereafter was abnormal (-3 SD). Neonatal hearing screening revealed bilateral deafness, which was confirmed by the auditory brain stem response at two months of age. Ophthalmological assessment was normal. Signs of neurological disabilities were obvious from about four months of age. MR imaging at four years of age showed microcephaly with large ventricles, subependymal calcifications and calcifications in the right frontal lobe. The grey matter appeared almost like band heterotopia with thick cortex and sparse gyri (figure 9). The cerebellum was normal.
The other girl (child 3) with severe multiple handicaps was born at term and had no signs of infection in the neonatal period. At five months of age she had developmental delay. Unilateral cerebral palsy was diagnosed at the neuropediatric clinic at 6 months of age and at that age, a hearing assessment revealed bilateral deafness. Ophthalmological assessment was normal. Computed tomography at 10 months of age showed unilateral pachygyria.

Serological analysis in the stored prepartum sera from the mother in early pregnancy show that she was IgG seronegative, but IgM positive. After delivery IgG antibodies were found indicative of a primary infection during gestation. Retrospectively considered, the mother was found to have experienced mononucleosis like symptoms early in pregnancy.

Child 2 was found to have developmental delay detected at four months of age. When his hearing was assessed at eight months, he was found to be deaf. He had a delay in gross motor function and could not walk before two years of age. MRI at one year and nine months of age showed abnormal signals in most white matter, focal pachygyria around the right Sylvian fissure, with local heterotopias, possibly also on the left side. He has developed coordination dysfunction. There is no evidence of decreased intellectual ability, however there are signs of a decreased attention span. Child 4 was assessed because of unilateral cerebral palsy and epilepsy. Signs of cerebral palsy manifested at six months of age and epilepsy presented at 12 months of age. There was no evidence of cognitive impairment or hearing deficit in this child. MRI at one year and ten months of age showed thick cortex in the right hemisphere, especially around the Sylvian fissure, and heterotopic gray matter in adjacent white matter. The right cerebral peduncle was thin.

### 4.3 RESULTS OF STUDY III

The Parents’ evaluations and attitudes were generally approving or very approving to neonatal CMV screening across the groups, regardless of whether their own infant had tested positive or whether the parent concerned belonged to the control group.

The parents of the infants with a false positive screening test were more worried about their child’s current and future health than the parents of the controls, while the difference was not statistically significant for the parents of true positive infants.

There were no differences between the parents of true positive infants, the parents of false positive infants and the parents of the controls with regard to anxiety as measured by STAI-S, or stress as measured by IES-R. The results are presented in table 5.
Table 5. Parental attitudes to screening and parental distress indicators grouped according to how the relevant infant tested for CMV infection at the screening and the differences between the groups.

<table>
<thead>
<tr>
<th>Instrument Items</th>
<th>True positive</th>
<th>False positive</th>
<th>Controls</th>
<th>(p)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study specific questionnaire</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>What was your experience of being asked to participate in CMV screening? a)</td>
<td>4.07</td>
<td>3.65</td>
<td>4.03</td>
<td>0.14</td>
</tr>
<tr>
<td>What is your attitude in general to screening infants for rare diseases? a)</td>
<td>4.21</td>
<td>4.31</td>
<td>4.58</td>
<td>0.26</td>
</tr>
<tr>
<td>What is your general opinion of screening for rare, but untreatable conditions in infants? a)</td>
<td>3.64</td>
<td>3.36</td>
<td>3.92</td>
<td>0.12</td>
</tr>
<tr>
<td>How would you evaluate the pre-screening information provided? a)</td>
<td>3.79</td>
<td>3.42</td>
<td>3.56</td>
<td>0.52</td>
</tr>
<tr>
<td>Are you worried about the infants current physical state of health? b)</td>
<td>3.50</td>
<td>3.80</td>
<td>3.23</td>
<td>0.021</td>
</tr>
<tr>
<td>Are you worried about your infant’s future health? b)</td>
<td>2.93</td>
<td>3.12</td>
<td>2.43</td>
<td>0.037</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Sum of items</strong></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>STAI-S</strong></td>
<td>20 items c)</td>
<td>35.76</td>
<td>37.61</td>
<td>33.83</td>
</tr>
<tr>
<td><strong>IES-R</strong></td>
<td>Intrusion (8 items) d)</td>
<td>18.07</td>
<td>19.27</td>
<td>16.88</td>
</tr>
<tr>
<td></td>
<td>Avoidance (8 items) d)</td>
<td>14.36</td>
<td>15.41</td>
<td>14.53</td>
</tr>
<tr>
<td></td>
<td>Hyperarousal (6 items) d)</td>
<td>9.71</td>
<td>10.18</td>
<td>10.29</td>
</tr>
<tr>
<td></td>
<td>Overall level of traumatic stress d)</td>
<td>42.14</td>
<td>44.86</td>
<td>41.71</td>
</tr>
</tbody>
</table>

* Anova main group effect for three groups. Parents of true positive, false positive and control infants

a) 1 = very negative, 2 = negative; 3 = neither negative/nor positive; 4 = positive; 5 = very positive
b) 1 = not at all; 2 = to some extent; 3 = rather; 4 = considerably
c) 1 = not at all; 2 = to some extent; 3 = rather; 4 = very much
d) 0 = not at all; 1 = to some extent; 2 = rather; 3 = considerably; 4 = very much

4.4 RESULTS OF STUDY IV
4.4.1 Clinical manifestation in the infants

The majority of the infants were born at term. In addition to the infant with a congenital infection, one boy (B) was born preterm in gestational week 36. The presenting sign in seven of the infants was that of seizures between 9 and 37 days after birth. All children
exhibited abnormalities on EEG. Herpes simplex virus DNA was found in the CSF of all the infants with the exception of the infant with a congenital HSV infection. In this boy, the diagnosis was established by virus isolation from vesicles. Cerebral involvement was indicated by the high protein content in the CSF and pronounced brain damage was evident from CT. Only two of the infants displayed mucocutaneous involvement: these infants with skin vesicles were born after a maternal primary infection and were the most severely impaired of the children studied. No one of the mothers with a primary infection had any signs of infection at delivery. Hence there was no possibility of prevention.

Six infants were born as a result of a reactivated HSV infection in the mother and one was born as a result of a postnatally acquired infection. Only three of the mothers with a recurrent HSV infection had a history of genital herpes and two had signs of this at delivery.

### 4.4.2 Permanent disabilities

The cognitive ability of the studied children was greatly affected. An overview of the cognitive impairments is shown in table 6. Only two children had an average intellectual ability, and two others had intellectual function at the lower level of the normal range. Five children had mental retardation, of whom two had mild mental retardation, while three had severe mental retardation. In addition, with the passage of time one of the children (B) with intellectual function at the lower level of the normal range seemed to lose abilities gained. Upon conducting a repeated neuropsychological assessment in this child at the age of five years, the results revealed severe mental retardation and autistic behaviour. MRI at that time showed parenchymal loss in the left hemisphere (figure 10).

![Figure 10. MRI. Parenchymal loss in the left hemisphere.](image)

Attention deficits were found in seven of the children studied. Only two children were considered to have impaired social skills when the initial assessment was conducted, but all children but one had difficulties with expressive speech. Because of a relapse a third child developed autistic behaviour later on. The majority of children seemed to feel well and did not display symptoms of anxiety or depression. Two children were considered to have poor wellbeing. One of them was a girl whose school situation did not meet her educational needs. She had not received any aid for communication problems despite having pronounced difficulties in verbal language and motor
dysfunction because of bilateral cerebral palsy. The other child was severely compromised with severe mental retardation and highly limited motor ability because of bilateral cerebral palsy.

The children that did not participate in the study were between 6 and 10 years of age. All four attended normal school classes and only one of them had extra educational support.

Eight children included in the study had cerebral palsy and three of the children that did not take part in the neuropsychological follow-up had cerebral palsy.

<table>
<thead>
<tr>
<th>Disability</th>
<th>Children included in the study</th>
<th>Not included</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CNS (N=7)</td>
<td>Diss (N=1)</td>
<td>Congenital (N=1)</td>
</tr>
<tr>
<td>CP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mental retardation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild mental retardation (IQ ≤70)</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Severe mental retardation (IQ &lt;50)</td>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Intellectual function in the lower level of normal range</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Educational support</td>
<td>6</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Attention deficit</td>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Difficulty with social interaction</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Difficulty with expressive speech</td>
<td>7</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Decreased well-being (anxiety)</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*) One child lost for follow-up

Neurodevelopmental outcome of the children were not well correlated with the extent of cerebral damage as visualised by computed tomography at 7-28 days after onset of signs.
5 DISCUSSION

5.1 DISCUSSION PAPERS I-III

Congenital CMV infection is the most common intrauterine infection causing childhood morbidity (14, 33). In the USA, where there is a relatively high birth prevalence (0.5-2.0%), cCMV infection is estimated to cause more disabilities than other known etiologies, such as Down syndrome, fetal alcohol syndrome, and spina bifida (13, 104). Congenital CMV infection is the leading cause of non-genetic sensorineural hearing loss in children (15, 21, 24, 81, 83) and an important cause of neurological impairment (17, 18, 23, 33). According to a study performed in the 1970s the birth prevalence in Sweden was 0.5% and long-term morbidity was identified in 18% of the children (22). The first study was undertaken in order to get up-to-date information of the birth prevalence in a Swedish population (southern Stockholm) by screening for CMV DNA on the DBS. Another aim of study 1 was to investigate the proportion of CMV-related hearing deficits in the population studied, by comparing results from newborn hearing screening with results from neonatal CMV screening. The results revealed a birth prevalence of 0.2% (95% CI 0.1%-0.3%) and, in this cohort, the risk of hearing deficits was about 20 times higher in infected infants than in uninfected ones.

An investigation of the maternal seroprevalence was also conducted. The results revealed that 72% of pregnant women in early pregnancy were IgG seropositive to CMV. Thus, about 30% (1818) of 6060 are susceptible to primary CMV infection during pregnancy. According to the expected rate of primary CMV infection, of about 1% among susceptible pregnant women, about 18 women would be estimated to sustain a primary infection in a group the size of ours. Thus, a transmission rate of 40-50% would result in 7-9 infants with congenital CMV infection. In our study, seven infants were born as a result of primary CMV infection and five as a result of recurrent infection.

As mentioned previously, hearing loss is a well-known consequence of cCMV infection. In this cohort, hearing deficit occurred in one infant in 12, thus it’s occurrence was in accordance with other studies (20, 33, 84). However, less is known about the spectrum of neurological sequelae following cCMV infection. The damage can take many different forms and differences in neurological outcome evolve due to damage to the fetal brain at different gestational ages (42). Congenital CMV infection has the capacity to disturb normal central nervous system development during proliferation and migration (50, 51). Zones of active myelination may also be vulnerable to damage by CMV virus (17, 60). The gestational age at the time of CMV infection has important implications for the subsequent neurological outcome in the child (42) because the susceptibility of the brain to CMV infection is believed to decrease with gestational age (42). However, the pathogenetic mechanisms in the development of sequelae are poorly understood. The injury may evolve due to damage of viral infection or immunopathology.

Cerebral cortical malformations have been clinically linked to cCMV infection and reported in several case reports (62-65), in addition to which, they have been mentioned in reviews concerning cerebral cortical malformations (17, 61). Research designed to study pathogenetic mechanisms has also been undertaken. In stem cell research human
CMV (hCMV) has been shown to inhibit induction of neural differentiation and induce apoptosis in infected cells (47) a finding that supports the teratogenic potential of cCMV infection. In animal models, the CMV virus replicates in neuronal progenitor cells in the subventricular area (33). In order to evaluate the contribution of cCMV in children with neurological disabilities on the basis of cerebral cortical malformations, study II was conducted. The findings revealed that four out of 26 children with neurological disabilities and migrational disturbances attending the neuropediatric clinic over a 20 year period had a cCMV infection, thus more than expected from the birth prevalence (0.2-0.5%) in Sweden (22, 136).

There are several known factors that may cause cerebral cortical malformations (51, 61), but in a considerable proportion of children with migrational disturbances, the etiology remains unknown. These malformations result in neurodevelopmental anomalies (52) such as cerebral palsy, mental retardation and severe epilepsy (51, 55, 57, 59). In clinical practise a better knowledge of the etiology of neurological impairments is important, but it is often difficult to achieve. For instance, the opportunity to inform parents of the etiology of their child’s cerebral cortical malformations is of the utmost importance in counselling the parents of children with such malformations about their child’s prognosis and the likelihood of other children that they might have inheriting the same neurological problems. In case of cerebral cortical malformations, the time of their onset can be no later than the fifth month of gestation as the migration takes place in earlier gestational months, thus complications in the perinatal period, such as asphyxia can be excluded (50). Genetic aberrations and inborn errors of metabolism are known etiological factors (51) as well as exposure to teratogenic exogenous factors during the migration of neural cells to the cortex (61). The finding in study II highlights the need to consider cCMV in the diagnosis of children with cortical malformations of unknown etiology.

Without screening or an increased knowledge amongst medical practitioners and in the general population, many cCMV infections will escape detection, even in severely neurologically compromised children. Neonatally symptomatic infants are more likely to experience multiple and more disabling sequelae (16, 18, 23), but the majority of children with cCMV related morbidity are asymptomatic at birth because of the much higher proportion of infants infected who do not develop signs of infection in the neonatal period (18). There is a need to be aware of the character of cCMV infection: in particular, that it can cause morbidity with delayed onset, and therefore it should be considered as a potential causative agent in children with hearing deficits, neurological impairment and visual impairment of unknown origin. The possibility of establishing a retrospective diagnosis is afforded by the ability to analyse the DBS, seeking CMV DNA (17, 60, 62, 89-91). The method may also be used in screening because of the feasibility of conducting large-scale investigations. The analysis of DBS has been shown to have high sensitivity and specificity in earlier investigations (89-91, 126), but the sensitivity has been reported to vary between 70% and 100% (92). Hence, studies using screening of CMV DNA on DBS may report lower prevalences than studies using screening by viral culture of urine. Furthermore, the sensitivity and specificity could be influenced by degradation of the viral DNA during storage, contamination between adjacent cards and the particular sequence of viral DNA chosen for use in detection if it (92). Analysis of CMV DNA on a DBS followed by viral culture of urine in study I yielded discordant results in 9 cases out of 21 considered to have a
positive screening. Thus, only 12 out of 21 positive DBS samples was confirmed by virus isolation in urine suggesting either difficulties associated with the specificity or the possibility that CMV DNA detection in neonatal blood is a more sensitive diagnostic tool than virus isolation in urine. This finding highlights the need to further evaluate the diagnostic potential and usefulness of CMV DNA analysis on DBS as a tool for retrospective diagnosis and screening.

The diagnosis of congenital CMV infection in relation to postnatal infection must be thorough as the risk of sequelae is only valid for congenital CMV infection (33). Hence, it is necessary to base the diagnosis on analysis of the DBS in all infants/children more than 2 weeks old. Since, the specificity and sensitivity of the diagnostic ability of CMV DNA analysis on DBS have not yet been established, different diagnostic tools should be combined whenever possible.

Hence, we found hearing deficit in an expected number of infants at birth (20, 33, 81, 84) and the proportion of cCMV infections in children with cerebral cortical malformations was considerable. The childhood morbidity found in these studies and in others (18, 19, 22-24, 81) point to the urgent need for prevention of cCMV infection. The importance of a vaccine for the prevention of sequelae associated with cCMV infection has been evident for decades (137). The benefits of vaccination are apparent, for example, the widespread use of a vaccine against rubella has dramatically decreased the number of cases of congenital rubella syndrome (11, 138). The capability of herpes viruses to establish latency and to be reactivated are amongst the challenges in vaccine development (31). Another concern is that preconceptional CMV immunity does not protect against congenital infections as a result of infections with a new CMV strain or reactivation of latent CMV infection (15, 16, 44). Nor does pre-existing maternal immunity protect from symptomatic disease (14, 22, 23, 33, 78, 79).

The need for the development of other interventions to reduce the disease burden of congenital cytomegalovirus infection is evident. Knowledge about the risk factors associated with congenital CMV infection may help to reduce the incidence of CMV infection among pregnant women. As contact with cytomegalovirus laden excretions from asymptomatic toddlers places seronegative pregnant women at serious risk of developing primary CMV infection, pregnant women would be able to prevent infection by hygienic practises if they were better informed about the potential risks (13, 19).

Neonatal hearing screening in combination with neonatal CMV screening, has been proposed in an attempt to enhance the outcome of children with late onset CMV-related hearing loss by ensuring that those at danger of developing such hearing loss and communication-related problems are provided with close monitoring and receive early interventions (24, 103, 104). Early rehabilitation and cochlear implantations (CI) in children with hearing loss from cCMV infection have been shown to enhance the possibilities of achieving spoken language (72, 139), and might consequently also improve social development (71). Furthermore, the benefit of providing antiviral treatment to newborn infants with hearing deficits is becoming apparent as it seems to preserve hearing and prevent subsequent deterioration in hearing loss (83).

However, screening of potentially harmful diseases in asymptomatic infants raises ethical and psychosocial concerns. As far as genetic screening is concerned, the issue of ethical and psychosocial consequences linked to obtaining informed consent has been
thoroughly evaluated (140). Likewise, neonatal screening for a congenital CMV infection has to be considered from the viewpoint of obtaining informed consent as asymptomatic infants who may be predisposed to the development of sequelae will be screened. Capacity to give informed consent will only be achieved if the parents understand the natural course of the infection, the risks and benefits associated with screening, the meaning of a false positive test, if they are able to consider the options and finally communicate their choices. Study III was designed to explore parental attitudes and psychological reactions to neonatal CMV screening. The results revealed that parents were generally approving of screening of this kind. As one would anticipate, a positive screening test resulted in heightened worry about the infant’s current and future health. However a significantly heightened worry about the health of their infant was detected only in the parents of false positive infants when they were compared to controls. This possibly arose because of the limited power to detect differences between the relatively few parents of positively tested infants and the control parents. Nevertheless, a similar result has been found in studies concerning metabolic diseases, genetic diseases and congenital hypothyreosis (102, 141, 142), revealing that the parents of false positive infants compose a vulnerable group that might continue to be worried in spite of receiving information that their infant has received a normal confirmatory test. Neither the symptoms of anxiety, nor the severity of stress discriminated between the parents of the infants who tested positive at screening from the control parents. Follow-up routines may contribute to relief from uncertainty and anxiety among parents and should be offered to the parents of infants with true positive screening as well as a false positive screening test.

Knowledge of the pathogenesis of long-term sequelae is of fundamental importance to guide prevention and treatment strategies. Late onset sequelae related to congenital CMV infection may be caused by chronic CMV infection, viral reactivation, immunological mechanisms or late presentation of damage already present at birth (94, 143). Furthermore, predictors of the long-term outcome, especially the neurodevelopmental outcome, in congenitally infected infants are essential for counselling the parents. Efforts to find factors of prognostic significance have revealed that neonatal symptomatic disease, pathological findings on neuroimaging and a high viral load detected in the blood or urine of infants are all linked to a higher risk of long-term sequelae (16, 103, 143-147). Among the symptomatic infants petechiae and intrauterine growth retardation have been identified as clinical predictors of hearing loss (148).

A correlation between a high viral load in the DBS and an increased risk of sensorineural hearing deficit has been found (103). This finding was also valid for study I, where the only infant with hearing deficit had higher number of genomic copies of CMV DNA on the DBS than the majority of infants in the study group (table 3, page 33). Another study, however, could not confirm the association between a high viral load and an increased risk of hearing deficit (149). This study showed that the positive predictive value of high viral load and risk of hearing deficit was low. However, a low viral burden predicted a good hearing outcome in asymptomatic infants. Since, a high viral load might, therefore, be a pathogenetic factor in hearing loss, infants deemed to be at risk may comprise a subgroup that could benefit from treatment. Data in a randomised controlled trial suggest that progressive hearing
deterioration in children with a high viral load can be prevented by antiviral therapy (83). In line with that hypothesis long-term antiviral treatment could afford even more benefits to the hearing outcome.

Antivirals with good bioavailability when administered orally are needed if long-term antiviral treatment is to be provided. Valganciclovir, the orally bioavailable prodrug of ganciclovir has been evaluated to determine its pharmacokinetic properties (95, 150, 151). In addition, a clinical trial is ongoing at present to evaluate the improvement of outcome with regard to hearing deficits in children treated with valganciclovir given orally for 6 weeks as opposed to for 6 months (152).

Treatment particularly for those with a high viral load and no evidence of hearing loss could be an option (33) in the future if antiviral agents with a high effectivity and low frequency of adverse effects are developed. However, based on current knowledge of the side-effects and benefits, treatment in order to prevent hearing loss cannot be recommended in asymptomatic infants (15, 37).

The investigations conducted have certain limitations. Study I is limited because the use of CMV DNA might be less sensitive (23) than virus isolation in urine used in a previous epidemiological study in Sweden (22). We also had a high number of positive results from DBS screening that were not confirmed by virus isolation in urine. Whether, these findings reflect low specificity or have any clinical importance remains to be determined.

Another limitation is the small sample size of infants with a cCMV infection. The risk of a hearing deficit was significantly higher among the infected infants than the uninfected ones, but the wide confidence interval limits the possibility of generalising and drawing conclusions about the magnitude of the increased risk.

The limitations in study II are related to its retrospective design. Not all children attending the neuropediatric clinic were investigated by neuroimaging, and therefore it is possible that the prevalence of cerebral cortical malformations was underestimated. Furthermore, the group of children was too small to generalise any conclusions about the prevalence of cCMV infection in children with cerebral cortical malformations, but the findings support the consideration of cCMV infection when seeking the cause in children with cerebral migrational disturbances.

Study III is also limited by small sample size of the parents of true positive infants and by the drop outs in this group. It cannot be excluded that the parents of infants with a positive screening test who did not participate in the study might have been more anxious than the participants. Another shortcoming is that the assessment was made a short time after the screening test was conducted making it impossible to draw conclusions about long-term effects. Some other studies with a long-term follow-up have revealed that a positive screening test had resulted in a negative effect on parent-child attachment (105).

In summary, congenital cytomegalovirus infection impacts considerably on children’s health, but a precise knowledge of its effect is limited. The opportunity to establish a retrospective diagnosis has improved considerably through the ability to analyse a DBS for CMV DNA. A congenital CMV infection should be considered and evaluated in children with a hearing deficit and/or cerebral cortical malformations of unknown etiology. Children known to have had a congenital CMV infection need to be monitored to identify any hearing deficit that develops, but also to ensure that
neurodevelopmental disabilities are not missed. Evaluations of whether the viral load proves useful in predicting hearing loss and whether antiviral therapy can be used to prevent hearing deficit remain to be made. Whether the introduction of a screening programme should be considered in the future depends on the benefits of improving the outcome of those at risk of developing hearing deficits or with such deficits at birth through antiviral treatment and other interventions.

Reliable estimates of the sequelae of congenital CMV infection can only be achieved by the long-term follow up of children identified by systematically performed screening, but an active surveillance program for children with a known CMV infection will also aid in increasing knowledge of the spectrum of disease and the common health burden. Currently, there is a voluntary Swedish national reporting system linked to an interactive database concerning infectious diseases during pregnancy, called INFREG (8).

5.2 DISCUSSION PAPER IV

Herpes simplex virus-induced encephalitis is a rare but devastating infection of the brain parenchyma affecting newborns, children and adults. Beyond the neonatal period, it is reported to occur in 1-4 cases per million individuals every year (153, 154). Approximately one third of these cases occur in children. The majority of neonatal HSV infections involving the CNS are caused by HSV-2, whereas after the neonatal period, herpes-induced encephalitis is caused by HSV-1 (28, 34, 153).

With reference to the incidence of neonatal HSV infections reported in the Stockholm area (112), on average two infants get infected with HSV in the neonatal period, in an area with approximately 25,000 births every year. Of these infections, more than 50% can be expected to involve the CNS (110), either as part of a disseminated disease or a disease localised to the CNS. Herpes infections in the newborn are potentially life-threatening, and children who survive are at great risk of developing long-term sequelae.

In the majority (85%) of cases, neonatal HSV infection is transmitted to the newborn during delivery by a mother with a genital HSV infection (26, 28, 34, 110) through viral shedding as a result of a primary infection or a recurrent infection. The greatest risk of transmission is when the mother has a primary genital HSV infection during the third trimester. The risk of transmission is lower in the case of recurrent infection, but considering that about 30,000 women who give birth every year in Sweden have a latent genital HSV infection with potential risk of reactivation, the numbers of children with sequelae related to neonatal HSV infections will be as high after recurrent infections as after primary ones (28).

Improvements in the outcome have been achieved by antiviral treatment, however early recognition and the institution of antiviral therapy are considered to be essential in reducing mortality and morbidity by preventing widespread viral dissemination and especially replication of the virus within the CNS (124). Antiviral treatment has decreased the mortality from about 85% in DISS disease and 50% in CNS disease to 29% and 4% respectively. Morbidity in disseminated disease has decreased from about 50% to 30%. Difficulties achieving a better prognosis in CNS disease have been noted and only about 30 % of those afflicted escape permanent disabilities (26, 110, 114) in spite of antiviral therapy.
Diffuse symptoms early in the course of the disease and an absence of vesicles are linked to the failure to initiate antiviral treatment prior to dissemination of virus and viral replication in the brain. The average time between the first symptoms of the disease and admission to hospital has been shown to be four days (110).

Prominent features of encephalitis are seizures and altered consciousness, but by the time these signs have presented themselves there is already an infection of the brain parenchyma (27, 116). Hence, much of the CNS damage that could give rise to future sequelae may occur prior to antiviral therapy being given.

The majority of studies evaluating long-term outcome after neonatal HSV-induced encephalitis have focused on neurological sequelae such as epilepsy, cerebral palsy and developmental delay or mental retardation (26, 27, 36, 116). After childhood encephalitis caused by HSV, neuropsychological dysfunction, developmental delay, behavioural impairment and language disturbances have been described (153). However, the neuropsychological outcome following childhood HSV-induced encephalitis has only been touched upon briefly by researchers and most the publications produced take the form of single case reports (67, 68). A more detailed evaluation of verbal and performance cognitive abilities, memory and attention abilities, communication, and social and emotional abilities has not, to the best of our knowledge, been performed in a cohort of children after neonatal HSV encephalitis. In study IV, children with neonatal HSV infection with involvement of the CNS are assessed to examine their neurodevelopmental outcome.

Among the children studied, cognitive ability was greatly affected. At the initial investigation, only two of the tested children had average intellectual ability and two had intellectual function at the lower level of the normal range. Five children had mental retardation, of whom two had mild and three had severe mental retardation. In addition, seven had a short attention span and eight had difficulties in expressive speech. Anxiety, behavioural disturbances or difficulties in social interaction were not a pronounced finding. The cerebral function of one infant with intellectual function at the lower level of the normal range deteriorated. Because he seemed to lose abilities that he had gained, a new evaluation was performed at the age of five years, and he was then shown to have severe mental retardation in combination with autistic behaviour.

Another five children with neonatal herpes encephalitis belonged to the cohort, but did not participate in the neuropsychological assessment. Parents of children with cognitive difficulties which impacted on daily living were more prone to give consent to their child’s participation in the study. Of the five children who did not take part in the investigation, one was lost to follow-up and the other four children attended a normal school, and only one of them needed extra educational support. Three of the children had cerebral palsy.

Thus in evaluating the results, the selection bias of there being more cognitive impairments in the children studied than in those omitted must be considered. In the cohort of 14 children, cognitive dysfunction was proved in seven, six of whom had mental retardation, the other had intellectual function in the lower level of the normal range. In summary, at least six of the 14 children manifested mental retardation (43%), seven of 14 manifested attention deficits (50%) and eight of 14 manifested difficulties with expressive speech (57%). Of the 14 children with neonatal herpes encephalitis, eleven in the cohort manifested cerebral palsy (78%).

Findings of cognitive impairments in combination with attention deficits and difficulties in expressive speech such as those seen here have implications for the
habilitation of children who have had neonatal herpes encephalitis. The children need to be met with expectations in accordance with their abilities and to facilitate cognitive function, external support through the provision of very concrete tools and in the use of particular teaching tasks is needed to reinforce the thinking process. A child with a short attention span needs frequent pauses during the learning process. Further, pharmacological therapy with stimulants such as methylphenidate can improve deficits in attention. Difficulties in expressive speech requires aids for communication such as pictures and sign language. The majority of the children had surprisingly good ability where social interaction was concerned, considering the extent of their neurological disability. However, in cases of autism spectrum disorders, there is a need for assistance to improve social ability and communication.

The high proportion of neurological sequelae in the CNS disease in spite of antiviral treatment is a major concern and points to the need for prevention. If genital HSV infection can be prevented, the majority of neonatal infections will also be prevented. The ultimate prophylaxis should be prevention of genital infection or prevention of viral shedding during reactivations by vaccination, but currently no effective prophylactic vaccine against genital HSV infections is available (39, 45). The advice to use a condom during pregnancy, especially in serodiscordant couples has also been proposed (29, 39). The possibility of preventing transmission to newborns during delivery when the mother has a genital HSV infection is limited because the mother’s infection usually goes unrecognised (35).

The chance of improving outcome through earlier institution of therapy is also limited. The majority of infants with SEM and DISS disease present during the second week of life, while infants with CNS disease present somewhat later, during the third week of life. The initial presenting signs may be diffuse. It is not possible to treat every infant with diffuse symptoms presenting during the first weeks of life with aciclovir in order to enhance the outcome of the few infants with HSV infections involving the CNS because many infants will receive treatment without HSV infection and be placed at unnecessary risk of adverse effects such as neutropenia (125). For instance, encephalitis caused by enteroviruses is far more common than neonatal herpes virus-induced encephalitis (125). However, in acutely ill infants younger than 1 month of age, neonatal HSV disease must be considered. If the suspicion cannot be ruled out aciclovir therapy should be initiated after appropriate viral cultures and specimens for PCR analysis have been obtained (124).

Another great concern is the risk of relapses of the encephalitis followed by deterioration in cerebral function. Relapses have been described previously after neonatal HSV-induced encephalitis, as well as after childhood encephalitis (27, 123, 153, 155-159) and one such case was also documented in study IV. The child concerned in our study reported with a confirmed deterioration in cognitive function, as measured by neuropsychological investigation, from low average intellectual function to severe mental retardation and autism.

Long-term oral suppression with acyclovir has been tried to decrease the risk of relapse after the cessation of intravenous therapy (124). Treatment has been shown to suppress cutaneous recurrences, but whether suppressive oral acyclovir treatment prevents CNS reactivations or only masks recurrences in the CNS remains unclear. In fact, failure of oral acyclovir to prevent CNS infection during treatment has been described (123). A phase III trial of acyclovir versus placebo to evaluate neurological outcome in infants with SEM and CNS disease given oral suppression is conducted by
the National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group (CASG) (123). According to an article by Kimberlin et al the results from the placebo controlled study will be presented in the year to come (108). Currently, oral acyclovir suppression on cessation of intravenous acyclovir cannot be recommended (108). The mechanisms related to relapse are poorly understood. As an alternative mechanism to the reactivation of virus in the CNS, secondary immune mediated processes triggered by the viral infection have been proposed (153, 155, 158).

Parents may be extremely worried of the knowledge that their infant has been affected by a HSV-induced CNS infection, given the high risk of adverse outcome, so it would be of great value to find reliable prognostic factors for a good prognosis of neurodevelopmental outcome. When HSV-2 infection, seizures and decreased consciousness are the presenting signs, the infection has been linked to a poorer prognosis (27, 110, 116). According to the results in study IV CT performed during the neonatal period did not provide reliable prognostic information on neurodevelopmental outcome. MRI (conventional or diffusion-weighted imaging) may be more sensitive and provide better prognostic information.

Infants may seem to develop well initially, but neurological impairment may be obvious during the first year of life because skills do not evolve at the expected time. Long-term follow-up with regard to developmental milestones and neurological dysfunction is essential in order to detect adverse development and institute appropriate interventions. The findings of a high proportion of cognitive impairment including deficits in attention point to the need for a thorough neuropsychological assessment in children that have suffered from neonatal herpes virus encephalitis. In this high-risk group of children, a neuropsychological investigation is recommended as early as possible, and the assessment should be repeated if there is a suspicion of loss of gained abilities. In conclusion, neuropsychological investigation is a useful tool for measuring outcomes of cerebral function and for habilitation purposes, and can also be used for the detection of relapses and for the evaluation of various treatment strategies.
6 CONCLUSIONS

Congenital CMV infection is the most common intrauterine infection with a known impact on child health, occurring in 0.2% of newborns in a Swedish cohort.

The risk of hearing deficits is significantly increased in children with a congenital CMV infection.

Congenital CMV infection should be considered in the diagnostic evaluation of children with hearing deficits and cerebral cortical malformations of unknown etiology.

Children with a known CMV infection should be afforded close monitoring of hearing and be followed-up with regard to developmental milestones.

A retrospective diagnosis of congenital CMV infection can be achieved through analysis of the dried blood spot sample. However, if possible, different diagnostic tools should be combined with DBS analysis because the specificity and sensitivity of the DBS has not been fully established yet.

Short-term outcome with regard to parental worry, anxiety and stress in conjunction with neonatal CMV screening reveal that severe parental distress is not an obvious consequence of well-organised CMV screening.

Parents of false positive infants compose an especially vulnerable group in screening and need to be followed-up as well as parents of true positive infants.

The risk of cognitive impairments, including deficits in attention following neonatal HSV infection involving the central nervous system, is high in spite of antiviral treatment.

Neuropsychological assessment is essential in the habilitation of the child with neonatal HSV encephalitis and is a tool to monitor deterioration of cerebral function related to relapses.
7 FUTURE PERSPECTIVES

The disease burden arising from a congenital cytomegalovirus infection is potentially preventable, but knowledge of the prevalence and spectrum of permanent sequelae caused by congenital CMV infection is limited, and consequently the public health burden of the disease can be ignored (13, 18). A first step to increase the consciousness of the impact can be achieved through a national surveillance program for infants with symptoms in the neonatal period and children with neurological sequelae and hearing deficits diagnosed retrospectively.

Another obvious area of improvement is the identification of subgroups of children that could benefit from antiviral treatment. Especially those infants with a high viral load, as it has been proposed that they compose a subgroup with a high risk of late onset hearing loss (103, 144, 146) have to be further evaluated. However, a better understanding of the pathogenetic mechanisms behind long-term sequelae is needed to enhance outcome, as yet it is not known whether the virus, hostfactors or the host-pathogen interplay are most important for the development of long-term sequelae. The limitation of viral load during a sensitive period of development would be beneficial if viral load is involved in the pathogenesis of sequelae and antiviral agents may play a crucial role in enhancing outcome. On the other hand, if the inflammatory response is involved in the development of sequelae, treatment to decrease inflammation may be the key to improve outcome.

Thus randomised controlled studies with a long-term follow-up are needed to evaluate the outcome of different treatment strategies. The duration of the follow-up is important, especially if it is to be possible to fully identify neurological sequelae because they first manifest themselves when a skill fails to develop at the expected time point.

A better knowledge of the natural history of congenital CMV infection may be of assistance in making rational decisions about prevention. One prevention-related target is the minimization of infection from asymptomatic toddlers to pregnant women by education about hygienic measures.

If treatment options with antiviral agents improve, opening up the opportunity to preserve hearing in infants with asymptomatic CMV infection, screening will need to be considered. In the context of screening, it is important to evaluate parental psychological reactions in a long-term fashion in order to draw conclusions about the impact on parent-child attachment.

A surveillance system for neonatal HSV infections should provide useful information on their incidence, natural course and morbidity. Outcome has improved over recent decades by using increased doses of intravenous acyclovir over longer periods of time than was previously the case. However, sequelae following neonatal herpes virus encephalitis remain unacceptably high in spite of treatment (26, 110, 114). Furthermore, there is a risk of deterioration beyond the acute infection because of relapses.

In the future, different treatment options have to be evaluated in randomised controlled trials in order to enhance neurodevelopmental outcome and prevent relapses. In this context, it is important to characterise the mechanisms involved in the relapses.
Viral reactivations in the CNS as well as immunological mechanisms have been suggested.

There is also a need to use neuropsychological testing in the evaluation of neuropsychological outcome following childhood encephalitis in research settings. Neuropsychological testing is a tool for evaluating different treatment strategies and for evaluation of cerebral function in suspected relapses related to HSV encephalitis.
Medfödd (kongenital) cytomegalovirus (CMV) infektion är den vanligaste kände infektionen, som kan drabba foster och ge upphov till handikapp hos barnet. Den anses vara en av de vanligaste orsakerna till medfödd hörselskada men olika neurologiska handikapp och synnedsättning kan också förekomma.

Gravida kvinnor löper risk att smittas med CMV genom kontakt med urin och saliv från småbarn som utsöndrar viruset. Smitta kan även överföras via sexuell kontakt. Cirka 1% av alla gravida kvinnor som inte har haft en CMV infektion beräknas bli smittade under graviditeten. I dessa fall är risken för att infektionen förs över till fostret 30-50%. Gravida kvinnor som har haft en CMV infektion före graviditeten kan få en ny infektion med en annan CMV virus stam eller reaktivera CMV viruset. I dessa fall är risken för fosterinfektion endast 1-3%.

Majoriteten (85%) av barn, som föds med CMV infektion saknar tecken på infektion under nyföddhetsperioden vilket medför att antalet barn med handikapp orsakade av kongenital CMV infektion sannolikt är underskattad.

Herpes simplex virus (HSV) infektion i nyföddhetsperioden är en ovanlig men potentiellt livshotande infektion med hög risk för neurologiska handikapp. Smittan från mor till barn sker vanligen under förlossningen. Prognosen har förbättrats med hjälp av tidigt insatt antiviral behandling, men risken för neurologiska handikapp är oacceptabelt hög trots behandling om viruset har hunnit smitta hjärnan innan behandlingen inleds.

Huvudsyftet med den här avhandlingen är att öka kunskapen om handikapp till följd av CMV eller HSV infektion vilka har förvärvats under fosterlivet eller förlossningen. Studierna har visat att 2 barn av 1000 föds med CMV infektion och att risken för medfödd hörselskada i den undersökta gruppen var cirka 20 gånger högre hos infekterade jämfört med oinfekterade barn. Hos barn med neurologiska handikapp på basen av en utvecklingsavvikelse av hjärnan kallad ”migrationsstörning” hade flera barn än förväntat (4/26) haft kongenital CMV infektion. Fyndet stöder misstanken om att CMV virus har förmåga att störa nervcellernas migration (förflyttning) från området runt hjärnventriclearna (de vätskefyllda hålrummen i hjärnan) till hjärnbarken och därmed orsaka missbildning av hjärnan och neurologiska handikapp. Forskning på stamceller i laboratorium har tidigare visat att CMV kan hämma neurologiska stamcellers förmåga att mogna och dela sig samt att viruset orsakar celldöd.

Eftersom de flesta barn med kongenital CMV infektion saknar symtom i nyföddhetsperioden är neonatal CMV screening den enda möjligheten att diagnostisera majoriteten av barn som riskerar att utveckla en hörselskada. Vi har därför undersökt och jämfört föräldrarnas psykologiska reaktioner vid falskt positivt, sant positivt och negativt CMV screening. Det framkom att föräldrar till barn med falskt positivt screening fortsatte att vara oroliga för sitt barns nuvarande och framtidiga hälsa efter att de fått besked om att det uppföljande urinprovet visade att barnet inte var infekterat. Det fanns däremot ingen ökad tendens till stress eller ångest hos föräldrar till barn med positivt screening jämfört med föräldrar till barn med negativ screening.

En grupp barn med neonatal herpesvirus infektion, som engagerat hjärnan undersöktes avseende neuropsykosologiska funktionsnedsättningar. Resultaten visade att en hög andel barn hade begåvningshandikapp, koncentrationssvårigheter samt språkliga

I bedömningen av barn med genomgången neonatal HSV infektion engagerande hjärnan är neuropsykologisk undersökning viktig för att barnet skall kunna möta sina förutsättningar. Neuropsykologisk undersökning är också ett hjälpmedel för att bedöma misstanke om försämring i hjärnfunktionerna på grund av att viruset reaktiveras.
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