Consequences of congenital cytomegalovirus infection in early childhood

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Chapter 1

General Introduction

General Introduction

Cytomegalovirus

Human cytomegalovirus (CMV), formally human herpesvirus 5 in viral taxonomy, belongs to the beta-herpesvirinae subfamily. It is the largest of the herpes viruses with a capsid diameter of approximately 130 nanometers [1] and it has the largest genome, consisting of about 235 kilobase pairs and around 165 genes. [2-4] The icosahedral nucleocapsid of this virus, containing the double stranded linear DNA genome, is encapsulated by a proteinaceous matrix, the tegumen, which in turn is enclosed by a lipid envelope embedding viral glycoproteins. [4-6]

Cytomegalovirus infection

CMV has a broad cellular tropism; an infection with CMV can spread to virtually all organs due to this broad range of target cell types. [7-9] The liver, gastrointestinal tract, lungs, retina and brain are predominant sites of clinical manifestations in immunocompromised hosts. [10]

After a primary infection with CMV, life-long latency is established. [8] Latency occurs predominantly in the monocyte-myeloid lineage, [11-13] although endothelial and neuronal progenitor cells have also been suggested as sites of latency. [14]

Reactivation of the latent virus can occur when the immune response becomes impaired; for example in patients treated with immunosuppressive drugs or in HIV-infected patients and presumably in the case of an immune system 'stunned' by a sudden shock in healthy individuals who need sudden admission to intensive care units. [7]

Reinfection with a different strain of CMV can also occur in persons who have already experienced a primary infection. The term recurrent infection can be used to cover both reinfection and reactivation. [15]

A CMV infection is usually mild or asymptomatic in healthy individuals, although it can cause a mononucleosis-like syndrome with persistent fever, malaise, jaundice, atypical lymphocytes and elevated liver transaminases in some individuals. [16, 17]

In immunocompromised persons or immunosuppressed transplant patients, CMV can cause serious morbidity and mortality, including end-organ disease such as hepatitis, gastroenteritis, colitis, pneumonitis, retinitis and infection of the nervous system. [7, 11, 15]

Epidemiology of cytomegalovirus

CMV infection is a common infection worldwide. The most important risk factor for a higher seroprevalence is age, because the chance of exposure to CMV increases over time. [18] The seroprevalence was found to be 20 to 30% higher among non-whites compared to whites and most studies found a higher seroprevalence in females than in males. [18] Furthermore, the seroprevalence among persons of lower socio-economic status was 10 to 30% higher compared to persons of higher socio-economic status. [18] The seroprevalence among women of reproductive age, ranging from 45 to 100%, tended to be highest in South America, Africa and Asia and lowest in Western Europe and the United States. [18]

In the Netherlands, the CMV seroprevalence among women of reproductive age was found to be much higher (96%) in women of non-European origin compared to native Dutch women and women of European origin (57%). European women working in childcare had higher seroprevalences (68%) than those with other professions (42%). [19] This higher occupational risk was also demonstrated in another Dutch study, with a higher seroprevalence in female day care personnel (57%) compared to women not working in day care (40%). [20]

Transmission of cytomegalovirus

CMV may be found in body fluids such as oropharyngeal secretions, cervical and vaginal excretions, semen, breastmilk, tears, urine, feces and blood. [17] This means that CMV can be transmitted via saliva, sexual contact, breastfeeding, blood transfusion or organ transplantation. [21] However, intimate contact is necessary for horizontal transmission of CMV. [17] The basic reproductive number (R_0) was estimated by mathematical modelling to be between 1.7 and 2.4, which means that an infected person transmits CMV on average to two susceptible individuals. [22, 23] The R_0 was found to be associated with ethnicity and socio-economic status, with a higher R_0 in non-Hispanic Blacks (4.1), Mexican Americans (3.7) and in persons with lower income (2.7). [22]

Young children have been identified as an important source of maternal infection. [24] Young children tend to shed the virus in urine and saliva for months with high, fairly stable, viral loads. [25] Children attending day care centers shed more frequently than children not attending day care [26], and younger age was significantly associated with higher viral loads [25]. Most maternal infections are probably acquired by mothers getting these fluids in their eyes, nose or mouth. [27]

Both after primary infection of a pregnant woman, as well as after reactivation of the virus or reinfection with a different strain of CMV, vertical transmission of CMV to the fetus can take place, resulting in a congenital infection. [28, 29]
Postnatally, newborns and infants can be infected by breastmilk from a seropositive mother. [30, 31] The breast is a common site of reactivation in postpartum women [17] and 93-96% of seropositive women shed CMV DNA in breastmilk. [32, 33] Between 37 and 59% of infants who received CMV-positive breastmilk became infected with CMV. [32, 33] In addition, a child may be infected with CMV via cervical excretions during its passage through the birth canal. [34]

Congenital cytomegalovirus infection

History of congenital cytomegalovirus infection

Congenital CMV infection was initially called “generalized cytomegalic inclusion disease” because of the observed intranuclear inclusions surrounded by a clear halo in the cells, [35, 36] producing the typical “Owl’s Eye” appearance. These large inclusion-bearing cells, found in the kidney of a stillborn infant with congenital syphilis, were first documented in 1881 by Ribbert. [37] He described these protozoan-like cells in a report in 1904, after a colleague reported finding similar cells in the lungs, kidneys and liver of an 8-month-old fetus with congenital syphilis. [38] Following this, multiple cases of stillborns or deceased newborns with petechiae, hepatosplenomegaly and intracranial calcifications were described and in all of them, cells with typical intranuclear inclusions were found. [39]

The term “generalized cytomegalic inclusion disease” was suggested in 1950 by Wyatt and colleagues, who described the pathology and morphological features of six cases and reviewed 66 cases from the literature. [40] They suggested that the etiological agent of the disease was a specific virus. In the same year, similar findings were reported by Smith and Vellios. [41] Later on, particles suggestive of a virus were observed in the clear halo around the intranuclear inclusion, using electron microscopy, in a case of cytomegalic inclusion disease. [42] In 1956, human cytomegalovirus was successfully isolated from the salivary gland and kidney of two deceased patients with cytomegalic inclusion disease. [43-45]

Epidemiology

Congenital CMV infection (cCMV) is the most common congenital infection in the USA and many other developed countries. [46] In a meta-analysis, including studies from around the world, the overall birth prevalence of cCMV has been estimated at 0.64%. [47] In industrialized countries the birth prevalence ranged from approximately 0.2% to 2.4%. [47, 48] The birth prevalence in the Netherlands has been estimated at 0.54%. [49] In developing countries, where maternal seroprevalence ranged from 84% to 100%, the birth prevalence varied between 0.6% and 6.1%. [50] It was demonstrated that 29% of the variance in birth prevalence of cCMV was explained by the maternal seroprevalence. [47] The fact that the birth prevalence of cCMV increases with increasing maternal seroprevalence means that in countries with high CMV seroprevalence, most cases of cCMV are due to non-primary maternal infections. [51] Moreover, even in countries with a maternal seroprevalence of around 50% it was estimated that the proportion of cCMV attributable to non-primary infections is 70%. [48, 52]

Transmission

As mentioned before, CMV can be transmitted vertically through the placenta. In the case of a primary maternal CMV infection, the transmission rate from mother to unborn child is about 32%. [47] The annual seroconversion rate in seronegative pregnant women was estimated to be 2.3%, with a range between 1% and 7%. [7, 53, 54] As discussed earlier, young children are presumably a major source of CMV infections in these women, since the saliva and urine of these young children can contain high viral loads. [25, 26, 55] The annual seroconversion rates were found to be higher in specific risk groups, including parents of CMV shedding children (24%) and day care providers (8.5%). [53]

The maternal to fetal transmission rate increases with advancing gestation. [51, 56] The intrauterine transmission rate following primary CMV infection was 5.2% in the preconception period (range: 0-16.7%) and 16.4% in the periconception period (range: 4.6-45%). [57] During pregnancy, transmission rates were observed of 36.5% in the first trimester (range: 22.2-42.2%), 40.1% in the second trimester (range: 26.9-44.9%) and 65% in the third trimester (range: 30.8-77.6%). [57, 58]
In the case of a recurrent infection in a seropositive woman, the vertical transmission rate is estimated to be 1.4%. [47] Reinfection with a different strain of the virus could be the result of intimate contact with a young child [27] and reactivation may possibly be associated with the hormonal changes in pregnancy [17]. It was observed that in seropositive pregnant women the prevalence of CMV shedding increased with advancing gestation. [26]

Fetal disease

During pregnancy, cCMV can sometimes be detected by prenatal imaging. Abnormal findings in fetuses with cCMV that can be detected by ultrasound include cerebral abnormalities (microcephaly, ventriculomegaly, brain calcifications, occipital horn cavities, subependymal cysts, and abnormal gyration), intra-uterine growth restriction, hyperechogenic bowel, hepatomegaly, ascites and pericardial effusion. [57-61] Moreover, cCMV is associated with fetal death. [62] In two studies cCMV was found in 9% to 15% of the examined stillborn fetuses. [63, 64]

Symptoms at birth

A congenital CMV infection can lead to clinically apparent symptoms and signs at birth. The classical picture of cytomegalic inclusion disease is characterized by involvement of multiple organs, in particular the reticuloendothelial and central nervous system, with or without ocular or auditory damage. [65] Clinical findings such as preterm birth, being small for gestational age, microcephaly, petechiae, purpura, jaundice, hepatosplenomegaly, and neurological findings, such as hypotonia, lethargy, poor suck and seizures are common in symptomatic children with cCMV. [65-67] Laboratory abnormalities including thrombocytopenia, elevated liver transaminases and conjugated hyperbilirubinemia, are likely to normalize within weeks. [67] Possible ophthalmologic findings after birth comprise chorioretinitis, optic atrophy and retinal hemorrhage. [67] In addition, sensorineural hearing loss may be present at birth. Abnormal findings on cerebral ultrasound, CT or MRI, for example intracranial calcifications and ventriculomegaly, are also frequently found in children with symptomatic cCMV. [66]

It is estimated that 11.0% to 12.7% of live-born children with cCMV are classified as being symptomatic at birth. [47, 68] However, the large difference in definitions between studies hinders comparison and interpretation of this data. [47] For example, the prevalence of symptomatic cCMV in one study would increase from 10% to 22% if intra-uterine growth restriction had been included in the case definition. [50] When the severity of the symptoms is taken into account, this may also influence the prevalence of symptomatic cCMV. For example, when transient symptoms such as petechiae and thrombocytopenia and anemia were included in the definition, 22.8% of the children in one study would be classified symptomatic, compared to 10.3% if only severe central nervous system involvement was taken into account. [58] Unfortunately there is, as yet, no international agreement on the case definition of symptomatic cCMV.

The prevalence of symptoms at birth also depends on the method of diagnosis. Dreher et al. showed that children with cCMV who were detected by screening had less symptoms at birth compared to the population that was detected based on clinical suspicion and who were subsequently referred to a medical specialist. Even though this is quite obvious, it is important to be aware that these two groups are distinct populations, presumably with a different natural history, when interpreting different studies on cCMV.

Mortality
Neonatal mortality in children with cCMV is estimated to occur in 4% of symptomatic infants. [68] Two studies showed that the greater part of cCMV-related mortality occurred in children younger than 12 months of age (68% and 72% of all cCMV deaths) and mortality was especially frequent in children younger than 1 month of age (26% and 31% of all cCMV deaths). [69, 70]

Long term impairment
Permanent sequelae of cCMV are predominantly sensory and neurological in nature. They can include sensorineural hearing loss, chorioretinitis, optic atrophy, cerebral palsy, epilepsy, microcephaly, delayed psychomotor development and mental retardation. [71]

Initially it was thought that children who were born to mothers with a primary CMV infection during pregnancy generally had not only more, but also more severe long-term impairment compared to children who were born to mothers who already had antibodies against CMV. [72, 73] The risk of one or more sequelae at almost 5 years of age was shown by Fowler et al. to be 25% (31/125) in the primary-infection group compared to 8% (5/64) in the recurrent-infection group. [72] However, this is the only study that showed this association. Recent studies demonstrate that long-term impairment, including hearing loss, occurs equally frequently in children born to women with a primary CMV infection and in those born to women with a recurrent CMV infection. [71, 74-76]

The clinical outcome of a child is certainly related to the trimester during which its mother acquires a primary CMV infection. [77] Children born to a mother who is infected in the first half of pregnancy (4-22 weeks) have a higher risk of a significant handicap compared to those infected later in pregnancy. [56] One third (32%, 11/34) of children with cCMV born after a first trimester maternal infection had central nervous system sequelae, compared to 15% (6/40) of those born to mothers infected after 13 weeks of gestational age. [78]

In the next sections, a general overview is given of the different types of long-term impairment caused by cCMV. In these sections, an extensive but not all-encompassing selection of studies on the prevalence of these impairments is presented in the accompanying tables. In these tables, a distinction has been made between screening based and non-screening based studies, as these populations might be different. More details about these studies are displayed in the table at the end of this section (Table 1.8).

Hearing impairment

Hearing impairment is the most frequently detected long-term impairment in children with cCMV. [68, 79, 80] A systematic review estimated that sensorineural hearing loss (SNHL) occurred in 12.6% of all children with cCMV. [81] Of children who were symptomatic at birth 32.8% experienced hearing loss, while hearing loss was seen in 9.9% of children with cCMV who were asymptomatic at birth. [81] The other way around, it was found that 8% of 3 to 5 year old children with permanent bilateral hearing impairment had cCMV. [82] Moreover, of the children with profound permanent bilateral hearing impairment (> 90 decibels) 23% were diagnosed with cCMV. [82]

Delayed-onset of hearing loss and fluctuation or progression of hearing loss is frequently observed in children with cCMV. [46, 79, 83-85] Delayed-onset of hearing loss can occur up to and even after the age of 6 years. [84, 86, 87] In a study with a follow-up until 15 years of age, 86.6% of asymptomatic children and 95.3% of symptomatic children with cCMV and hearing loss were detected at or before six years of age. [84] The median age at detection of the delayed-onset hearing loss was 33 months in the symptomatic children and 44 months in the asymptomatic group. [84] Because of the delayed onset of the hearing loss, up to half of the children with hearing loss due to cCMV will be missed by neonatal hearing screening. [86]

Hearing loss due to cCMV can be either unilateral or bilateral. The proportion of bilateral hearing loss ranges between 25% and 94%, [88] with an average of about 60%. It was estimated that about 70% of the hearing loss is bilateral in symptomatic children compared to just over 40% in asymptomatic children. [81] The severity of hearing loss can be mild, moderate, severe or profound. [79]

In Table 1.1 an overview of several studies on the prevalence of hearing loss in children with cCMV is presented to give an impression of the prevalence of hearing loss, the used study designs, the duration of follow-up and the method of detection of cCMV. Most of the presented studies were performed in Europe and North America. It is clear from the table that the variation in prevalence between the studies is quite large. Part of this variation might be explained by the difference in the definition of hearing loss. The threshold used to define hearing loss is different in most studies, but commonly ranges between 20 and 40 decibels. [88] In addition, some studies also included hearing loss at only one frequency (8000 Hz) [89], while other studies did not include high-frequency hearing loss that occurred only at 8000 Hz. It is noteworthy that most of the recent studies (published after 1997) include no or only very small control groups.

Table 1.1 - Prevalence of hearing impairment in children with and without cCMV.

Author Reference	Year	Country Region	Period	Follow-up	Method (n)	Overall n / N - %	Sympt. n / N - %	Asympt. n / N - %	Control n / N - %
Screening based studies									
Reynolds [89]	1974	USA Alabama	1967-1970	3 y	urine/ saliva	9/16 56.3%			2/12 16.7%
Hanshaw [90]	1976	USA New York	1967-1970	3,5 - 7 y	IgM/urine (8644)			5/40 12.5%	3/44 6.8%
Saigal [80]	1982	Canada Ontario	1974-1975	3 y	urine (15212)	7/33 21.2%	1/(3)*	6/(38)*	0/44 0%
Kumar [91]	1984	USA Ohio	1971-1974	7,5 y	urine			4/17 23.5%	1/17 5.9%
Ahlfors [92]	1984	Sweden Malmo	1977-1982	0,5 - 4 y	urine (10328)	4/42 9.5%	2/(8)*	2/(34)*	0/49 0%
Williamson [93]	1992	USA Texas	1983-1989	1,5 / 2 y	urine			9/59 15.3%	0/26 0%
Fowler [79]	1997	USA Alabama	1980-1995	5 y	urine/ saliva			22/307 7.2%	0/277 0%
Ahlfors [71]	1999	Sweden Malmo	1977-1986	6 y	urine (16474)	5/46 10.8%	3/(22)*	2/(54)*	0/30 0%
Fowler [86]	1999	USA Alabama	1980-1996	6 y	urine/ saliva	60/388 15.4%	(53) 36.4%	(335) 11.3%	
Boppana [76]	1999	USA Alabama	1991-1997	3,5 y	urine/ saliva		9/42 21.4%		
Dahle [84]	2000	USA Alabama	1966-1999	5 y (0 - 19 y)	urine/ saliva	133/860 15.5%	85/209 40.7%	48/651 7.4%	
Coats [94]	2004	USA Texas	1982-1992	4 / 11 y S / As	urine	40/125 32.0%	29/42 69.0%	11/83 13.3%	0/21 0%
Numazaki [95]	2004	Japan Sapporo	1977-2002	7 y	urine (11938)			2/17 11.8%	
Foulon [96]	2008	Belgium Brussel	1996-2006	2¾ y	urine (14021)	13/60 21.7%	1/3 33.3%	12/57 21.1%	
Engman [97]	2008	Sweden Stockholm	2003-2004	4 y	DBS (6060)	1/12 8.3%			
Non-screening based studies									
Stagno [98]	1977	USA Alabama	1967-?	4,5 m	screen + referred	10/59 16.9%	3/8 37.5%	7/51 13.7%	2/41 4.8%
Pass [99]	1980	USA Alabama	1965-1979	4 y (¾ - 14 y)	urine testing		7/23 30.4%		
Williamson [100]	1982	USA Texas	1968-1980	5,5 y	urine testing		11/17 64.7%		
Kylat [66]	2006	Canada Toronto	1987-2000	2 y	registry		17/38 44.7%		
Ancora [101]	2007	Italy Bologna	1997-2003	3,5 y	referred	9/56 16.1%			
Goderis [87]	2016	Belgium Flanders	2007-2014	1 - 6 y	urine testing	98/379 25.9%	77/123 62.6%	21/256 8.2%	

Sympt (S): symptomatic; Asympt (As): asymptomatic; y: year; m: month
*percentage lost-to-follow-up is unclear

Visual impairment

There is relatively limited information available concerning visual impairment in cCMV. An overview of some studies on visual impairment in children with cCMV is presented in Table 1.2.

Some case reports have been published on rare ophthalmological abnormalities, including cyclopia and anophtalmia, in children with cCMV. [102, 103] Besides the ocular findings in some smaller cohorts, only two large studies have focused on the visual impairment in children with cCMV. [94, 104] In these studies, visual impairment was seen in approximately 15% of children with a symptomatic cCMV infection compared to less than 1% in children with cCMV who were asymptomatic at birth. [94, 104] Visual impairment in these children was caused by macular scarring, optic atrophy and cortical visual impairment. [94]

Chorioretinitis or retinal scarring was seen in approximately 20% of symptomatic and 2% to 4% of asymptomatic children with cCMV. [94, 104] The retinal scarring is suggestive of past episodes of chorioretinitis. [94] Optic atrophy was also more common in symptomatic children (7-11%) than in asymptomatic children (<1%). None of these problems were seen in the very small control group (n = 21). [94] Strabismus occurred more frequently in symptomatic children with cCMV (around 25%) compared to asymptomatic children with cCMV (1-2%) and the control group (5%). [94, 104] Another study, which focused on children with cCMV and hearing loss and cochlear implants, found that the prevalences of ocular abnormalities in these children were similar to those in the symptomatic children in the previously mentioned studies. [105]

Table 1.2 - Prevalence of visual impairments in children with and without cCMV

Author Reference	Year	Country, Follow-up, Method * Type of visual impairment	Overall n (%)	Sympt. n (%)	Asympt. n (%)	Control n (%)
		Screening based studies				
Hanshaw	1976	USA, 3.5 - 7 y, screening			n = 44	n = 44
[90]		Chorioretinitis			1 (2.3)	0 (0.0)
Pass	1980	USA, 4 y, screening		n = 23		
[99]		Chorioretinitis		4 (17.4)		
		Optic atrophy		2 (8.7)		
Coats	2004	USA, 4/11 y (S/As), screening		n = 42	n = 83	n = 21
[94]		Visual impairment (VA < 0.1)		7 (16.7)	0 (0.0)	0 (0.0)
		Subnormal vision (VA 0.1 - 0.5)		2 (4.8)	1 (1.2)	0 (0.0)
		Retinal scarring		9 (21.4)	2 (2.4)	0 (0.0)
		Optic atrophy		3 (7.1)	0 (0.0)	0 (0.0)
		Strabismus		12 (28.5)	1 (1.2)	1 (4.7)
		Cortical visual impairment		4 (9.5)	0 (0.0)	0 (0.0)
		Non-screening based studies				
Ramsay	1991	England, 3.5 y, lab registry	n = 65			
[106]		Chorioretinitis	3 (4.6)			
		Cortical blindness	4 (6.2)			
Anderson	1996	USA, 5 y, screening + referred		n = 113	n = 332	
[104]		Visual impairment (VA < 0.4)		17 (15.0)	2 (0.6)	
		Chorioretinitis		22 (19.5)	13 (3.9)	
		Optic atrophy		12 (10.6)	1 (0.3)	
		Pigmentary retinopathy		8 (7.1)	10 (3.0)	
		Strabismus		26 (23.0)	5 (1.5)	
		Nystagmus		3 (2.7)	3 (0.9)	
Kylat	2006	Canada, 2 y, registry		n = 38		
[66]		Visual impairment (VA < 0.1)		4 (11.0)		
		Cortical visual impairment		3 (7.9)		
Teär Fahnehjelm	2015	Sweden, 8 y, DBS tested (hearing loss + cochlear implant)	n=26			n = 13
[105]		Visual impairment (VA ≤ 0.3)	5 (19.2)			0 (0.0)
		Macula scarring	5 (19.2)			0 (0.0)
		Optic atrophy	1 (3.8)			0 (0.0)
		Strabismus	5 (19.2)			1 (7.7)

VA: visual acuity, Sympt. (S): symptomatic, Asympt. (As): asymptomatic; y: year
* More details about the studies are presented in Table 1.8

Neurological impairment

In addition to the risk of hearing impairment and visual problems, cCMV is a risk factor for other neurological impairments, such as epilepsy, cognitive deficits and motor deficits, including cerebral palsy. [107] These more general neurological impairments are presented in this section. Table 1.3 displays the prevalences of general neurological impairments in children with cCMV, with and without symptoms at birth. Neuro-behavioral impairments are discussed in the next section.

Cerebral palsy, epilepsy or seizures, and psychomotor retardation have often been reported in children with cCMV, especially in those with symptoms at birth. The prevalence of neurological impairment tends to be higher in the studies that were non-screening based. This is probably related to selection bias in these studies, because children with disabilities are more readily recognized and then tested for cCMV. Furthermore, some differences in prevalence could be explained by the use of different case definitions. For example, some studies define developmental delay as a developmental quotient (DQ) more than 2 standard deviations (SD) below the mean (DQ < 70) [95], while others use a developmental quotient below 85 (-1 SD) [101]. Sometimes no case definitions were provided by the authors. [76, 108]

When looking at the overall long-term outcome, mild impairment (including unilateral SNHL, mild bilateral SNHL, mild motor impairment or developmental or language delay in the absence of hearing loss or other problems) was seen in 7% (11/154) of children with cCMV detected by neonatal screening. [74] These problems were mostly diagnosed between two and five years of age. [74] Moderate impairment, which included moderate or severe bilateral SNHL without any other identified problem, mild bilateral SNHL and mild cerebral palsy, or moderate learning difficulties, occurred in 5% (7/154) of children with an onset before the age of one year. [74] Severe impairment included multiple problems, moderate to severe cerebral palsy or severe learning difficulties and these were found in 9/154 (6%) of children. These severe problems always presented before the age of one year. [74]

Based on these two studies, in the group of children with symptoms at birth the average of mild, moderate and severe impairment was 10.5%, 5.3% and 26.3% respectively, whereas in the asymptomatic children the average of these problems was 6.7%, 4.4% and 3.0% respectively. [74]

Table 1.3 - Prevalence of neurological impairment in children with and without cCMV

Author Reference	Year	Country, follow-up, method * Type of impairment	Overall n (%)	Sympt. n (%)	Asympt. n (%)	Control n (%)
		Screening based studies				
Pass [99]	1980	USA, 4 y, screening		n = 23		
		Psychomotor delay		4 (17.4)		
		Seizures		5 (21.7)		
		Spasticity		6 (26.1)		
Ahlfors [92]	1984	Sweden, 0,5-4 y, screening		n = 8	n = 35	n = 49
		Psychomotor retardation		1 (12.5)	1 (2.8)	0 (0.0)
		Cerebral palsy		1 (12.5)	0 (0.0)	0 (0.0)
Ivarsson [109]	1997	Sweden, 2 y, screening	n = 32			n = 51
		Development scale <normal	6/32 (18.8)			8/51 (15.7)
Boppana [76]	1999	USA, 3,5 y, screening		n = 42		
		Seizure		2/41 (4.9)		
Ahlfors [71]	1999	Sweden, 7 y, screening		n = 16	n = 44	n = 39
		Mental retardation		2 (12.5)	2 (4.5)	0 (0.0)
		Cerebral palsy		2 (12.5)	2 (4.5)	0 (0.0)
		Epilepsy		0 (0.0)	1 (2.3)	0 (0.0)
Numazaki [95]	2004	Japan, 6 y, screening			n = 21	
		Mental Retardation (DQ < 70)			0 (0.0)	
Zhang [110]	2007	China, 6 y, screening			n = 49	n = 50
		DQ 70-89			13 (26.5)	7 (14.0)
		DQ < 70			4 (8.2)	1 (2.0)
Townsend [74]	2013	UK, 5 y, screening	n = 87	n = 5	n = 82	n = 111
		Mild impairment	7 (8.1)	1 (20.0)	6 (7.3)	3 (2.7)
		Moderate impairment	4 (4.6)	1 (20.0)	3 (3.7)	0 (0.0)
		Severe impairment	3 (3.4)	2 (40.0)	1 (1.2)	0 (0.0)
Townsend [74]	2013	Sweden, 7 y, screening	n = 67	n = 14	n = 53	n = 39
		Mild impairment	4 (6.0)	1 (7.1)	3 (5.7)	1 (2.6)
		Moderate impairment	3 (4.5)	0 (0.0)	3 (5.7)	0 (0.0)
		Severe impairment	6 (9.0)	3 (21.4)	3 (5.7)	0 (0.0)
		Non-screening based studies				
Williamson [100]	1982	USA, 5.5 y, testing		n = 17		
		Cerebral palsy		7 (41.2)		
		Microcephaly		10 (58.9)		
Ramsay [106]	1991	England, 3.5 y, lab registry		n = 65		
		Cerebral palsy		14 (21.5)		
		Severe psychomotor retardation		6 (9.2)		
Kylat [66]	2006	Canada, 2 y, registry		n = 38		
		Bayley < -2 SD / cognitive deficit		27 (71.1)		
		Developmental delay		32 (84.2)		
		Bedridden or wheelchair bound		6 (15.7)		
Ancora [101]	2007	Italy, 3.5 y, referred	n = 56	n = 17	n = 39	
		DQ <85	8 (14.3)	7 (41.2)	1 (2.6)	
Karltorp [107]	2014	Sweden, 7.8 y, testing (HL)	n = 26			n = 13
		Cerebral palsy	2 (7.6)			0 (0.0)

Sympt: symptomatic, Asympt: asymptomatic, DQ: development quotient, HL: hearing loss, *see Table 1.8

Neuro-behavioral impairment

Besides the often reported neurological problems related to cCMV, there also seems to be a relation with neuro-behavioral impairment such as attention deficit hyperactivity disorder (ADHD) and autism spectrum disorders (Table 1.4). These conditions have been reported in two Swedish cohort studies, [71, 107] but as early as in the 1980's some cases of children with cCMV and autism were reported. [111-114] Recently, more studies supported this potential link between cCMV and autism spectrum disorder. [115-119]

It is not clear whether social and emotional problems are more common in children with cCMV than in children without cCMV. One study found no difference in social quotient between asymptomatic children and controls. [89] In another study more behavioral problems, based on two different rating systems, were seen in asymptomatic children with cCMV compared to the control group. However, in this study no differences were seen for the different subscales of hyperactivity, learning, impulsive behavior, and psychosomatic and anxiety disorders. [80] More difficulties were seen in social skills and emotional problems in children with cCMV, hearing loss and a cochlear implant compared to the control group. [107]

Table 1.4 - Prevalence of neurobehavioral impairment in children with and without cCMV

Author Reference	Year	Country, follow-up, method * Type of impairment	Overall n (%)	Sympt. n (%)	Asympt. n (%) or mean ± SD	Control n (%)or mean ± SD
		Screening based studies				
Reynolds	1974	USA, 3 y, screening			n = 18	n = 18
[89]		Mean Social Quotient			123	134
Ahlfors	1999	Sweden, 7 y, screening		n = 16	n = 44	n = 39
[71]		Developmental disorder		1 (6.3)	4 (9.1)	0 (0.0)
		ADHD		1 (6.3)	1 (2,3)	0 (0.0)
		Autism/Asperger		0 (0.0)	2 (4.5)	0 (0.0)
Zhang	2007	China, 6 y, screening			n = 49	n = 50
[110]		Adaptive capacity (DQ)			87.5 ± 12.9	92.0 ± 10.9
		Personal-social skills (DQ)			93.4 ± 12.9	97.6 ± 11.7
		Global development (DQ) - sign			88.6 ± 13.1	94.0 ± 11.4
Milewska	2010	Poland, 6-6.5 y, screening			n = 38	
[120]		Increased emotional sensitivity			14 (36.8)	
		Problem with school maturity			6 (15.8)	
		Non-screening based studies				
Karltorp	2014	Sweden, 7.8 y, testing (HL)	n = 26			n = 13
[107]		ADHD	2 (7.6)			0 (0.0)
		Autism Spectrum Disorder	4 (15.4)			0 (0.0)

Sympt: symptomatic, Asympt: asymptomatic, y: year, ADHD: attention deficit hyperactivity disorder, HL: hearing loss, DQ: development quotient, * More details about the studies are presented in Table 1.8

Cognitive impairment

Cognitive impairment was reported in about 30 to 50% of children with symptomatic cCMV. [76, 99, 100] However, these studies often consisted of a small number of patients. Cognitive deficits were reported to occur much less frequently in asymptomatic children (6.5%). [68] However, it is not clear whether children with asymptomatic cCMV have a lower IQ than children without cCMV (see Table 1.5). Most of the studies reported no significant differences in intelligence quotient (IQ) between asymptomatic cCMV-positive children and controls. [80, 89, 91, 121, 122] Nonetheless, other studies found a significant difference in IQ between asymptomatic children with cCMV and the control group. [90, 110]

Table 1.5 - Prevalence of cognitive impairment in children with and without cCMV

Author Reference	Year	Country, follow-up, method * Type of impairment	Overall n (%) or mean ± SD	Sympt. n (%) or mean ± SD	Asympt. n (%) or mean ± SD	Control n (%) or mean ± SD
		Screening based studies				
Reynolds	1974	USA, 3 y, screening			n = 18	n = 18
[89]		IQ < 90			7 (38.9)	4 (22)
		IQ < 70			1 (5.5)	1 (5.5)
Hanshaw	1976	USA, 3.5 - 7 y, screening			n = 44	n = 44
[90]		IQ < 90			12 (27.3)	6 (13.6)
		IQ < 80			7 (15.9)	0 (0.0)
Pass	1980	USA, 4 y, screening		n = 23		
[99]		IQ < 80		10 (43.4)		
		IQ < 50		9 (39.1)		
Saigal	1982	Canada, 3 / 5 y, screening			n = 41	n = 44
[80]		Mean IQ (Stanf.-Binet) 3 y - NS			97.0±16.5	100.6±15.6
		Mean IQ (Stanf.-Binet) 5 y - NS			107.8±16.6	106.5±9.5
Kumar	1984	USA, 7.6 y, screening			n = 15	n = 17
[91]		Mean IQ (WISC) - NS			89.5 ± 11.7	85.9
Ivarsson	1997	Sweden, 7 y, screening	n = 25			n = 41
[109]		Intellectual scale (WISC) - NS	5.8 ± 2.0			6.4 ± 1.6
		Intellectual scale < normal - NS	3/25 (12.0)			2/41 (4.8)
Boppana	1999	USA, 3.5 y, screening		n = 21		
[76]		IQ 50- 70		6 (28.6)		
		IQ < 50		0 (0.0)		
Numazaki	2004	Japan, 6 y, screening			n = 21	
[95]		IQ (WISC) 70 - 89			3 (14.3)	
Zhang	2007	China, 6 y, screening			n = 49	n = 50
[110]		Mean IQ (WPPSI)- sign			89.4 ± 12.8	95.3 ± 10.5
		IQ (WPPSI) 70 - 89			15 (30.6)	9 (18.0)
		IQ (WPPSI) < 70 - NS			4 (8.2)	1 (2.0)

Table 1.5 - Prevalence of cognitive impairment in children with and without cCMV

Author Reference	Year	Country, follow-up, method * Type of impairment	Overall n (%) or mean ± SD	Sympt. n (%) or mean ± SD	Asympt. n (%) or mean ± SD	Control n (%) or mean ± SD
Townsend	2013	UK, 5 y, screening	n = 69			n = 108
[74]		Mean IQ (WPPSI) – NS	111.7±15.7			113.0±13.6
		Mean Verbal IQ – NS	102.6±17.8			106.0±13.6
		Mean Performance IQ – NS	119.3±14.2			118.1±14.1
		Non-screening based studies				
Williamson	1982	USA, 5.5 y, testing		n = 17		
[100]		Subnormal IQ		9 (52.9)		
Conboy	1986	USA, 9.2 y, testing?			n = 18	n = 18
[121]		Mean IQ (WISC) - NS			100 ± 17.1	100 ± 12.3
		Mean Verbal IQ - NS			96 ± 15.3	98 ± 13.9
		Mean Performance IQ - NS			103 ± 17.4	101 ± 11.7
		Mental processing (K-ABC) - NS			98 ± 13.5	97 ± 11.9
Temple	2000	USA, 4.8 y, testing?			n = 49	n = 69
[122]		Mean IQ (WPPSI) - sign			82.5 ± 16.4	88.6 ± 17.7
		Mean Verbal IQ - NS			81.1 ± 14.3	86.6 ± 15.5
		Mean Performance IQ - NS			87.2 ± 17.4	92.8 ± 18.7
Temple	2000	USA, 8.6 y, testing?			n = 60	n = 104
[122]		Mean IQ (WISC-R) - NS			92.7 ± 15.7	91.6 ± 14.1
		Mean Verbal IQ - NS			92.1 ± 16.2	90.7 ± 14.7
		Mean Performance IQ - NS			94.7 ± 15.4	94.3 ± 14.1
Farkas	2011	Israel, 3 y, referred (normal US)	n = 21	n = 1	n = 20	n = 21
[124]		Cognitive z-score (11-42m, n=14)	0.50 ± 0.68			0.31 ± 0.82
		Cognitive z-score (43-83m, n=7)	0.49 ± 0.43			1.15 ± 0.86
		Cognitive z-score (all, n = 21)	0.49 ± 0.59			0.59 ±0.91

Sympt: symptomatic, Asympt: asymptomatic, sign: statistically significant difference, NS: not statistically significant difference; US: fetal ultrasound, IQ: intelligence quotient, WISC: Wechsler Intelligence Scale for Children, WPPSI: Wechsler Preschool and Primary Scale of Intelligence, K-ABC: Kaufman Assessment Battery for Children * More details about the studies are presented in Table 1.8

Motor impairment

Table 1.6 shows the motor impairments that were observed in children with and without cCMV. Motor deficits, psychomotor abnormalities and neuromuscular disorders have been reported in approximately 30% of children with symptoms at birth. [66, 71, 99, 101, 106] Mild motor problems, including ataxia, mild hypotonia and an awkward gait were also seen in children with symptomatic cCMV. [106]

The asymptomatic group has been studied less often. The prevalence of motor problems in asymptomatic children appears to be lower (between 2.6% and 18.2%) than in symptomatic children with cCMV. [71, 101] Yet, other studies found no difference in motor skills between asymptomatic cCMV-positive children and children without cCMV. [110, 124]

In children with hearing loss and cCMV the gross motor skills were poorer than in children with cCMV without hearing loss and than in cCMV negative children. [125] In this study, no differences were seen in motor skills between symptomatic and asymptomatic children. [125] In another group of children with cCMV and hearing loss, some motor milestones (head control, unsupported sitting and unaided walking) were reached at a later age than by controls. [126] This was also seen in children with cCMV, hearing loss and cochlear implants. [107] In these children balance was also frequently impaired (83%) and vestibular responses were often abnormal (90%). [107] Other recent studies reported abnormal vestibular responses in children with cCMV, both in children with and without hearing loss. [126, 127] Even though this finding could be readily explained by the vestibular infection that has been shown in children with cCMV, [128, 129] it is important to note that a cochlear implant itself can lead to vestibular impairment. [126, 130]

Table 1.6 – Prevalence of motor impairment in children with and without cCMV

Author reference	Year	Country, follow-up, method * Type of impairment	Overall n (%) or mean ± SD	Sympt. n (%) or mean ± SD	Asympt. n (%) or mean ± SD	Control n (%) or mean ± SD
Screening based studies						
Ivarsson	1997	Sweden, 7 y, screening	n = 30	n = 5	n = 30	n = 43
[109]		Abnormal Stott test - NS	2 (6.7)			1 (2.3)
Ahlfors	1999	Sweden, 7 y, screening		n = 16	n = 44	n = 39
[71]		Motor delay (Stott < -3 / unable)		3 (31.6)	8 (18.2)	1 (2.6)
Boppana	1999	USA, 3,5 y, screening		n = 42		
[76]		Motor abnormalities		6 (14.3)		
Zhang	2007	China, 6 y, screening			n = 49	n = 50
[110]		Motor skills (DQ) - NS			92.0 ± 12.8	95.8 ± 11.5
Milewska	2010	Poland, 6-6.5 y, screening			n = 38	
[120]		Poor visual-motor integration			3 (7.9)	
Non-screening based studies						
Kylat	2006	Canada, 2 y, registry		n = 38		
[66]		Mild motor deficit		9 (23.7)		
		Moderate motor deficit		12 (31.6)		
		Severe motor deficit		10 (26.3)		

Table 1.6 – Prevalence of motor impairment in children with and without cCMV

Author reference	Year	Country, follow-up, method * Type of impairment	Overall n (%) or mean ± SD	Sympt. n (%) or mean ± SD	Asympt. n (%) or mean ± SD	Control n (%) or mean ± SD
		Non-screening based studies				
Ancora	2007	Italy, 3.5 y, referred	n = 56	n = 17	n = 39	
[101]		Motor delay	6 (10.7)	5 (29.4)	1 (2.6)	
Zagolski	2008	Poland, 3 m, urine testing	52 ears	20 ears	32 ears	80 ears
[127]		No vestibular evoked potential	12 (23.1)	12 (60.0)	0 (0.0)	0 (0.0)
		No caloric response	16 (30.7)	12 (60.0)	4 (12.5)	0 (0.0)
Farkas	2011	Israel, 3 y, referred (normal US)	n = 21	n = 1	n = 20	n = 21
[124]		Motor z-score (11-42m, n = 14)	1.14 ± 1.02			0.81 ± 0.99
		Motor z-score (43-83m, n = 7)	-.07 ± 0.47			0.37 ±0.56
		Motor z-score (n = 21)	0.48 ± 0.79			0.49 ±0.70
Karltorp	2014	Sweden, 7.8 y, testing	n = 26			n = 13
[107]		*Children with HL and CI*				
		Mean age of walking	19 m			12 m
		Delayed age of walking	17/23 (73.9)			0/13 (0.0)
		Pathological head movement	21/25 (84.0)			2/13 (15.4)
		Balance (M-ABC) < p 10	15/18 (83.3)			1/9 (11.1)
		Abnormal caloric test	9/10 (90.0)			not tested
		Oral motor problems:	5/26 (19.2)			1/13 (7.7)
Bernard	2015	France, 5 y, referred (AC)	n = 52	n = 30	n = 22	n = 58
[126]		*Children with HL (92.3%)*				
		Mean age of head control	5.1 m			2.8 m
		Mean age of unsupported sitting	10.2 m			6.4 m
		Mean age of unaided walking	24 m			13.6 m
		Abnormal vestibular test	48 (92.3)	29 (96.7)	19 (86.4)	
		Vestibular abnormal - frequency	47 (90.4)			
		Vestibular abnormal - tract	45 (86.5)			

Sympt: symptomatic, Asympt: asymptomatic, y: year, m: months, AC: Audiological Center; HL: hearing loss, CI: cochlear implant, US: fetal ultrasound, M-ABC: Movement-ABC, DQ: development quotient
* More details about the studies are presented in Table 1.8

Speech and language impairment

It is obvious that hearing loss can lead to speech and language impairment. However, the speech and language development of children with hearing loss and cCMV was shown to be poorer than that of children with hearing loss due to other causes. [82, 107, 131] Few studies focused on speech and language problems in a cohort of cCMV positive children in which only a few children had hearing loss (see Table 1.7). [71,120,124]

In children with symptomatic cCMV speech delay was reported frequently (79%) while only 45% of the study population had moderate to severe hearing loss. [66] Another study of children with symptomatic cCMV reported that 3 of the 14 children with delayed expressive language skills (verbal dyspraxia or dysarthria) had no hearing loss. [100]

Some studies found no difference in speech and language development between asymptomatic children and controls. [91, 124] However, another study in which only 5% of children had hearing loss, reported abnormalities in speech development in 32% of children with an asymptomatic or mildly symptomatic cCMV. [120]

Table 1.7 - Prevalence of language impairment in children with and without cCMV

Author reference	Year	Country, follow-up, method * Type of impairment	Overall n (%)	Sympt. n (%)	Asympt. n (%)	Control n (%)
		Screening based studies				
Kumar	1984	USA, 7.6 y, screening (23.5% HL)			n = 15	n = 17
[91]		Speech-language delay			8 (53.3)	10 (58.9)
Ahlfors	1999	Sweden, 7 y, screening (8.3% HL)	n = 60	n = 16	n = 44	n = 39
[71]		Language problem	3 (5.0)	0 (0.0)	3 (6.8)	0 (0.0)
Zhang	2007	China, 6 y, screening (?% HL)			n = 49	n = 50
[110]		Language development - sign			81.6 ± 14.1	90.1 ± 11.8
Milewska	2010	Poland, 6 y, screening (5.3% HL)			n = 38	
[120]		Speech development delay			12 (31.6)	
		Non-screening based studies				
Williamson	1982	USA, 5.5 y, testing (64.7% HL)		n = 17		
[100]		Expressive language delay		14 (82.4)		
Kylat	2006	Canada, 2 y, registry (57.9% HL)		n = 38		
[66]		Speech delay		30 (78.9)		
Farkas	2011	Israel, 3 y, referred (4.7% HL)	n = 21	n = 1	n = 20	n = 21
[124]		Language z-score (11-42m, n=14)	1.34 ± 0.97			1.03 ± 0.90
		Language z-score (43-83m, n = 7)	0.16 ± 0.55			0.26 ± 0.52
		Language z-score (n = 21)	0.78 ± 0.86			0.64 ±0.67
Karltorp	2014	Sweden, 7.8 y, testing (100% HL)	n = 26			n = 13
[107]		Children with HL and CI				
		Language impairment	2 (7.7)			0 (0.0)

Sympt: symptomatic, Asympt: asymptomatic, y: year, HL: hearing loss, CI: cochlear implant

* More details about the studies are presented in Table 1.8

As indicated previously, Table 1.8 contains more details about the specific study designs that were included in tables 1.2 to 1.7.

Table 1.8 - Information on the different study designs presented in the articles

Author	Year	Country / Region	Period	Follow-up	Method
Ahlfors [92]	1984	Sweden Malmo	1977- 1982	6m - 4y	urine screening
Ahlfors [71]	1999	Sweden Malmo	1977- 1986	7 y	urine screening
Ancora [101]	2007	Italy Bologna	1997- 2003	42 m	referred
Anderson [104]	1996	USA Alabama	1966- 1991	4.8 y	screening/ referred
Bernard [126]	2015	France Paris	2000- 2013	5 y	testing?
Boppana [76]	1999	USA Alabama	1991- 1997	41 m	urine/saliva screening
Coats [94]	2004	USA Texas	1982- 1992	4/11/9 y S/As/co	urine screening
Conboy [121]	1986	USA Alabama	tested in 1984	6.5-12.5 y	urine testing?
Farkas [124]	2011	Israel Tel Aviv	2001- 2007	34 m (11-83 m)	referred
Hanshaw [90]	1976	USA New York	1967- 1970	3.5 - 7 y	IgM/urine screening
Ivarsson [109]	1997	Sweden Malmo	1977- 1982	7 y	urine screening
Karltorp [107]	2014	Sweden Stockholm	2002- 2012	7.8/6.0 y CMV/co	DBS testing
Kumar [91]	1984	USA Ohio	1971- 1974	7.6/7.4 y CMV/co	urine screening
Kylat [66]	2006	Canada Toronto	1987- 2000	2 y	referred/ registry
Milewska [120]	2010	Poland Warsaw	2000	6-6.5 y	urine screening
Numazaki [95]	2004	Japan Sapporo	1977- 2002	6 y	urine screening
Pass [99]	1980	USA Alabama	1965- 1979	4 y (9 m-14 y)	urine test
Ramsay [106]	1991	England and Wales	1983- 1987	3.5 y (1-6.5 y)	lab registry
Reynolds [89]	1974	USA Alabama	1967- 1970	38 m	urine/saliva screening
Saigal [80]	1982	Canada Ontario	1974- 1975	3 / 5 y	urine screening
Stagno [98]	1977	USA Alabama	1967- ?	?	screening/ referred
Teär Fahnehjelm [105]	2015	Sweden Stockholm	2002- 2012	8.3 / 5.6 y CMV/co	DBS testing
Temple [122]	2000	USA Alabama	?	4.7 / 8.7 y CMV 4.9 / 8.6 y co	urine testing?
Townsend [74]	2013	UK London	1979- 1986	5 y	throat swab screening
Townsend [74]	2013	Sweden Malmo	1977- 1985	7 y	urine screening
Williamson [100]	1982	USA Texas	1968- 1980	5.5 y (1-10 y)	urine testing
Zagolski [127]	2008	Poland Krakow	?	3 m	urine testing
Zhang [110]	2007	China Qinba	1997- 2000	6 y	urine screening

S: symptomatic, As: asymptomatic, CMV: congenital CMV infection, co: control group, m: month, y: year

Prevention of congenital CMV infection

The previous section demonstrated that cCMV can lead to substantial long-term impairment, especially in children with symptoms at birth. Nevertheless, even though cCMV is the most common congenital infection, awareness concerning cCMV is generally low. Among women of reproductive age in many countries (e.g. the Netherlands, USA, Canada, Japan and Singapore), only about 15-20% of women have heard of cCMV. [27, 132-136] It is worrying that pregnant women do not know about cCMV, but it is extremely disquieting that medical students, midwives and doctors generally know so little about it. [137-140]

In order to minimize the prevalence and consequences of cCMV it would be best to prevent this disorder occurring. Increasing the low awareness of cCMV is the first step in prevention; people need to know about cCMV before they can take any action against it. In this section, different options for the prevention of (damage by) cCMV will be discussed.

Primary prevention

Prevention of maternal CMV infection during pregnancy
If it were possible to prevent an active infection with CMV during pregnancy then the virus could not be transmitted to the unborn child. Prevention of a primary infection during pregnancy in a seronegative woman could be achieved by a safe and effective vaccine or by prevention of transmission of the virus to the mother, for example by hygienic measures. In seropositive women the aim would be to prevent reinfection as well as reactivation. The risk of reinfection could potentially be lowered by hygienic measures or by a vaccine that would be effective against different strains of CMV. Reactivation of CMV during pregnancy may possibly be prevented by a vaccine that enforces cellular immunity against CMV. [141, 142] However, the relative contribution of reinfections and reactivations to recurrent CMV infections in seropositive women is currently still unclear.

- *Vaccination*
People have been calling for an effective vaccine for many years [143] and even though the development of a vaccine against CMV started in the 1970s [144] and the development of such a vaccine has been ranked as the highest priority by the Institute of Medicine in 2000 [145], there is still no effective vaccine registered. [146, 147]
Already a MF59-adjuvanted recombinant glycoprotein B (gB) subunit vaccine has been shown to be 50% effective in preventing primary infection in CMV-seronegative young mothers in a Phase II double blind placebo-controlled randomized trial. [148] In addition, this vaccine has been demonstrated to reduce the duration of viremia after kidney or liver transplantation when it was given to patients before the transplantation in a Phase II double blind placebo-controlled randomized trial. [149]
It has been shown that a vaccine can boost the immune response in seropositive women [150], which is potentially beneficial in the prevention of intrauterine transmission in cases of reactivation or reinfection, although this remains to be proven. More vaccine candidates have been developed, [151] nonetheless none of these vaccines has been registered.
It has been calculated that if a vaccine with an efficacy of 60% would be available for universal vaccination of female adolescents this would be cost effective. [152] Moreover, a vaccine which protects 50% to 60% of the population could potentially lead to the

interruption of endemic transmission through herd immunity, because of the low estimated basic reproductive number of around 2 in developed countries. [22, 23] If CMV would be globally eradicated, this would also prevent CMV-disease in immunocompromised patients.

- *Hygienic advice*

It has already been mentioned that CMV can be transmitted through body fluids by intimate contact between persons. [17] Preventing contact with these fluids by conventional hygienic measures could, to some extent, lower the risk of primary infection or reinfection. [71]
A number of relatively simple hygienic measures for pregnant women could partially reduce the risk of cCMV. These measures include: Avoid kissing a child on the mouth. Avoid sharing foods, drinks, eating utensils and cutlery with a young child. Avoid putting anything in the mouth that has just been in the mouth of a child (e.g. pacifier). Washing hands after changing a diaper or handling other body fluids (e.g. cleaning a runny nose). [153]
It has been demonstrated that providing information about CMV infection and its complications, together with instructions detailing protective behaviors, was effective in preventing seroconversion in pregnant women. [154-157]

Secondary prevention

Prevention of intrauterine transmission

If a primary CMV infection is diagnosed during pregnancy, the risk of intra-uterine transmission might be influenced by, for example, medication. This would mean that all pregnant women would need to be screened in order to detect the majority of primary maternal CMV infections. At present, only occasionally a primary maternal infection is detected as a result of clinical symptoms in the mother. Moreover, there is currently no effective intervention to reduce the transmission risk.
During the last few years a lot of attention has been given to the potential efficacy of CMV-specific hyperimmune globulin administered after a primary infection has been diagnosed. Initially, this intervention seemed promising in a prospective, non-randomized study. [158] However, a later double-blinded randomized placebo-controlled trial reported no statistically significant effect on the fetal infection rate. [159]
Furthermore, antiviral agents could potentially reduce the intrauterine transmission of CMV. Only one study, in HIV-infected mothers receiving either valaciclovir prophylaxis or placebo, showed that the prevalence of cCMV was lower in the valaciclovir arm. However, the difference with the placebo arm was not statistically significant. [160] Still, this potential beneficial effect justifies further investigation.

Tertiary prevention

After a fetus has been infected with CMV, it might be possible to influence the severity of the disease and the long-term outcome in these children, either during pregnancy or postnatally. First of all, the children with cCMV have to be identified, for example by maternal and subsequent fetal screening, by ultrasound or by neonatal screening.

Prevention of disease or reduction of disease severity - prenatally

CMV-specific hyperimmune globulin was also investigated as a potential treatment of established fetal infection to prevent disease. A number of observational studies on hyper-immune globulin therapy demonstrated a reduction in the percentage of children with

symptoms at birth or with poor outcome. [158, 161, 162] However, since no randomized placebo-controlled trials have been performed, it is not clear if this treatment is beneficial for fetuses with cCMV.
It is possible that anti-viral agents could prove to be beneficial in the near future. Maternal administration of valacyclovir was shown to lead to therapeutic concentrations in the fetal blood. [163] The first results of a phase II open-label study on the effects of high-dose valacyclovir treatment in pregnant women with a proven intrauterine CMV infection after primary infection are encouraging, but further investigation is needed. [164]

Prevention of disease or reduction of disease severity - postnatally
Early recognition of cCMV can be beneficial because this enables early intervention. When a child is diagnosed with cCMV it might be possible to prevent future sequelae or deterioration of present sequelae. Progression of hearing loss was less frequent in children with cCMV and neurological symptoms at birth who were treated with (val)ganciclovir within one month after birth compared to those who were not treated. [165-167] In addition the treated children had better outcomes on the language-composite component and receptive-communication scale at 24 months of age. [167] It is currently not known if this antiviral treatment could also be beneficial in other children with cCMV, for example children with only hearing loss at birth, or possibly even asymptomatic children.
Even if a child is asymptomatic at birth, an early diagnosis could be advantageous for their development. As has been discussed earlier, cCMV can lead to hearing impairment in the first years of life. It can be difficult to recognize hearing loss in young children, especially in case of unilateral hearing loss, and therefore the diagnosis of late-onset hearing loss can be considerably delayed. This delayed detection could have negative effects on language development. If it is known that a child has cCMV at birth, regular hearing screening could enable prompt diagnosis and early treatment of hearing loss, which is valuable for speech and language development. [168, 169]

- *Universal screening*
Universal screening is the screening of all children, independent of specific risk factors. With universal screening virtually all children with cCMV are expected to be identified. However, this will also impose a risk of false positive results. In order to prevent missing children with cCMV and to keep the number of false positives as low as possible a test would have to be highly sensitive and highly specific. Many studies looked at the sensitivity and specificity of different types of tests. Urine and saliva testing seem to give the best results [170-172], although these may have considerable logistic difficulties. Testing dried blood spots however, is logistically convenient for screening and depending on the method used has reasonably acceptable test characteristics. [173]

- *Targeted screening*
Another option is targeted screening, in which screening is performed only in children with a high risk of cCMV, for example screening of children with sensorineural hearing loss at birth. Because children with hearing loss have a ten times higher risk of cCMV, [174] fewer children need to be screened in order to identify one child with cCMV. This will decrease the absolute amount of false positives because the total amount of children screened is smaller.

Outline of this thesis

The aim of this thesis was to determine the long-term consequences of congenital CMV infection in the Netherlands.

It is already clear that cCMV can lead to a broad range of neurological impairments. Especially hearing loss and overt and severe neurological problems have been extensively studied and much data are available on long-term outcome of children with symptomatic cCMV infection as these are obviously identified at birth and subsequently followed up. However, because the long-term consequences for the large group of children with asymptomatic cCMV are less frequently studied, no conclusion can currently be drawn concerning their long-term outcome. Moreover, methodological issues might have led to less reliable estimates of long-term impairments. Many of the studies included no or only small control groups and there was a lack of consensus on definitions. As previously mentioned, there may be differences between study populations based on screening and populations based on referral after clinical suspicion. In addition, the frequently used prospective study design has the advantage of uniform and structured data collection, but it may also lead to information bias when the parents, doctors and investigators are aware of the condition of the children.

We decided to perform a nation-wide, screening based, retrospective cohort study with a control group to gain more insight into the whole range of clinical consequences, as well as the impact of cCMV on the daily life of children and parents and the economic consequences. This study is called the CROCUS study, an acronym for **C**onsequences and **R**isk factors **O**f congenital **C**ytomegalovir**US** infection. The aim of this study was to determine the long-term consequences of cCMV in the Netherlands in children up to six years of age.

The information on the burden of disease of cCMV gained from this study is important, especially when considering preventive measures. An estimate of the prevalence of cCMV and the prevalence of the long-term consequences of cCMV, together with estimates of the overall and subgroup-specific prevalence of CMV infection in the Netherlands, will provide input for mathematical models. This will enable analyses of the potential effects of various vaccination strategies against CMV in the Netherlands. Moreover, the insights gained from this study can be used in considering the potential benefits and disadvantages of neonatal screening in the Netherlands.

Whereas many studies throughout the world reported CMV seroprevalences, the nation-wide seroprevalence of CMV in the Netherlands is still unknown. A national serum bank together with demographic data could be used to estimate the seroprevalence and identify risk factors for higher seroprevalence, which could consequently be helpful to evaluate future preventive strategies. The results of such a national seroprevalence study in the Netherlands are presented in **Chapter 2.**

In **Chapter 3** the design of the large, retrospective, nation-wide, screening based study is presented. In addition, this chapter includes the birth prevalence estimated by this study as well as the participation rate of this study.

The long-term clinical impairments of children with and without cCMV, up to the age of six years, are presented in **Chapter 4**. It includes data on hearing, visual, neurological, cognitive, motor and speech-language impairment in both symptomatic and asymptomatic children. This chapter provides an estimate of the clinical consequences of cCMV.

In **Chapter 5** the consequences of cCMV on the daily life of children and their parents are presented. These include child development as assessed by the parents, the school performance of children, the quality of life of both children and their parents, the amount of care that is needed by the children and the consequences of their child's impairment for the parents.

In **Chapter 6** the financial consequences of cCMV are discussed. The costs for children with and without cCMV are estimated up to six years of age, based on the care that was provided to these children. This will be the first study to estimate the costs of cCMV directly.

Finally, **Chapter 7** contains the general discussion on the topics that are discussed in this thesis. Besides the implications of this research for preventive measures and the current care of children with cCMV, suggestions for future studies will be made.

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