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Consequences of congenital cytomegalovirus infection in early childhood

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Citation

Korndewal, M. (2017, January 24). *Consequences of congenital cytomegalovirus infection in early childhood*. Retrieved from <https://hdl.handle.net/1887/45778>

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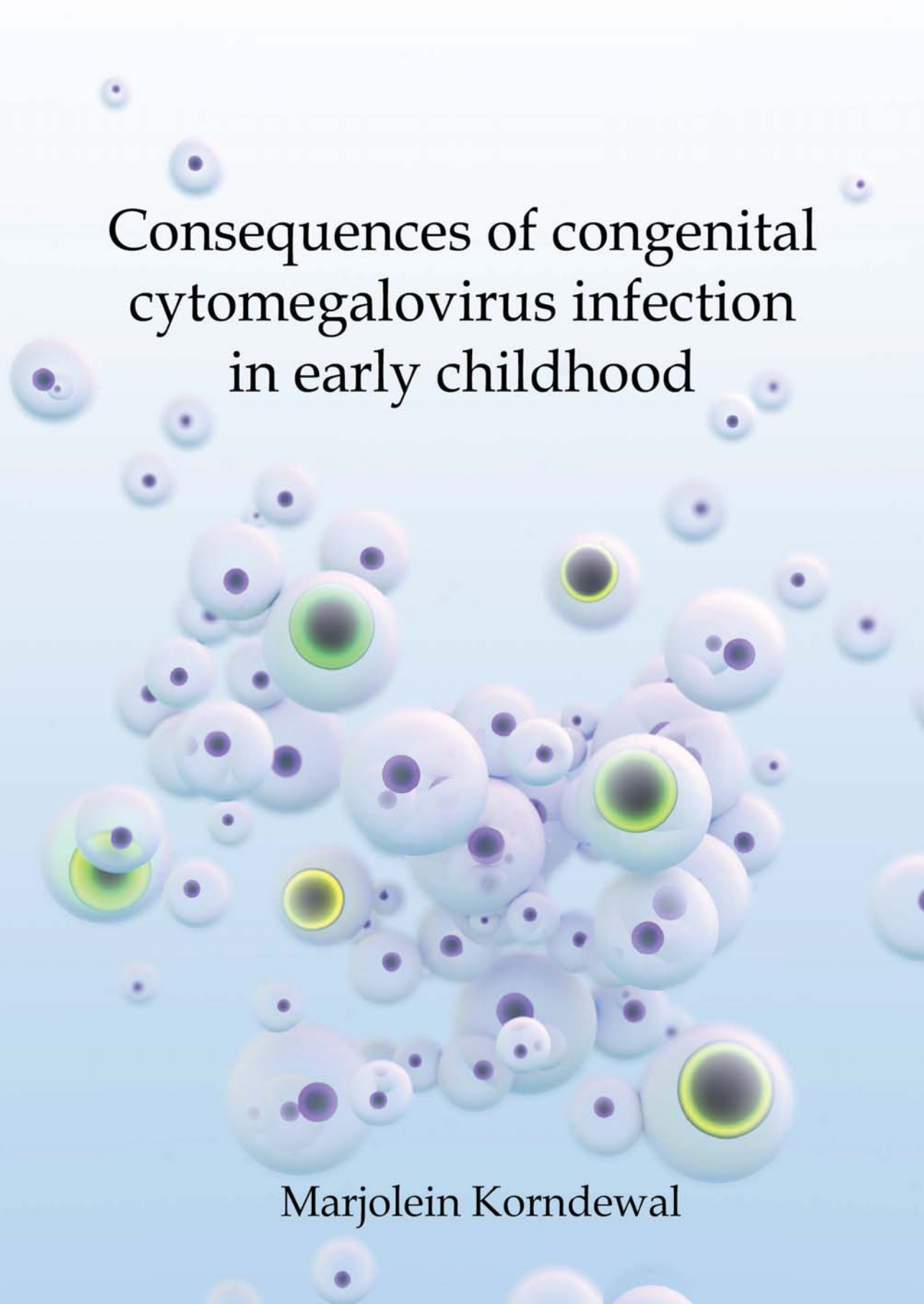


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Author: Korndewal, Marjolein

Title: Consequences of congenital cytomegalovirus infection in early childhood

Issue Date: 2017-01-24



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PhD Thesis, University of Leiden, The Netherlands

ISBN: 978-94-6332-127-3

Cover design: Marijn Vink and Bob Embregts, Asura Motion Design, Zeist, the Netherlands
Printing: GVO drukkers & vormgevers, Ede, the Netherlands

The publication of this thesis was financially supported by the Leiden University Medical Center and the National Institute for Public Health and the Environment.

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Consequences of congenital cytomegalovirus infection in early childhood

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof. mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op dinsdag 24 januari 2017
klokke 15.00 uur
door

Marjolein Jolanda Korndewal

geboren te Baarn

in 1982

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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 Malmö	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 Malmö	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 anophthalmia, 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 *	Overall n (%)	Sympt. n (%)	Asympt. n (%)	Control n (%)
Screening based studies						
Hanshaw [90]	1976	USA, 3.5 - 7 y, screening Chorioretinitis			n = 44 1 (2.3)	n = 44 0 (0.0)
Pass [99]	1980	USA, 4 y, screening Chorioretinitis Optic atrophy		n = 23 4 (17.4) 2 (8.7)		
Coats [94]	2004	USA, 4/11 y (S/As), screening Visual impairment (VA < 0.1) Subnormal vision (VA 0.1 - 0.5) Retinal scarring Optic atrophy Strabismus Cortical visual impairment		n = 42 7 (16.7) 2 (4.8) 9 (21.4) 3 (7.1) 12 (28.5) 4 (9.5)	n = 83 0 (0.0) 1 (1.2) 2 (2.4) 0 (0.0) 1 (1.2) 0 (0.0)	n = 21 0 (0.0) 0 (0.0) 0 (0.0) 0 (0.0) 1 (4.7) 0 (0.0)
Non-screening based studies						
Ramsay [106]	1991	England, 3.5 y, lab registry Chorioretinitis Cortical blindness	n = 65 3 (4.6) 4 (6.2)			
Anderson [104]	1996	USA, 5 y, screening + referred Visual impairment (VA < 0.4) Chorioretinitis Optic atrophy Pigmentary retinopathy Strabismus Nystagmus		n = 113 17 (15.0) 22 (19.5) 12 (10.6) 8 (7.1) 26 (23.0) 3 (2.7)	n = 332 2 (0.6) 13 (3.9) 1 (0.3) 10 (3.0) 5 (1.5) 3 (0.9)	
Kylat [66]	2006	Canada, 2 y, registry Visual impairment (VA < 0.1) Cortical visual impairment		n = 38 4 (11.0) 3 (7.9)		
Teär Fahnehjelm [105]	2015	Sweden, 8 y, DBS tested (hearing loss + cochlear implant) Visual impairment (VA ≤ 0.3) Macula scarring Optic atrophy Strabismus	n=26 5 (19.2) 5 (19.2) 1 (3.8) 5 (19.2)			n = 13 0 (0.0) 0 (0.0) 0 (0.0) 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 Psychomotor delay Seizures Spasticity		n = 23 4 (17.4) 5 (21.7) 6 (26.1)		
Ahlfors [92]	1984	Sweden, 0,5-4 y, screening Psychomotor retardation Cerebral palsy		n = 8 1 (12.5) 1 (12.5)	n = 35 1 (2.8) 0 (0.0)	n = 49 0 (0.0) 0 (0.0)
Ivarsson [109]	1997	Sweden, 2 y, screening Development scale <normal	n = 32 6/32 (18.8)			n = 51 8/51 (15.7)
Boppana [76]	1999	USA, 3,5 y, screening Seizure		n = 42 2/41 (4.9)		
Ahlfors [71]	1999	Sweden, 7 y, screening Mental retardation Cerebral palsy Epilepsy		n = 16 2 (12.5) 2 (12.5) 0 (0.0)	n = 44 2 (4.5) 2 (4.5) 1 (2.3)	n = 39 0 (0.0) 0 (0.0) 0 (0.0)
Numazaki [95]	2004	Japan, 6 y, screening Mental Retardation (DQ < 70)			n = 21 0 (0.0)	
Zhang [110]	2007	China, 6 y, screening DQ 70-89 DQ < 70			n = 49 13 (26.5) 4 (8.2)	n = 50 7 (14.0) 1 (2.0)
Townsend [74]	2013	UK, 5 y, screening Mild impairment Moderate impairment Severe impairment	n = 87 7 (8.1) 4 (4.6) 3 (3.4)	n = 5 1 (20.0) 1 (20.0) 2 (40.0)	n = 82 6 (7.3) 3 (3.7) 1 (1.2)	n = 111 3 (2.7) 0 (0.0) 0 (0.0)
Townsend [74]	2013	Sweden, 7 y, screening Mild impairment Moderate impairment Severe impairment	n = 67 4 (6.0) 3 (4.5) 6 (9.0)	n = 14 1 (7.1) 0 (0.0) 3 (21.4)	n = 53 3 (5.7) 3 (5.7) 3 (5.7)	n = 39 1 (2.6) 0 (0.0) 0 (0.0)
Non-screening based studies						
Williamson [100]	1982	USA, 5.5 y, testing Cerebral palsy Microcephaly		n = 17 7 (41.2) 10 (58.9)		
Ramsay [106]	1991	England, 3.5 y, lab registry Cerebral palsy Severe psychomotor retardation		n = 65 14 (21.5) 6 (9.2)		
Kylat [66]	2006	Canada, 2 y, registry Bayley < -2 SD / cognitive deficit Developmental delay Bedridden or wheelchair bound		n = 38 27 (71.1) 32 (84.2) 6 (15.7)		
Ancora [101]	2007	Italy, 3.5 y, referred DQ <85	n = 56 8 (14.3)	n = 17 7 (41.2)	n = 39 1 (2.6)	
Karltorp [107]	2014	Sweden, 7.8 y, testing (HL) Cerebral palsy	n = 26 2 (7.6)			n = 13 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 $\pm$ SD	Control n (%) or mean $\pm$ SD
Screening based studies						
Reynolds [89]	1974	USA, 3 y, screening Mean Social Quotient			n = 18 123	n = 18 134
Ahlfors [71]	1999	Sweden, 7 y, screening Developmental disorder ADHD Autism/Asperger		n = 16 1 (6.3) 1 (6.3) 0 (0.0)	n = 44 4 (9.1) 1 (2.3) 2 (4.5)	n = 39 0 (0.0) 0 (0.0) 0 (0.0)
Zhang [110]	2007	China, 6 y, screening Adaptive capacity (DQ) Personal-social skills (DQ) Global development (DQ) - sign			n = 49 87.5 $\pm$ 12.9 93.4 $\pm$ 12.9 88.6 $\pm$ 13.1	n = 50 92.0 $\pm$ 10.9 97.6 $\pm$ 11.7 94.0 $\pm$ 11.4
Milewska [120]	2010	Poland, 6-6.5 y, screening Increased emotional sensitivity Problem with school maturity			n = 38 14 (36.8) 6 (15.8)	
Non-screening based studies						
Karltorp [107]	2014	Sweden, 7.8 y, testing (HL) ADHD Autism Spectrum Disorder	n = 26 2 (7.6) 4 (15.4)			n = 13 0 (0.0) 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 $\pm$ SD	Sympt. n (%) or mean $\pm$ SD	Asympt. n (%) or mean $\pm$ SD	Control n (%) or mean $\pm$ SD
Screening based studies						
Reynolds [89]	1974	USA, 3 y, screening IQ < 90 IQ < 70			n = 18 7 (38.9) 1 (5.5)	n = 18 4 (22) 1 (5.5)
Hanshaw [90]	1976	USA, 3.5 - 7 y, screening IQ < 90 IQ < 80			n = 44 12 (27.3) 7 (15.9)	n = 44 6 (13.6) 0 (0.0)
Pass [99]	1980	USA, 4 y, screening IQ < 80 IQ < 50		n = 23 10 (43.4) 9 (39.1)		
Saigal [80]	1982	Canada, 3 / 5 y, screening Mean IQ (Stanf.-Binet) 3 y - NS Mean IQ (Stanf.-Binet) 5 y - NS			n = 41 97.0 $\pm$ 16.5 107.8 $\pm$ 16.6	n = 44 100.6 $\pm$ 15.6 106.5 $\pm$ 9.5
Kumar [91]	1984	USA, 7.6 y, screening Mean IQ (WISC) - NS			n = 15 89.5 $\pm$ 11.7	n = 17 85.9
Ivarsson [109]	1997	Sweden, 7 y, screening Intellectual scale (WISC) - NS Intellectual scale < normal - NS	n = 25 5.8 $\pm$ 2.0 3/25 (12.0)			n = 41 6.4 $\pm$ 1.6 2/41 (4.8)
Boppana [76]	1999	USA, 3.5 y, screening IQ 50- 70 IQ < 50		n = 21 6 (28.6) 0 (0.0)		
Numazaki [95]	2004	Japan, 6 y, screening IQ (WISC) 70 - 89			n = 21 3 (14.3)	
Zhang [110]	2007	China, 6 y, screening Mean IQ (WPPSI)- sign IQ (WPPSI) 70 - 89 IQ (WPPSI) < 70 - NS			n = 49 89.4 $\pm$ 12.8 15 (30.6) 4 (8.2)	n = 50 95.3 $\pm$ 10.5 9 (18.0) 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 $\pm$ SD	Sympt. n (%) or mean $\pm$ SD	Asympt. n (%) or mean $\pm$ SD	Control n (%) or mean $\pm$ SD
Townsend [74]	2013	UK, 5 y, screening Mean IQ (WPPSI) – NS Mean Verbal IQ – NS Mean Performance IQ – NS	n = 69 111.7 $\pm$ 15.7 102.6 $\pm$ 17.8 119.3 $\pm$ 14.2			n = 108 113.0 $\pm$ 13.6 106.0 $\pm$ 13.6 118.1 $\pm$ 14.1
Non-screening based studies						
Williamson [100]	1982	USA, 5.5 y, testing Subnormal IQ		n = 17 9 (52.9)		
Conboy [121]	1986	USA, 9.2 y, testing? Mean IQ (WISC) - NS Mean Verbal IQ - NS Mean Performance IQ - NS Mental processing (K-ABC) - NS			n = 18 100 $\pm$ 17.1 96 $\pm$ 15.3 103 $\pm$ 17.4 98 $\pm$ 13.5	n = 18 100 $\pm$ 12.3 98 $\pm$ 13.9 101 $\pm$ 11.7 97 $\pm$ 11.9
Temple [122]	2000	USA, 4.8 y, testing? Mean IQ (WPPSI) - sign Mean Verbal IQ - NS Mean Performance IQ - NS			n = 49 82.5 $\pm$ 16.4 81.1 $\pm$ 14.3 87.2 $\pm$ 17.4	n = 69 88.6 $\pm$ 17.7 86.6 $\pm$ 15.5 92.8 $\pm$ 18.7
Temple [122]	2000	USA, 8.6 y, testing? Mean IQ (WISC-R) - NS Mean Verbal IQ - NS Mean Performance IQ - NS			n = 60 92.7 $\pm$ 15.7 92.1 $\pm$ 16.2 94.7 $\pm$ 15.4	n = 104 91.6 $\pm$ 14.1 90.7 $\pm$ 14.7 94.3 $\pm$ 14.1
Farkas [124]	2011	Israel, 3 y, referred (normal US) Cognitive z-score (11-42m, n=14) Cognitive z-score (43-83m, n=7) Cognitive z-score (all, n = 21)	n = 21 0.50 $\pm$ 0.68 0.49 $\pm$ 0.43 0.49 $\pm$ 0.59	n = 1	n = 20	n = 21 0.31 $\pm$ 0.82 1.15 $\pm$ 0.86 0.59 $\pm$ 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 $\pm$ SD	Sympt. n (%) or mean $\pm$ SD	Asympt. n (%) or mean $\pm$ SD	Control n (%) or mean $\pm$ SD
Screening based studies						
Ivarsson [109]	1997	Sweden, 7 y, screening Abnormal Stott test - NS	n = 30 2 (6.7)	n = 5	n = 30	n = 43 1 (2.3)
Ahlfors [71]	1999	Sweden, 7 y, screening Motor delay (Stott < -3 / unable)		n = 16 3 (31.6)	n = 44 8 (18.2)	n = 39 1 (2.6)
Boppana [76]	1999	USA, 3,5 y, screening Motor abnormalities		n = 42 6 (14.3)		
Zhang [110]	2007	China, 6 y, screening Motor skills (DQ) - NS			n = 49 92.0 $\pm$ 12.8	n = 50 95.8 $\pm$ 11.5
Milewska [120]	2010	Poland, 6-6.5 y, screening Poor visual-motor integration			n = 38 3 (7.9)	
Non-screening based studies						
Kylat [66]	2006	Canada, 2 y, registry Mild motor deficit Moderate motor deficit Severe motor deficit		n = 38 9 (23.7) 12 (31.6) 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 $\pm$ SD	Sympt. n (%) or mean $\pm$ SD	Asympt. n (%) or mean $\pm$ SD	Control n (%) or mean $\pm$ SD
Non-screening based studies						
Ancora [101]	2007	Italy, 3.5 y, referred Motor delay	n = 56 6 (10.7)	n = 17 5 (29.4)	n = 39 1 (2.6)	
Zagolski [127]	2008	Poland, 3 m, urine testing No vestibular evoked potential No caloric response	52 ears 12 (23.1) 16 (30.7)	20 ears 12 (60.0) 12 (60.0)	32 ears 0 (0.0) 4 (12.5)	80 ears 0 (0.0) 0 (0.0)
Farkas [124]	2011	Israel, 3 y, referred (normal US) Motor z-score (11-42m, n = 14) Motor z-score (43-83m, n = 7) Motor z-score (n = 21)	n = 21 1.14 $\pm$ 1.02 -.07 $\pm$ 0.47 0.48 $\pm$ 0.79	n = 1	n = 20	n = 21 0.81 $\pm$ 0.99 0.37 $\pm$ 0.56 0.49 $\pm$ 0.70
Karltorp [107]	2014	Sweden, 7.8 y, testing <i>Children with HL and CI</i> Mean age of walking Delayed age of walking Pathological head movement Balance (M-ABC) < p 10 Abnormal caloric test Oral motor problems:	n = 26 19 m 17/23 (73.9) 21/25 (84.0) 15/18 (83.3) 9/10 (90.0) 5/26 (19.2)			n = 13 12 m 0/13 (0.0) 2/13 (15.4) 1/9 (11.1) not tested 1/13 (7.7)
Bernard [126]	2015	France, 5 y, referred (AC) <i>Children with HL (92.3%)</i> Mean age of head control Mean age of unsupported sitting Mean age of unaided walking Abnormal vestibular test Vestibular abnormal - frequency Vestibular abnormal - tract	n = 52 5.1 m 10.2 m 24 m 48 (92.3) 47 (90.4) 45 (86.5)	n = 30 29 (96.7)	n = 22 19 (86.4)	n = 58 2.8 m 6.4 m 13.6 m

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

Cytomegalovirus infection in the Netherlands: seroprevalence, risk factors, and implications

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Journal of Clinical Virology 2015 Feb; 63: 53-58

Abstract

Background

Cytomegalovirus (CMV) infections occur worldwide and are usually asymptomatic in healthy individuals. In fetuses and immunocompromised persons, they can cause severe disease and disabilities.

Objective

To determine the CMV seroprevalence and risk factors for CMV infection in the Netherlands.

Study design

In a cross-sectional population-based study (PIENTER-2, 2006-2007), sera and questionnaire data were collected from 6386 individuals. Sera were tested for CMV-specific IgG antibodies using enzyme-linked immunosorbent assay (ELISA).

Results

The CMV seroprevalence in the general population (6 months - 79 years) was 45.6%. Age and country of origin were the most prominent independent risk factors. The seroprevalence was significantly lower in native Dutch and Western individuals (41.5%) than in non-Western individuals (76.7%). Multivariable logistic regression analysis showed that age, lower educational level, first-generation migrancy, and among native Dutch/Western individuals, female gender and having contact with young children, were independently associated with CMV seropositivity. The geometric mean concentrations of antibodies increased with age and were higher in women than in men.

Conclusion

CMV seroprevalence in the Netherlands is relatively low compared to other countries. This is in line with our finding of a higher seroprevalence among migrants compared to the native population. The higher seroprevalence in women and individuals who have contact with young children is especially important for women of reproductive age. Preventing CMV infection in these women, through counseling on hygiene or possible future vaccination, may lead to a decrease of congenital CMV infections.

Background

Cytomegalovirus (CMV) is a common cause of infections worldwide. Like other herpes viruses, primary infection is followed by lifelong latency, with episodes of reactivation when the virus can be transmitted again. Transmission is possible via saliva, urine, blood, sexual contact, breastfeeding, and organ transplantation. In addition, CMV can be transmitted vertically through the placenta [1].

Both primary and recurrent CMV infections are usually asymptomatic in healthy individuals but can lead to serious complications in organ or stem-cell transplant recipients and other immunocompromised individuals [2].

The most important burden of disease comes from CMV infections during pregnancy that can lead to infection of the unborn child. Worldwide, between 0.2% and 1.0% of all newborns have a congenital CMV infection, which can cause severe and long-lasting disability including sensorineural hearing loss and cognitive or motor developmental delay [1].

A congenital CMV infection may be caused by a primary maternal infection or by a recurrent maternal infection due to reactivation of latent virus or reinfection with another CMV strain. Primary infection is more likely than recurrent infection to be transmitted to the fetus and to cause more severe disability. Still, recurrent infections account for almost 60% to 95% of congenital CMV infections, given population seroprevalences between 30% and 95% [3,4].

Since most CMV infections are asymptomatic in healthy individuals, serological testing is the only reliable way to estimate the prevalence of a prior infection in a population. Most seroprevalence studies have focused on selected subpopulations. A meta-analysis among women of reproductive age showed worldwide CMV seroprevalences ranging from 45% to 100%. The seroprevalences are lowest in Western Europe and the United States and highest in South America, Africa, and Asia [5].

Large nation-wide population-based seroprevalence studies are essential to reliably assess the CMV prevalence and risk factors for infection in order to direct future preventive measures and estimate their cost-effectiveness [6].

Objectives

The aim of this study was to determine CMV seroprevalence in the general population of the Netherlands using a national serum bank and furthermore to define risk factors for contracting CMV infections based on demographic and epidemiologic data.

Study design

Study population and design

This study used information from the PIENTER2 project, a cross-sectional population-based serum bank established in 2006-2007. Details of the study design have been previously published. [7] Briefly, 40 municipalities equally distributed over five geographic regions of the Netherlands were randomly selected proportional to their population size. An age-stratified sample was drawn from the population register, and the migrant population was oversampled in 12 of the 40 municipalities.

A total of 19,781 individuals, including 2558 non-Western migrants, were invited to complete a questionnaire and donate a blood sample. Serum samples were obtained from 6386 individuals (32%). Sufficient serum to test for CMV antibodies was available from 6382 participants. The study was approved by the local medical ethics committee (Almere, ISRCTN 20164309), and all participants, or their legal representatives, gave written informed consent.

Antibody assay

An ELISA, ETI-CYTOK-G PLUS (DiaSorin, Saluggia, Italy), was used to detect CMV-specific IgG antibodies. A concentration above 0.4 IU/ml was considered seropositive for a CMV infection, according to the manufacturer's instructions. Antibody concentrations were included in the analysis, and for that purpose, concentrations above the upper limit of detection (>10 IU/ml) were registered as 10 IU/ml (169 individuals).

Definition of variables

In accordance with the CBS (Dutch Statistics) definitions, a non-Western migrant is defined as a person originating from Turkey or a country in Africa, South America, or Asia (except Indonesia and Japan). A Western migrant is someone from Japan or a country in Europe (except Turkey), North America, Oceania, or Indonesia. First-generation migrants are foreign-born residents of the Netherlands who have at least one foreign-born parent. Second-generation migrants are born in the Netherlands but have at least one foreign-born parent. If both parents were foreign-born, a participant's origin was based on the mother's country of birth [8].

Educational level was classified as low (no education or only primary education), middle (junior technical school, lower general or intermediate vocational secondary education), or high (higher vocational or higher general secondary education, pre-university or university education). If the participant was a child (<15 years of age), the educational level of the mother was used.

Women of childbearing age are defined as women 20–45 years old. Household members are persons living in the same house as a participant. Contacts are non-household persons with whom a participant spoke the day before filling in the questionnaire.

Statistical analysis

Overall CMV seroprevalence and 95% confidence intervals (CI) were calculated for persons older than 6 months of age. Children younger than 6 months were excluded because their antibodies tend to be maternally derived.

The overall seroprevalence was weighted proportional to the Dutch reference population as recorded on 1 January 2007, taking gender, age, ethnic origin, and degree of urbanization into account.

Unweighted seroprevalences were calculated for three subgroups: native Dutch and other Western individuals, non-Western individuals, and women of childbearing age.

Logistic regression analysis was performed to study risk factors for CMV seropositivity in the general Dutch population. It was stratified by origin, since considerable differences were observed between Dutch/Western participants and non-Western participants. Variables included age, gender, educational level, generation of migrants (first or second), number of persons per household, a child under 4 years of age in the household (yes/no), number of contacts, and contact with children under 4 years of age (yes/no). Logistic regression analysis, adjusted for age and gender, was conducted for each risk factor separately, and multivariable analysis was performed for all risk factors together.

Both analyses were also performed for Dutch women of childbearing age and their Western migrant counterparts. The subgroup of non-Western childbearing women was too small to be included in the analysis ($n = 101$).

Geometric mean concentrations (GMCs) of CMV antibodies were calculated for CMV-seropositive individuals in the overall population and in the three subgroups. Multivariable linear regression analysis was limited to seropositive individuals, to study whether any variables were independent predictors of CMV antibody concentration levels. The regression coefficients of the linear regression were exponentially transformed to the GMC ratio (the ratio of GMC between two categories).

Data analyses were performed using SAS, version 9.1.3 (SAS Institute Inc., Cary, NC, USA).

Results

Study population

After exclusion of children under 6 months of age, the analysis includes 6249 persons, aged 6 months to 79 years, of whom 5205 (83.3%) were of Dutch/Western origin and 1044 (16.7%) of non-Western origin.

The Dutch/Western individuals differed from non-Western in mean age ($37.6 \text{ years} \pm 23.6 \text{ SD}$ vs. $24.3 \text{ years} \pm 24.0 \text{ SD}$) and educational level, with 40.6 vs. 21.9% defined as high, 50.8 vs. 40.7% as middle, 7.5% vs. 31.8% as low, and 1.1% vs. 5.6% missing.

CMV seroprevalence

Based on our study population, CMV seroprevalence in the general population of the Netherlands was 45.6%. It was lower in individuals of native Dutch origin (40.1%) than in Western migrants (57.3%) and non-Western migrants (76.7%). Among all migrants, seroprevalence was higher in the first generation than the second generation of residents in the Netherlands (Table 2.1).

Table 2.1 - CMV seroprevalence in the Dutch population, aged 6 months to 79 years, in relation to geographic origin

Geographic origin	Number	Seroprevalence	
		%	95% CI
Overall study population	6249	45.6	41.9 - 49.3
Native Dutch	4772	40.1	38.7 - 41.5
Western	433	57.3	52.6 - 61.9
1st generation migrant	152	69.7	62.4 - 77.0
2nd generation migrant	281	50.5	44.7 - 56.4
Non-Western	1044	76.7	74.2 - 79.3
1st generation migrant	664	87.2	84.7 - 89.7
2nd generation migrant	380	58.4	53.5 - 63.4
Non-Western (per country)			
Morocco / Turkey	338	84.0	80.1 - 87.9
Surinam / Aruba / Netherlands Antilles	351	69.5	64.7 - 74.3
Other non-Western countries	355	76.9	72.5 - 81.3

The seroprevalence in the general population increased steadily with age (Figure 2.1).

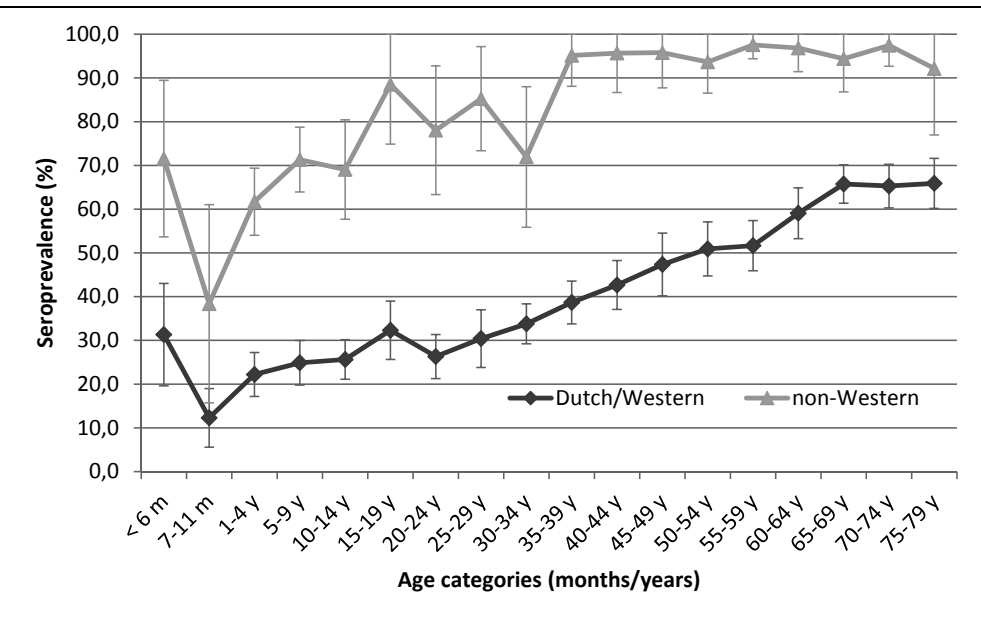


Figure 2.1 - Weighted CMV seroprevalence (with 95% CI) per age category for individuals of Dutch/Western origin (n = 5313) and non-Western origin (n = 1069), aged 0 - 79 years.

Women of reproductive age

In women of reproductive age (20-45 years), seroprevalence was much higher in non-Western individuals (85.1%; 95% CI: 78.1 - 92.2) than in Dutch/Western individuals (36.9%; 95% CI: 33.9 - 40.0).

In native Dutch women, seroprevalence increased with age from about 30% at 20-24 years of age to more than 40% at 40-44 years of age. An increase of 0.58% per year (95% CI: 0.16 - 1.01) was found based on linear regression analysis.

Risk factors for seropositivity

The odds of seropositivity, adjusted for gender and age, was 8.26 (95% CI: 6.97 - 9.80) times higher in non-Western individuals compared to Dutch/Western individuals.

- Dutch and Western origin (Table 2.2a)

In logistic regression analysis, adjusted for age and gender, and in multivariable analysis, the odds for CMV seropositivity was statistically significantly higher in the following groups: older age, women, persons with a low educational level, first-generation migrants, and persons who reported contact with young children (not living in the same household). Due to the high correlation between age and seropositivity, the crude seroprevalence in persons who have no contact with young children is higher compared to persons who have contact with young children, even though an inverse association is found in regression analysis. This discrepancy between crude seroprevalence and odds ratios, adjusted for age and gender, is also noticeable within subgroups of household size and persons living with or without a young child in the household.

- Non-Western origin (Table 2.2b)

For non-Western individuals an independent positive association for CMV seropositivity was found with older age and lower educational level. The odds of seropositivity was almost three times higher (2.97; 95% CI: 2.15-4.12) in first-generation migrants than in second-generation migrants.

- Women of reproductive age

The multivariable logistic regression analysis among Dutch/Western women (n = 950) revealed no statistically significant associations between any of the studied risk factors and CMV seropositivity, except for age.

Table 2.2a - Seroprevalence and risk factors for seropositivity among Dutch/Western persons

Characteristics	number	seroprev %	logistic regression**		multivariable logistic regression	
			OR	95% CI	OR	95% CI
Overall population	5205	41.5				
Age						
6 m-9 y	830	21.8	1 (ref)		1 (ref)	
10-19 y	629	28.0	1.38*	1.08 - 1.75	1.28	0.96 - 1.69
20-29 y	643	28.9	1.41*	1.11 - 1.79	1.52*	1.16 - 1.99
30-39 y	646	36.8	2.04*	1.62 - 2.57	2.09*	1.64 - 2.66
40-49 y	603	45.3	2.91*	2.31 - 3.67	3.04*	2.33 - 3.95
50-59 y	609	52.7	3.90*	3.10 - 4.91	4.04*	3.06 - 5.32
60-69 y	703	62.2	5.82*	4.65 - 7.28	5.88*	4.40 - 7.85
70-79 y	542	64.6	6.61*	5.19 - 8.42	6.59*	4.83 - 8.97
Gender						
male	2348	38.7	1 (ref)		1 (ref)	
female	2857	43.9	1.26*	1.12 - 1.42	1.26*	1.12 - 1.42
Education						
	57 missing					
high	2113	36.2	1 (ref)		1 (ref)	
middle	2646	42.9	1.14*	1.01 - 1.29	1.17*	1.04 - 1.33
low	389	59.9	1.75*	1.38 - 2.22	1.85*	1.44 - 2.38
Group of migrants						
Native Dutch	4772	40.1	1 (ref)		1 (ref)	
1st generation Western	152	69.7	3.03*	2.10 - 4.35	3.22*	2.23 - 4.65
2nd generation Western	281	50.5	1.54*	1.20 - 1.99	1.57*	1.22 - 2.03
Number in household						
	37 missing					
1 or 2 persons	2183	52.1	1 (ref)		1 (ref)	
3 or 4 persons	2137	34.2	1.05	0.90 - 1.22	1.00	0.85 - 1.19
>4 persons	848	32.7	1.19	0.97 - 1.45	1.13	0.91 - 1.40
Child*** in household						
no	4287	44.5	1 (ref)		1 (ref)	
yes	918	27.8	1.04	0.87 - 1.24	0.97	0.78 - 1.20
Number of contacts						
< 8 persons	2252	44.4	1 (ref)		1 (ref)	
≥ 8 persons	2953	39.4	1.06	0.94 - 1.20	1.09	0.96 - 1.24
Contact with child***						
no	4050	42.4	1 (ref)		1 (ref)	
yes	1155	38.6	1.23*	1.06 - 1.42	1.25*	1.06 - 1.46

* statistically significant ($p < 0.05$); ** corrected for age and gender (age is corrected for gender and gender is corrected for age only); *** child younger than 4 years of age; seroprev: seroprevalence;

Table 2.2b - Seroprevalence and risk factors for seropositivity among non-Western persons

Characteristics	number	seroprev %	logistic regression**		multivariable logistic regression	
			OR	95% CI	OR	95% CI
Overall population	1044	76.7				
Age						
6 m-9 y	516	65.9	1 (ref)		1 (ref)	
10-19 y	101	71.3	1.30	0.81 - 2.08	1.62	0.95 - 2.76
20-29 y	69	81.2	2.29*	1.22 - 4.32	3.66*	1.79 - 7.48
30-39 y	69	84.1	2.79*	1.43 - 5.47	2.85*	1.36 - 5.99
40-49 y	37	94.6	9.02*	2.15 - 38.0	8.18*	1.86 - 36.0
50-59 y	105	94.3	8.65*	3.72 - 20.1	6.19*	2.49 - 15.4
60-69 y	96	95.8	11.9*	4.29 - 32.9	8.62*	2.78 - 26.8
70-79 y	51	96.1	13.0*	3.11 - 54.0	11.4*	2.39 - 54.4
Gender						
male	489	77.5	1 (ref)		1 (ref)	
female	555	76.0	0.83	0.61 - 1.12	0.83	0.60 - 1.15
Education 58 missing						
high	229	62.9	1 (ref)		1 (ref)	
middle	425	76.0	2.33*	1.60 - 3.41	2.59*	1.73 - 3.87
low	332	85.8	4.07*	2.62 - 6.33	3.92*	2.45 - 6.27
Group of migrants (non-Western)						
1st generation migrant	664	87.2	1 (ref)		1 (ref)	
2nd generation migrant	380	58.4	0.34*	0.24 - 0.47	0.31*	0.22 - 0.45
Number in household 44 missing						
1 or 2 persons	243	87.2	1 (ref)		1 (ref)	
3 or 4 persons	434	72.4	1.40	0.83 - 2.36	1.22	0.68 - 2.16
>4 persons	323	74.3	1.76*	1.02 - 3.04	1.35	0.73 - 2.49
Child*** in household						
no	722	81.4	1 (ref)		1 (ref)	
yes	322	66.1	0.84	0.61 - 1.17	0.92	0.63 - 1.34
Number of contacts						
< 8 persons	667	79.0	1 (ref)		1 (ref)	
≥ 8 persons	377	72.7	0.75	0.55 - 1.03	0.93	0.65 - 1.33
Contact with child***						
no	874	79.3	1 (ref)		1 (ref)	
yes	170	63.5	0.61*	0.42 - 0.88	0.78	0.51 - 1.18

* statistically significant ($p < 0.05$); ** corrected for age and gender (age is corrected for gender and gender is corrected for age only); *** child younger than 4 years of age; seroprev: seroprevalence

Geometric Mean Concentration

The geometric mean concentration (GMC) among seropositive individuals increased with age by 0.85% per year (95% CI: 0.75-0.96). The GMCs were lower in males than females from the age of 15 to 19 years onwards (Figure 2.2). The GMC ratio, in multivariable analysis, for women compared to men was 1.24 (95% CI: 1.17 - 1.32) among Dutch/Western individuals and 1.13 (95% CI: 1.03 - 1.24) among non-Western individuals.

Across both genders, GMCs were higher among non-Western individuals compared to Dutch/Western individuals, with a GMC ratio of 1.19 (95% CI: 1.11 - 1.27).

Among Dutch /Western individuals, the GMC ratio was 1.17 (95% CI: 1.04 - 1.33) for persons who lived in a households with young children compared to those who did not.

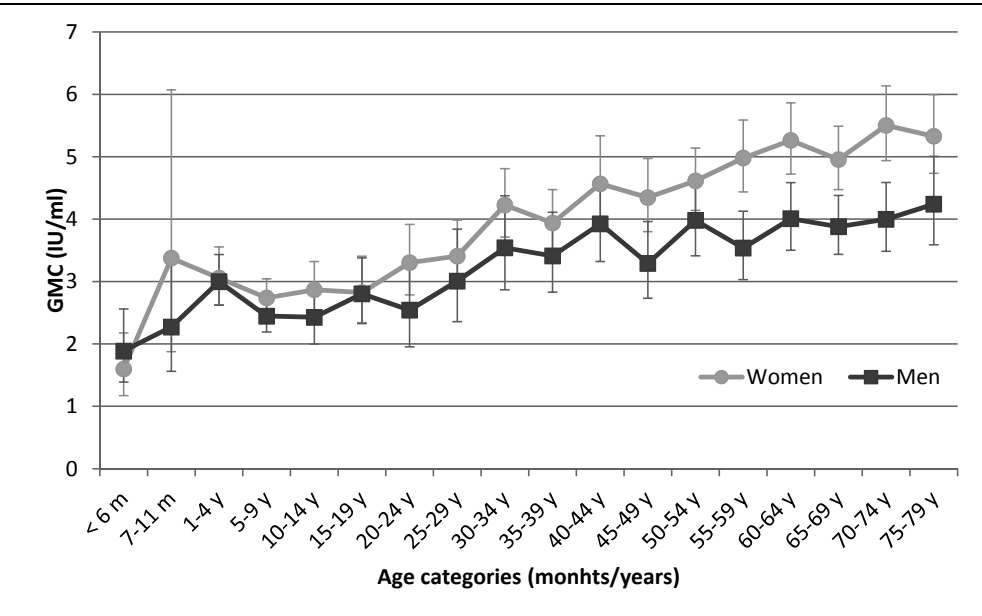


Figure 2.2 - Geometric mean concentration (with 95% CI) per age category by gender among CMV-seropositive individuals in the general population of the Netherlands (n = 2963).

Discussion

This large population-based study showed that the CMV seroprevalence in the general population of the Netherlands (2006-2007) is 45%. This is somewhat lower than that found in the USA (58.9% in 1988-1994) [9], Australia (57% in 2002) [10], and Portugal (77% in 2002-2003) [11]. These differences may be partly explained by differing study populations (e.g. different age distribution) and possible changes in seroprevalence over time.

Our large sample size and the population-wide weighted sampling ensure sufficient statistical power to enable reliable risk factor analysis. Given the relatively low response rate of 32%, the study population might not be fully representative, but data were collected from many subgroups of the general population, unlike most seroprevalence studies.

Age and country of origin were the most prominent independent risk factors for CMV seropositivity in this study. Additionally, low educational level and, among Dutch/Western individuals, female gender and contact with young children were independently associated with a higher CMV seroprevalence.

The association between non-Western country of origin and seropositivity probably reflects the higher infection rate in those countries, which also explains the higher seroprevalences among first-generation compared to second-generation migrants. Differences might be related to different lifestyles. For example, sharing the same plate or cup can facilitate CMV transmission among family members and close relatives. Also, a higher frequency of breastfeeding among Mediterranean first-generation mothers [12] and longer breastfeeding among Turkish women may contribute to increased seroprevalence in their young children [13].

Other factors aside, the increase of seroprevalence with age is well known. This may result from cumulative exposure to CMV throughout life, but seroprevalence measured in older people might also reflect CMV infections that occurred in childhood. Studies in Spain and Japan found decreased seroprevalence with age [14, 15], perhaps related to changes in wealth, socio-economic status, and hygiene. Other studies showed no changes in CMV seroprevalence over time [16, 17].

The geometric mean concentration (GMC) of CMV antibodies in seropositive individuals increases steadily with age. This phenomenon may reflect viral reactivation or reinfection with another CMV strain, boosting the humoral immunity. Yet again, the titer difference among the age categories could be caused by a higher pressure of infection during the childhood of older individuals. The cross-sectional design of our study precludes exploring these trends over time. Longitudinal data would be essential to investigate the increase in seroprevalence and antibody titers with age and to distinguish between continuous exposure over time and prior infections.

Young children constitute a well-known source of CMV because they often excrete relatively large amounts of virus in their saliva and urine for a long time [18-22]. We found that household composition (number and age of household members) had little influence on CMV seroprevalence. Yet among Dutch/Western individuals, CMV seroprevalence was associated with exposure to young children outside the household. Higher GMCs were seen in persons living with children younger than 4 years of age. Moreover, in Dutch/Western individuals, both seroprevalence and GMC were higher in females than males. This might be related to traditional role patterns, with women more involved in childcare and therefore more exposed to CMV infection and re-infection. Exposure could take place both at home and at work, as demonstrated in other studies [23, 24]. The GMC difference between genders could also result from hormonal differences in general or specific to the menstrual cycle or pregnancies, as has been suggested for other herpes viruses [25, 26].

Finally, the association between seroprevalence and educational level are in line with the 10-30% higher seroprevalences in persons with a lower socioeconomic status compared to persons with a higher socioeconomic status, as reported in a meta-analysis by Cannon et al. [5]. They suggest that this finding is related to cultural differences and differences in household crowding, child rearing, child day-care attendance, and sexual activity [5].

The identified risk factors for CMV infection, including female gender and having contact with young children are especially important for women of reproductive age. Prevention of CMV infection could be partially achieved by avoiding exposure to children's urine and saliva. For example by handwashing after changing diapers, feeding and handling children's toys; not sharing foods, drinks and eating utensils; and cleaning objects that came into contact with urine or saliva. The feasibility of behavioral intervention has been demonstrated by studies among pregnant women showing that CMV acquisition could be prevented by counseling on better hygiene [27, 28].

The relatively low seroprevalence among Dutch/Western women of reproductive age (37%) means that more than 60% are seronegative and therefore at risk of a primary CMV infection during pregnancy, putting offspring at risk for severe disability caused by congenital CMV infection. In contrast, despite approximately 85% seropositivity in non-Western women of reproductive age, they are at risk of reactivation or reinfection, which likewise can cause a congenital CMV infection and its consequences. [3, 4].

Thus preventive measures against congenital CMV infections should be aimed both at women susceptible for primary CMV infection (mostly of Dutch/Western origin) and at seropositive women (mainly of non-Western origin). Accordingly, future vaccines should be able to prevent both primary and recurrent infections. The data from this study could be used to make more detailed estimates, by modeling, of the potential effects of a vaccination program within the various subgroups. Meanwhile, influencing behavior and increasing awareness of congenital CMV infection remains the most important means for prevention.

In conclusion, this study showed that CMV seroprevalence in the Netherlands is relatively low compared to other countries. However, seroprevalence is considerably higher in the growing subpopulation of non-Western residents, especially among first generation migrants. Other independent risk factors for CMV infection are older age, lower educational

level, and among Dutch/Western individuals, female gender and contact with children. Preventive measures, including hygienic counseling, should be focused mainly at women of reproductive age, in order to prevent CMV infection and subsequently congenital CMV infection with risk of severe disabilities.

Funding

The Dutch Ministry of Health, Sports and Welfare.

Competing interests

None declared.

Ethical approval

Medical Ethics Committee, Almere, ISRCTN 20164309.

Acknowledgements

Final editorial review by Lucy Phillips.

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Chapter 3

Disease burden of congenital cytomegalovirus infection at school entry age: study design, participation rate and birth prevalence

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Epidemiology and Infection, 2016 May; 144(7): 1520-1527.

Summary

Background

Congenital cytomegalovirus infection (cCMV) may lead to symptoms at birth and long term consequences. We present a nation-wide, retrospective cohort study on the outcome of cCMV up to the age of six years.

Methods

For this study we identified cCMV, using polymerase chain reaction (PCR), by analysing the dried blood spots (DBS), which are taken shortly after birth for neonatal screening. The group of children with cCMV was compared to a group of children who were cCMV negative at birth. Data were collected about their health and development up to six years of age.

Results

Parents of 73693 children were invited to participate, and 32486 (44.1%) gave informed consent for testing their child's DBS for CMV. Of the 31484 DBS that were tested, 156 (0.5%) were positive for cCMV. Among these, four children (2.6%) had been diagnosed with cCMV prior to this study.

Conclusion

This unique retrospective nation-wide study permits the estimation of long term sequelae of cCMV up to the age of six years. The birth prevalence of cCMV in this study was 0.5%, which is in line with prior estimates. The vast majority of children (97.4%) with cCMV had not been diagnosed earlier, indicating under diagnosis of cCMV.

Introduction

Cytomegalovirus (CMV) infection is endemic worldwide and is usually asymptomatic in healthy individuals. In immunocompromised patients however, it can cause serious complications. [1] Primary infections, reinfections and reactivations with CMV during pregnancy can lead to infection in the fetus. [2]

Congenital cytomegalovirus infection (cCMV) is the most prevalent congenital infection worldwide with a birth prevalence ranging from 0.4 to 2.0% of all newborns. [3] An estimated 10 to 15% of all congenitally infected infants have signs and symptoms at birth, including hepatosplenomegaly, icterus, petechiae, microcephaly and intracranial calcifications. [4] Long term sequelae, such as sensorineural hearing loss, cognitive and motor developmental delay and neurological problems, occur in 40 to 58% of children who were symptomatic at birth. In addition, about 10 to 15% of the children who were asymptomatic at birth develop long term sequelae. [4] These estimates of symptoms and sequelae are based on a meta-analysis by Dollard et al, which mostly included studies with a relatively short follow-up of no more than three years. Moreover, most of these studies focused only on hearing loss and general developmental delay. Most information on the long term outcome (more than five years) is presented by Townsend et al., based on a study of 176 children with cCMV from two large population-based screening cohorts in Sweden and the United Kingdom. [5] These previous studies on the burden of cCMV were based on the prospective follow-up of different cohorts of children with cCMV diagnosed at birth, usually born in a single region.

The CROCUS-study (**C**onsequences and **R**isk factors **O**f congenital **C**ytomegalovir**U**S) was designed specifically to address the limitations of a more selected study population. It aimed to evaluate the long term consequences of cCMV in the Netherlands in a nation-wide group of children, retrospectively diagnosed at the age of five years. The design of this study will enable us to look at a broad spectrum of outcome measures across the whole range of children with cCMV, from symptomatic to asymptomatic. In this paper, we present this unique study design, the participation rate and the birth prevalence of cCMV in the Netherlands.

Methods

Figure 3.1 displays the design of the CROCUS-study, comprising the study elements; identification of children with cCMV (1) and collection of study data (2); and the regular youth healthcare system for all children in the Netherlands provided by Regional Public Health Services.

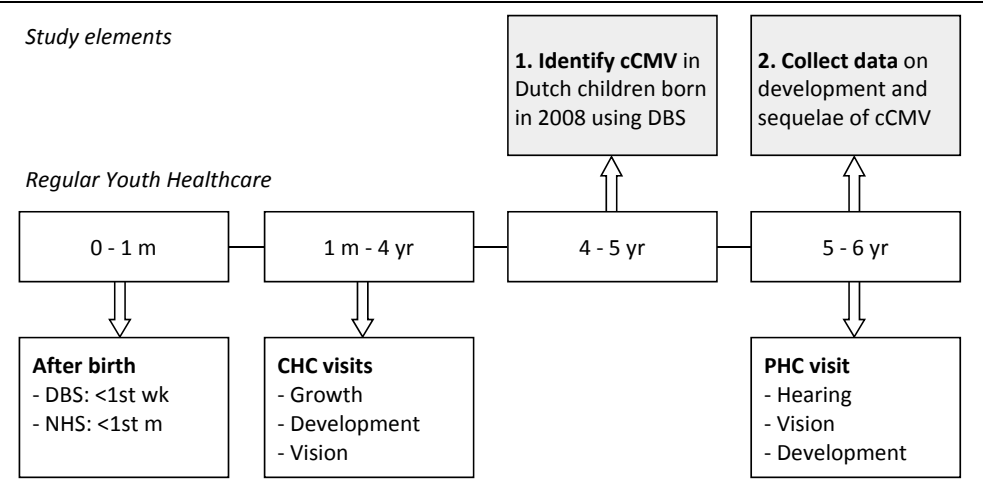


Figure 3.1 - Design of the CROCUS-study Design of the CROCUS study.
cCMV: Congenital cytomegalovirus; CHC: child health center; DBS: dried blood spot; NHS: neonatal hearing screening; PHC: preventive health check; wk: week; m: month; yr: year.

Identification of children with congenital CMV infection and selection of controls

All children born between 1 January 2008 and 30 September 2008, living in the Netherlands were eligible for entry into this study (n = 139543 births, Statistics Netherlands [6]). All children, living in regions covered by the Regional Public Health Services who were willing to participate in this study (n = 19; 68% of total), were invited to take part in this study between October 2012 and January 2013.

Parents of this cohort of children were asked for consent to retrospectively test their child’s dried blood spot (DBS) for CMV DNA by polymerase chain reaction (PCR). The DBS was collected after birth for neonatal screening for 18 conditions, such as Phenylketonuria, and was stored for five years. To test the DBS, a real-time multiplex PCR was chosen, incorporating two independent CMV target genes, i.e. UL55 and UL 123, as described by Boeckh et al. [7] This multiplex PCR had a 95% detection limit of 850 copies per milliliter and was carried out at the National Institute for Public Health and the Environment (RIVM). All DBS samples with single or double positive PCR results, and those of the selected cCMV negative control group, were tested again, using a PCR against a third distinct target region of the UL123 gene, at the Leiden University Medical Center (LUMC). [8]

A three times as large cCMV negative control group, matched for sex, month of birth and postal code region, was randomly selected for each child with cCMV. The parents of the children with cCMV and the selected cCMV negative controls were informed and asked to participate in the second part of study, which consisted of the collection of data on the child's health and development.

Data collection in the cCMV children and controls

All parents were asked to fill in questionnaires and to give their consent to retrieve data from the youth healthcare records, teachers, general practitioners and other healthcare providers.

Parental questionnaires on child development and quality of life

The Child Development Inventory (CDI) [9, 10], translated into Dutch [11], was used to assess development. The Pediatric Quality of Life™ (PedsQL™) [12-16] and Short-Form 12® (SF-12®) [17, 18] were used to assess health related quality of life for children and parents respectively. An additional questionnaire was developed to obtain information about the child's medical history and demographic features of the family.

Youth Healthcare

During the first four years of life the health, growth and development of children are regularly checked at the Child Health Center. At the intake, data are collected concerning pregnancy, delivery and the first weeks of life. Neonatal hearing screening is performed at infants' homes, usually within the first week of life, using oto-acoustic-emissions. Tests for early detection of visual disorders are performed repeatedly during the first three years of life. After the age of three years, a picture vision test and later the Landolt-C vision test are used to assess visual acuity. Data on development (focused on fine and gross motor skills, social skills and communication) are recorded in a child development chart (van Wiechen chart). Length or height, weight and head circumference are measured repeatedly and noted on a growth chart. If any of these findings deviate from normal values, further evaluation will be performed by a medical specialist or other healthcare provider.

At five or six years of age, a Preventive Health Check is performed by the school physician. This check includes hearing screening, a visual acuity test (Landolt-C) and measurement of height and weight. In some regions screening of motor skills is standard procedure, mostly using the Baecke Fassaert Motor Test; other regions perform extensive motor tests only when indicated. All these data from the Child Health Center visits and Preventive Health Check were collected for this study.

School

In the Netherlands, 95% of all regular primary schools use the same student tracking system (CITO), while at special needs primary schools a number of different methods are used. The results from the first two years of primary education were collected. School results were divided into quintiles (I, II, III, IV and V) in which I stands for the 20% highest scores or into quartiles (A, B, C and D/E) with A indicating the highest 25% scores.

General practitioner and healthcare providers

In the Netherlands the general practitioner provides primary medical care and keeps track of the entire medical history and medication use of a patient. If the child attended the general practitioner or other healthcare providers, these medical data were retrieved.

Outcome measures

The primary outcome of this study is sensorineural hearing loss up to the age of six years. This is defined as more than 40 decibels non-conductive hearing loss in at least one ear. Secondary outcome measures are visual impairment, motor impairment, cognitive impairment, quality of life and growth in the first six years of life. Visual impairment is defined as best-corrected visual acuity less than 0.3 in the better eye. Cognitive impairment, based on the CDI, is defined as a developmental age under minus two standard deviations. Values under minus two standard deviations for height, weight and head circumference are considered growth retardation or microcephaly.

Sample size calculation

The sample size calculation was based on the primary outcome of sensorineural hearing loss. We estimated sample size for unequal group sizes using a continuity correction, since the outcome is rare in the cCMV negative subgroup. To demonstrate a difference in hearing loss of 10% in the cCMV positive group and 0.1% in the cCMV negative group with a power (β) of 90% and two-sided alpha (α) of 5% we needed complete data from 83 cCMV positive and 166 cCMV negative children. Given the estimated response rate of 33% for DBS testing and 75% (cCMV positive) and 50% (cCMV negative) for approval for data collection, 25000 DBS needed to be tested, which meant that 75000 parents needed to be approached for this study.

Ethical and legal issues

This study was approved by the medical ethical committee of the Leiden University Medical Center in Leiden and is registered in the “Dutch Trial Register” (NTR 3582).

In accordance with good clinical practice guidelines, study data will be stored for 15 years. In the informed consent form, parents could give separate consent for DBS testing, approval of data collection, storage of the materials (DBS) for 15 years, and permission for future contact concerning additional research projects. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Response rate

Differences in response rate may lead to both an under- or overestimation of the disease burden of cCMV. To assess potential differences, socio-demographic characteristics, based on the postal code region, of responders and non-responders were compared, using data from Statistics Netherlands.

Results

DBS testing - Response rate and cCMV prevalence

Letters were sent to parents of 73693 children, born in the Netherlands between 1 January and 30 September 2008 (Figure 3.2). Of the parents of 34105 children who responded (46.3%), the majority (32486; 95.3%) gave informed consent to have their child's DBS tested for CMV.

DBS of in total 31484 children were tested for cCMV. DBS that had been obtained later than 21 days after birth were excluded from the study because diagnosis of cCMV would be uncertain in such a case, since the infection could have been acquired after birth. CMV DNA was detectable in 154 of the tested DBS, based on the triple target PCR confirmation. In addition, DBS of two children participating in the study were not available for testing at the time of the study, but they had been diagnosed cCMV positive elsewhere shortly after birth. This resulted in 156 confirmed cases of cCMV and a birth prevalence of 0.50% (95% confidence interval: 0.42 - 0.57).

There were few marked differences between the demographic backgrounds of inhabitants of the postal code areas of the groups who did and did not respond (Table 3.1). Among the postal code areas of the non-responders the proportion of migrants, especially non-Western migrants, as well as the proportion of households with lower incomes, were somewhat higher compared to the postal code areas of responders.

Table 3.1 - Differences in the group of responders and non-responders based on their postal code region.

Characteristic	Responders * (n = 32486)	Non-responders** (n = 41207)
Children (under five years of age)	6.2%	6.3%
Migrants (persons with foreign background)	17.9%	23.6%
Western migrants ***	8.3%	8.8%
Non-Western migrants ****	9.6%	14.9%
Average number of persons per household	2.4	2.3
Low household income (lowest 40%)	35.0%	38.6%
Middle household income (middle 40%)	41.5%	40.4%
High household income (upper 20%)	24.2%	21.6%

Data are presented as average percentage or number per group based on postal code region

* Parents who gave informed consent for dried blood spot (DBS) testing

** Parents who did not respond or gave no informed consent for DBS testing

*** Persons from Europe (except Turkey), North America, Oceania, Japan and Indonesia

**** Persons with a Turkish, African, Asian and Latin-American background

Data retrieval - Participation rate and cCMV diagnosis

Parents of the 156 children with cCMV were contacted to inform them of the diagnosis and to invite them to take part in the second part of the CROCUS-study. Parents of only four of these 156 children (2.6%) were aware that their child had cCMV prior to this phone call, at which time the children were five years old. Informed consent was given for 133 (85%) cCMV positive children to participate in the second part of the study. (Figure 3.2)

From the children who were confirmed CMV DNA negative at birth (n = 31330), a selection was made from those who could be matched to the cCMV positive children (n = 468). As soon as informed consent was obtained for two matched controls per cCMV positive case further inclusion of a third selected matched control was ceased. Parents of 365 of these children were asked for informed consent and this was given for 274 (75%) of these cCMV negative control children. (Figure 3.2)

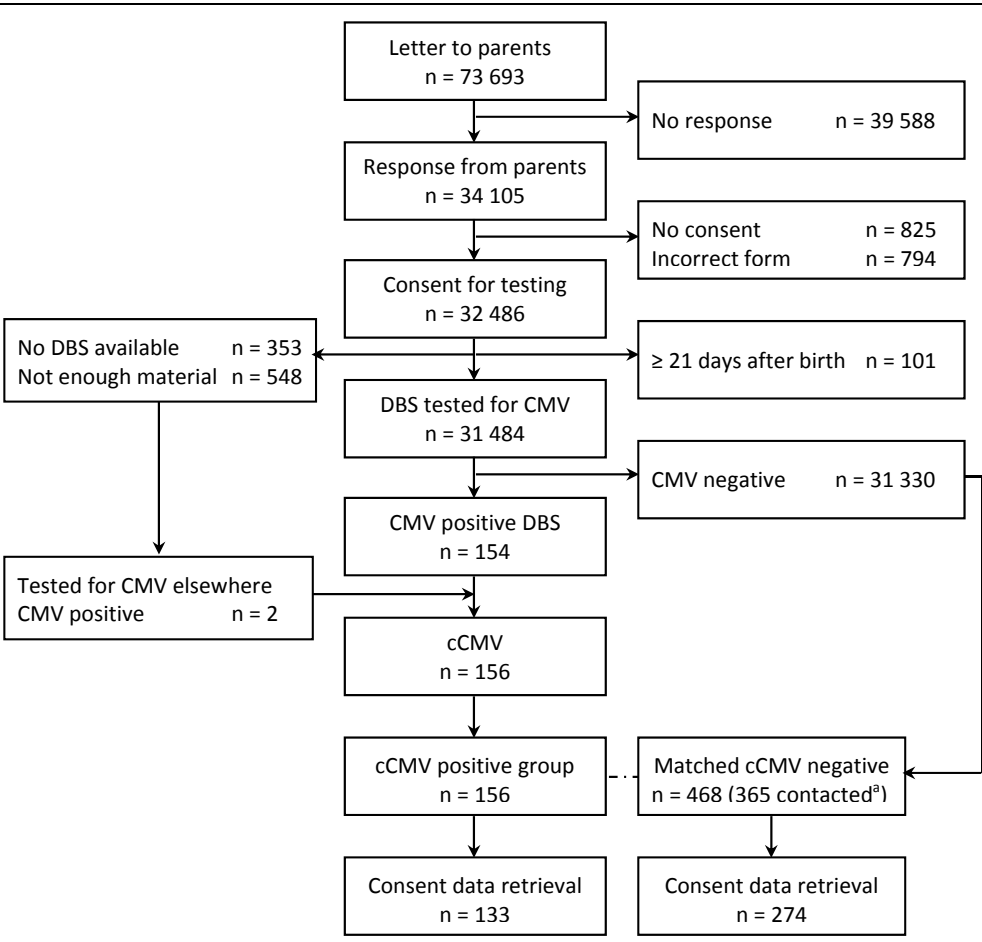


Figure 3.2 - Flowchart of the CROCUS study. ^a Inclusion of matched controls was ceased when informed consent was obtained for two matched controls. DBS, Dried blood spot; CMV, cytomegalovirus; cCMV, congenital CMV infection; n indicates number of children.

Discussion

This unique study design demonstrates that population-based long term outcome of cCMV can be studied in a relatively short time frame by using neonatal DBS to retrospectively identify exposed (cCMV positive) and unexposed (cCMV negative) children. Currently DBS are stored for five years in the Netherlands, but longer storage might be useful. This is particularly relevant for congenital diseases that might not always be possible to recognize at birth, such as CMV, rubella and toxoplasmosis. [19-21] It is clear from other studies that some long term consequences of cCMV become apparent later than five years after birth; for example hearing loss may first become obvious up to, or even after, the age of six years. [22, 23] Such a retrospective diagnosis may be of clinical and epidemiological relevance, and may guide future interventions.

The birth prevalence of cCMV of 0.5% found in this study is very similar to previous estimates in the Netherlands (0.54%) [24] and in Europe (0.5%) [25]. Birth prevalence and maternal seroprevalence are directly related [3, 26] and the found birth prevalence is also in line with the overall seroprevalence among women of childbearing age in the Netherlands of approximately 37%. [27]

Remarkably, only four (2.6%) of the 156 children with cCMV had been diagnosed prior to this study. This implies that the majority of children with cCMV are missed in the Netherlands, despite the fact that the Netherlands has an excellent health system which is highly accessible. The broad spectrum and lack of specificity of clinical signs related to cCMV might have contributed to this under diagnosis. Previous studies show that cCMV is symptomatic at birth in almost 13% of cases, and that long term consequences occur in about 17% of cases. However, these estimates are all based on prospective studies in which more extensive examinations and monitoring may lead to information bias with an overestimation of the symptoms, signs and sequelae attributed to cCMV. In addition, it has been shown that medical practitioners in the Netherlands have a relatively low level of awareness concerning cCMV. [28, 29] All these factors may contribute to the high rate of under diagnosis found in this study.

Although the retrospective design of this study avoids information bias for the majority of the study outcomes, it also has some disadvantages. The main drawback is the high probability of missing data, for example a complete physical exam after birth is often not registered and neonatal symptoms might therefore be missed. In addition, selection bias, such as differences in response rate between parents of children with and without health problems, is possible. Yet, this bias may go both ways and it could lead to either an over- or under estimation of the disease burden. There are actually some differences between parents who consent to the DBS testing and those who did not respond or did not consent. The seroprevalence of CMV in the Netherlands is higher in groups with lower income or non-Western origin, [27] therefore the cCMV birth prevalence could be somewhat higher in the non-responder group, which could lead to an underestimation of the disease burden.

Another potential bias is the use of DBS for CMV testing, which is dissimilar to many other studies. Testing the DBS for CMV is less sensitive than postnatal urine CMV testing and the sensitivity depends on the viral load. [30] Large differences in sensitivity have been described in different studies. [31, 32] Recently a pooled sensitivity of 84.4% and specificity of 99.9%, based on PCR methods with reported detection limits ranging from 450 to 9400 copies per milliliter, has been demonstrated in a meta-analysis. [33] Using a highly specific assay, with a sensitivity of around 80%, and taking the birth prevalence of 0.5% into account, about one in 1000 children had a false negative test result. Therefore, the chance is small that one of the children with a cCMV false negative test result was included in the relatively small cCMV negative control group, containing only 274 of the 31330 children with a cCMV negative test result. We assume that the children with cCMV, who have not been diagnosed by DBS testing in this study, mostly had low viral loads. Therefore, children who were cCMV positive in this study, probably had a slightly higher viral load, which is known to be associated with poorer long term outcomes [34-36], than the entire group of children with cCMV. This may lead to an overestimation of the disease burden.

Our study design, with data collection up to the age of six years, enables us to look at the whole range of children with cCMV, including mainly asymptomatic children, some children who might be retrospectively classified as symptomatic and those who were clearly symptomatic at birth. This means that this study will produce information on a wide diversity of sequelae related to cCMV. Besides the well-known complications such as hearing loss and cognitive developmental delay, it allows us to explore other outcomes that are possibly related to cCMV. However, this study is powered with hearing loss as the primary outcome and, even though the response rates were higher than expected and the results may give us an impression of potential problems related to cCMV, the sample size may not be sufficient to obtain statistically significant results on these other outcome measures.

In conclusion, this study confirms a birth prevalence of cCMV in the Netherlands of 0.5%. It clearly shows that cCMV is currently under diagnosed, since only four of the 156 children with cCMV had been diagnosed prior to this study. Many questions remain concerning cCMV. The information collected in this study on the long term consequences of all infants with cCMV, ranging from symptomatic to asymptomatic, can be used to clarify the relevance and need for preventive measures, including neonatal screening [37].

Acknowledgement

We would like to thank the following participating Regional Public Health Services for their contribution to this study: GGD Amsterdam, GGD Brabant-Zuidoost, Careyn Jeugd en Gezin and Rivas Zorggroep (former GGD Zuid-Holland Zuid), GGD Den Haag, GGD Flevoland, GGD Fryslân, GGD Gooi en Vechtstreek, GGD Groningen, GGD Hart voor Brabant, GGD Hollands-Midden, GGD IJsselland, GGD Limburg-Noord, GGD Midden-Nederland, GGD Rivierenland, GGD Rotterdam-Rijnmond, GGD West-Brabant, GGD Zaanstreek/Waterland, GGD Zeeland and GGD Zuid-Holland-West.

We would like to acknowledge the help of Petra Oomen, data manager of the Praeventis database for neonatal screening, for providing the parents' addresses and the data required to collect the dried blood spots.

We would like to thank Jeroen Cremer (RIVM) and Lisette Rusman (LUMC) for testing thousands of dried blood spots for cytomegalovirus. We acknowledge Dr. Hein Boot (†) for his valuable advice and extensive participation in the development of the cytomegalovirus PCR on dried blood spots.

Financial support

This research was funded by Strategic Research of the National Institute of Public Health and the Environment.

Conflict of interest

None.

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Chapter 4

Long-term impairment attributable to congenital cytomegalovirus infection

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Submitted

Abstract

Background

Congenital cytomegalovirus infection (cCMV) is the most common congenital infection worldwide, with a birth prevalence of 0·6% in industrialized countries. This study aimed to estimate the long-term impairment attributable cCMV.

Methods

This nationwide cohort study retrospectively assessed cCMV in children born in 2008 in the Netherlands, using polymerase chain reaction on 31,484 stored, neonatal dried blood spots. Extensive medical data of cCMV-positive children (n = 133) and matched cCMV-negative controls (n = 274) up to 6 years of age, obtained from general practitioners, medical specialists, and healthcare providers, were analyzed.

Findings

Moderate-to-severe long-term impairment was diagnosed in 24·8% (33/133) of all children with cCMV (53·8% in symptomatic and 17·8% in asymptomatic), compared to 12·0% (33/274) of children without cCMV. Sensorineural hearing loss was seen only in 5 cCMV-positive children (3·8%). Developmental delays were diagnosed more often in cCMV-positive children compared to cCMV-negative children; motor (12·0% vs. 1·5%), cognitive (6·0% vs. 1·1%), and speech-language (16·5% vs. 7·3%). Long-term impairment in multiple domains was more frequent in symptomatic and asymptomatic cCMV-positive children (19·2% and 8·4%, respectively) compared to cCMV-negative children (1·8%).

Interpretation

Children with cCMV were twice as likely to have long-term impairment up to 6 years of age compared to cCMV-negative controls, with an attributable risk of 12·8%. This risk difference was most marked in developmental domains and sensorineural hearing loss. These insights into the risk of cCMV-associated impairment can help optimize care and stimulate preventive measures.

Funding

Strategic Research of the National Institute of Public Health and the Environment.

Introduction

Congenital cytomegalovirus infection (cCMV) is the most common congenital infection worldwide, with a birth prevalence of around 0.6% in industrialized countries. [1] The only systematic review on the prevalence of permanent sequelae of cCMV was published by Dollard et al. in 2007. They found that 12.7% of children with cCMV had signs or symptoms at birth, including hepatosplenomegaly, jaundice, thrombocytopenia, microcephaly, and intracranial calcification, and that 40–58% of these children developed permanent sequelae, such as hearing loss and developmental delay. [2] Moreover, 13.5% of children asymptomatic at birth were estimated to develop long-term sequelae. [2] They concluded that overall, 17–20% of infected children suffer from permanent neurological or sensory sequelae. These figures are frequently cited as representing the disease burden of cCMV. This systematic review was based on prospective cohort studies with universal neonatal cCMV screening. Often, these prospective studies merely report data on cCMV-positive children, with small or even no control groups. This may have led to information bias and an overestimation of cCMV's attributable morbidity.

The aim of the CROCUS study (**C**onsequences and **R**isk factors **O**f **C**ongenital cytomegalovir**U**S infection) was to estimate the long-term consequences of cCMV up to the age of 6 years in the Netherlands. In this large, nationwide retrospective cohort study, the diagnosis of cCMV was established at the age of 5 years; therefore practically all collected data had been recorded before a diagnosis of cCMV was made. The medical data, from birth to the age of 6 years, of children with cCMV and a matched group of children without cCMV were collected and analyzed. The comparison between these groups provides insight into the disease burden attributable to cCMV.

Methods

Study design

Details on the CROCUS study's design have been published previously. [3] In short, children with and without cCMV were retrospectively identified. Subsequently medical data of all participating children with cCMV and a twice as large matched control group of children without cCMV were collected and analyzed.

Children living in the Netherlands and born between January 1 and September 30, 2008, were eligible for inclusion. After informed parental consent was obtained, between October 2012 and January 2013, cCMV was diagnosed retrospectively in stored neonatal dried blood spots using polymerase chain reaction (PCR) [4] between November 2012 and June 2014. A control group with negative cytomegalovirus PCR results was matched to the group with cCMV for sex, birth month, and postal code. A second, different, internally controlled quantitative real-time PCR performed in triplicate, with an assumed sensitivity of 84% [5], was used for confirmation in both groups [6–8].

Medical data of participating cCMV-positive and cCMV-negative children were collected, with informed consent of parents, between May 2014 and January 2015. Complete medical records were requested from their general practitioners. Information concerning hospital admissions, outpatient clinic visits, and provided care was also requested from all medical specialists and other healthcare providers who were mentioned by the parents. All records were entered anonymously into the study database prior to linking the information to the cCMV status.

The primary outcome of the study was sensorineural hearing loss up to the age of 6 years. Secondary outcomes included cognitive and motor developmental delay, and visual impairment. The sample size calculation was based on an expected prevalence of sensorineural hearing loss of 10% in cCMV-positive and 0.1% in cCMV-negative children, with a 90% power and a 5% two-sided alpha. Using a continuity correction, the sample size was estimated at 83 cCMV-positive and 166 cCMV-negative children.

The study was approved by the medical ethics committee of the Leiden University Medical Center in Leiden and is registered in the Dutch Trial Register (NTR 3582).

Definitions

Symptoms at birth

Clinically apparent abnormalities were defined as one or more clinically evident signs potentially related to cCMV that were recorded in the first 4 weeks of life, comprising prematurity, being small for gestational age, microcephaly, hepatomegaly or splenomegaly, generalized petechiae or purpura, seizures, and hypotonia.

The z-scores for birth weight and head circumference were calculated using the Dutch reference charts with Growth Analyser Research Calculation Tools (Version 4.0, Growth Analyser B.V., Rotterdam, the Netherlands), taking ethnicity and gestational age into account.

Children with aberrant laboratory values, cranial ultrasound or ophthalmologic abnormalities, or neonatal sensorineural hearing loss, in addition to clinically apparent abnormalities in the neonatal period, were defined as being symptomatic at birth. Children without any of the abovementioned symptoms or signs were considered asymptomatic at birth.

Long-term impairment

Sensorineural hearing loss was defined as ≥ 40 -decibels unilateral sensorineural hearing loss or bilateral hearing loss of ≥ 40 decibels in the better ear. Severity of hearing loss was categorized as moderate (41-70 decibels), severe (71-90 decibels), or profound (>90 decibels).

Visual acuity and diagnoses of ophthalmological disorders were obtained from reports of the ophthalmologist or optometrist. Visual impairment was defined as a visual acuity below 0.3. The diagnoses of neurological impairment were obtained from reports of medical specialists. Microcephaly was defined as a head circumference below -2 standard deviations for age at the last recorded measurement.

Cognitive developmental delay was defined as an intelligence quotient ≤ 70 if this was tested or it was based on a diagnosis by a medical specialist.

If results of a Movement Assessment Battery for Children [9] were available, a score below the fifth percentile was considered to be developmental delay. Otherwise, the physical therapist's report was used to determine the diagnosis and severity of motor developmental delay.

Problems concerning speech and language development, including severity, were determined on the basis of reports from a speech therapist or speech and hearing center. The Schlichting test [10] for language production and the Reynell test [11] for language comprehension were used if recorded. A score below -1.5 standard deviations was considered moderately to severely delayed.

The frequency of long-term impairment for each domain of impairment (hearing, visual, neurological, cognitive, motor and speech-language) was estimated by combining disorders considered moderate to severe that were diagnosed up to the age of 6 years. Overall long-term impairment was defined as the presence of one or more disorders in one or more domains. Children with disorders in two or more domains were considered to be more severely impaired. All diagnoses provided by specialists and other healthcare providers were registered and categorized by MK and checked by AO.

Missing data handling

The Netherlands has a well-organized healthcare system that is cost-free and easily accessible for children, with regular checks by the preventive youth healthcare. [12] All newborns are regularly checked in the first weeks of life by a physician or midwife. When specific problems are detected then, or later, by the general practitioner or the youth healthcare physician, children are referred to a medical specialist or other healthcare providers. It was assumed that few relevant outcomes were missed. Therefore the prevalence of long-term impairment was expressed as a percentage of the entire study population (Table 4.2 and 4.3). The same is true for the table on neonatal findings (Table 4.1). This table additionally displays the number of children whose neonatal clinical features were available.

Statistical analysis

Data are reported as number and percentage or mean and standard deviation. Data on signs and symptoms at birth were compared between children with and without cCMV. For long-term impairment, data were presented for cCMV-positive and cCMV-negative children and within these groups for those who were symptomatic or asymptomatic at birth. The cCMV-positive and cCMV-negative groups were compared for the prevalence of impairment in each of the 6 domains (hearing, visual, neurologic, cognitive, motor, and speech-language), for the prevalence of any impairment, and for the prevalence of impairment in more than one domain. The risk differences and the relative risk with 95% confidence intervals (95% CI) were calculated to compare groups. Statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

Baseline data

In this study, 31,484 neonatal dried blood spots were retrospectively tested, resulting in 156 (0.5%) children diagnosed with cCMV [3] (Figure 4.1). Medical data of 407 children were collected, including 133 children with cCMV and 274 children without cCMV. The mean age on January 1, 2014, was similar in both groups (5.5 ± 0.2 years). In cCMV-positive and cCMV-negative children, 56.4% and 54.4%, respectively, were males.

Three of the participating children had been diagnosed with cCMV prior to inclusion in this study: one had been diagnosed with cCMV antenatally and was treated postnatally with valganciclovir because of cranial ultrasound abnormalities; one with cerebral palsy and hearing loss was diagnosed at 20 months of age; and one child identified with hearing loss in the neonatal period was diagnosed at 2 years of age.

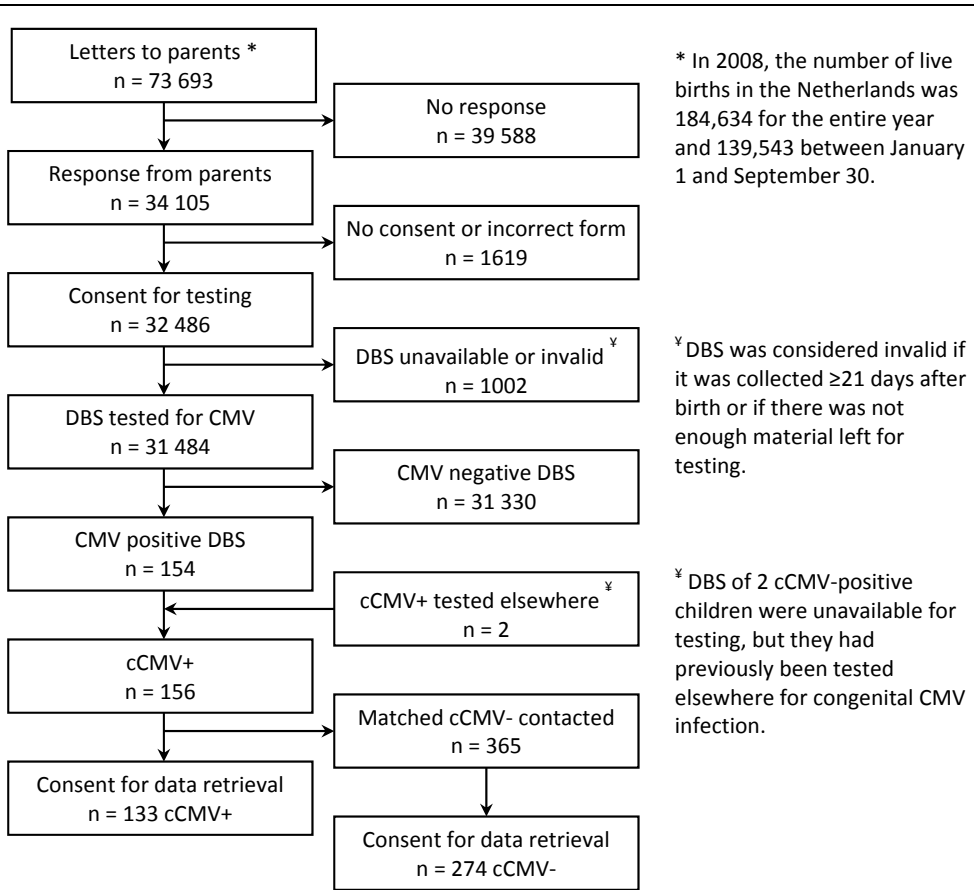


Figure 4.1 - Flowchart of the CROCUS study (adapted from Korndewal et al. [3])
DBS: dried blood spot; CMV: cytomegalovirus; cCMV: congenital CMV infection.

Symptoms at birth (Table 4.1)

Of the children with cCMV, 18.1% had clinically apparent abnormalities in the neonatal period. In the group of children without cCMV, 12.0% had clinically apparent abnormalities. When additional laboratory, cranial ultrasound or ophthalmological abnormalities, and sensorineural hearing loss were taken into account, 19.6% of children with cCMV and 12.4% of children without cCMV were symptomatic at birth.

Table 4.1 - Symptoms and signs in the neonatal period.

	cCMV-positive n = 133			cCMV-negative n = 274			Risk difference % (95% CI) [¥]
	n [†]	(%) [‡]	reported [¥]	n [†]	(%) [‡]	reported [¥]	
Preterm birth ¹	13	(9.8)	132	18	(6.6)	273	
Small for gestational age ²	5	(3.8)	130	10	(3.7)	271	
Microcephaly ³	7	(5.3)	61	7	(2.6)	130	
Hepatomegaly / Splenomegaly	0		31	0		55	
Petechiae / Purpura ⁴	0		31	0		55	
Hypotonia / Seizures ⁵	0		31	0		55	
Clinically apparent*	24	(18.1)	132	33	(12.0)	273	6.0 (-1.6 - 13.6)
Thrombocytopenia ⁶	0		8	0		15	
Neutropenia ⁷	0		2	0		2	
Elevated ALAT ⁸	0		1	0		1	
Conjugated hyperbilirubinemia ⁹	0		2	0		1	
Neonatal hearing loss ¹⁰	2 ^a	(1.5)	98	0 ^b		217	
Abnormal cranial ultrasound ¹¹	1 ^c	(0.8)	3	5 ^d	(1.8)	10	
Ophthalmologic abnormalities ¹²	0		1	0		-	
Symptomatic at birth**	26	(19.6)	133	34	(12.4)	273	6.9 (-0.7 - 14.9)

cCMV-positive: children with congenital CMV infection; cCMV-negative: children without cCMV

[†] Number of children (n) with signs or symptoms; [‡] Percentage (%) compared to the whole group.

[¥] Number of children in whom the clinical feature was assessed and available for this study.

[¥] Risk difference presented as percentage and 95% confidence interval (CI).

* One or more clinically evident signs or symptoms potentially related to cCMV.

** One or more signs or symptoms potentially related to cCMV.

1 Gestational age less than 37 weeks. 2 Weight less than -2 standard deviations for gestational age.

3 Head circumference less than -2 standard deviations for gestational age.

4 Generalized petechiae or purpura. 5 Hypotonia persisting more than 1 hour after birth or seizures.

6 Thrombocytes <100 * 10⁹/liter. 7 Neutrophil granulocytes <0.5 * 10⁹/liter.

8 Alanine-amino transferase (ALAT) >80 units/liter. 9 Conjugated bilirubin >2 milligrams/deciliter.

10 Sensorineural hearing loss ≥40 decibels, detected by neonatal hearing screening

11 Any abnormalities on cranial ultrasound. 12 Any permanent ophthalmologic abnormalities.

a Severe unilateral sensorineural hearing loss (SNHL) (n = 1), Moderate bilateral SNHL (n = 1).

b Conductive unilateral hearing loss <40 decibels (n = 1). c Germinal matrix cystic lesions (n = 1).

d Choroid plexus cyst (n = 2), subependymal cyst (n = 1), echodensities grade 1 (n = 1), subependymal hemorrhage grade 1 (n = 1).

Among the 20% of children with available data on physical examination after birth, no hepato- or splenomegaly, hypotonia, or generalized petechiae or purpura were recorded. No abnormalities were reported from the few children who had undergone blood tests. Three children were referred to a speech and hearing center for further testing after failing the neonatal hearing screening. Of these, two children with cCMV were diagnosed with sensorineural hearing loss; another child without cCMV had unilateral conductive hearing loss.

Long-term impairment (Table 4.2, Supplement 4.1)

Hearing impairment

Sensorineural hearing loss was present only in cCMV-positive children (3-8%). Profound unilateral hearing loss was seen in three children and bilateral hearing loss in two children. Hearing loss was present in both symptomatic and asymptomatic children. In two children, hearing loss was detected at the neonatal hearing screening. The other three children had late-onset hearing loss, which became apparent at the age of 2 ($n = 1$) or 5 years ($n = 2$).

Visual impairment

Unilateral visual impairment was present in two children: one cCMV-positive child had optic nerve atrophy, and one cCMV-negative child had congenital cataracts. In addition, two cCMV-positive children had cortical visual impairment. All children with visual impairment were classified as asymptomatic at birth. Other correctable visual disorders, such as refractive errors, strabismus, and amblyopia, were more frequent in the cCMV-negative group.

Neurological impairment

Cerebral palsy with polymicrogyria was present in two cCMV-positive children. Epilepsy, microcephaly, autism spectrum disorders, and attention deficit–hyperactivity disorder were diagnosed in both cCMV-positive and cCMV-negative children. Autism spectrum disorders were diagnosed somewhat more often in cCMV-positive children, whereas attention deficit–hyperactivity disorder was more frequent in cCMV-negative children.

Developmental delay

Cognitive impairment was more frequent in children with cCMV, especially in the symptomatic group.

Fine and gross motor impairment and balance impairment were more frequently seen in cCMV-positive children compared to the cCMV-negative group. In addition, sensory processing disorder and developmental coordination disorder occurred mostly in the cCMV-positive group.

Speech and language problems were common in both cCMV-positive and cCMV-negative children; nevertheless they were more prevalent in the cCMV-positive children.

More details of the long-term consequences of children with and without cCMV are provided in Supplement 4.1.

Table 4.2 - Long-term impairment, diagnosed in the first 6 years of life, of cCMV-positive and cCMV-negative children with or without symptoms at birth, per condition.

	cCMV-positive			cCMV-negative		
	All n = 133 n (%)	Sympt.‡ n = 26 n (%)	Asympt.† n = 107 n (%)	All n = 274 n (%)	Sympt.‡ n = 34 n (%)	Asympt.† n = 240 n (%)
Hearing impairment						
Sensorineural hearing loss ¹	5 (3.8)	2 (7.7)	3 (2.8)	0	0	0
unilateral	3 (2.3)	1 (3.9)	2 (1.9)	0	0	0
bilateral	2 (1.5)	1 (3.9)	1 (0.9)	0	0	0
moderate (40-70 decibels)	2 ears	1 ear	1 ear			
severe (71-90 decibels)	1 ear	1 ear				
profound (>90 decibels)	4 ears	1 ear	3 ears			
Visual impairment						
Optic nerve atrophy	1 (0.8)	0	1 (0.9)	0	0	0
Congenital cataract	0	0	0	1 (0.4)	0	1 (0.4)
Cortical visual impairment	2 (1.5)	0	2 (1.9)	0	0	0
*Refractive error	13 (9.8)	4 (15.4)	9 (8.4)	42 (15.3)	8 (23.5)	33 (13.8)
*Strabismus	2 (1.5)	0	2 (1.9)	9 (3.3)	4 (11.8)	5 (2.1)
*Amblyopia	3 (2.3)	0	3 (2.8)	10 (3.7)	2 (5.9)	8 (3.4)
Neurological impairment						
Cerebral palsy	2 (1.5)	1 (3.9)	1 (0.9)	0	0	0
Epilepsy	2 (1.5)	2 (7.7)	0	3 (1.1)	0	3 (1.3)
Microcephaly	2 (1.5)	2 (7.7)	0	3 (1.1)	0	3 (1.3)
Autism spectrum disorder	4 (3.0)	2 (7.7)	2 (1.9)	5 (1.8)	1 (2.9)	4 (1.7)
ADHD ²	1 (0.8)	1 (3.9)	0	7 (2.6)	0	7 (2.9)
Cognitive impairment						
Cognitive impairment ³	8 (6.0)	4 (15.4)	4 (3.7)	3 (1.1)	1 (2.9)	2 (0.8)
Motor impairment						
Fine motor impairment ⁴	13 (9.8)	5 (19.2)	8 (7.5)	2 (0.7)	0	2 (0.8)
Gross motor impairment ⁴	13 (9.8)	4 (15.4)	9 (8.4)	3 (1.1)	1 (2.9)	2 (0.8)
Balance impairment ⁴	8 (6.0)	1 (3.9)	7 (6.5)	1 (0.4)	1 (2.9)	0
Sensory processing disorder ⁵	4 (3.0)	2 (7.7)	2 (1.9)	1 (0.4)	0	1 (0.4)
Speech-language impairment						
Language disorder ⁶	14 (10.5)	6 (23.1)	8 (7.5)	10 (3.7)	1 (2.9)	9 (3.8)
Speech disorder ⁷	16 (12.0)	6 (23.1)	10 (9.4)	15 (5.5)	3 (8.8)	12 (5.0)
Oral motor skill difficulties ⁸	6 (4.5)	2 (7.7)	4 (3.7)	9 (3.3)	1 (2.9)	8 (3.4)
Auditory processing disorder	2 (1.5)	2 (7.7)	0	1 (0.4)	0	1 (0.4)

The figures in the table represent numbers and percentages unless stated otherwise.

cCMV-positive: children with congenital CMV infection (cCMV); cCMV-negative: children without cCMV

‡ Symptomatic at birth. † Asymptomatic at birth. * Reversible impairment that was not included in the summary outcome measure for long-term impairment.

Table 4.2 - Long-term impairment, diagnosed in the first 6 years of life, of cCMV-positive and cCMV-negative children with or without symptoms at birth, per condition.

1 Sensorineural hearing loss ≥ 40 decibels. 2 Attention deficit-hyperactivity disorder. 3 Cognitive impairment according to the medical specialist or an intelligence quotient ≤ 70 based on the Snijders-Oomen Nonverbal intelligence test (SON) or the Wechsler Preschool and Primary Scale of Intelligence (WPPSI). 4 Motor impairment according to the physical therapist or a score below the fifth percentile on the movement Assessment Battery for Children (movement-ABC). 5 Sensory processing disorder or developmental coordination disorder. 6 Any moderate-to-severe problem in language development according to the speech therapist or a score ≤ 1.5 SD on the Reynell test (language comprehension) or Schlichting test (language production). 7 Any moderate-to-severe problem in speech development according to the speech therapist, including articulation disorder, phonological disorder, voice disorders, and lisping. 8 Any moderate-to-severe problem in oral motor skills, including stuttering, low muscle tone, open mouth posture, and problems with swallowing.

Long-term impairment per domain (Table 4.3, Figure 4.2)

When all outcome measures were combined, 24.8% of the cCMV-positive children had one or more long-term impairments, compared to 12.0% in the cCMV-negative group. Within the cCMV-positive group, the percentage was much higher among symptomatic children (53.8%) than those without symptoms at birth (17.8%) (Figure 4.2). No clear difference was observed between symptomatic and asymptomatic cCMV-negative children (Table 4.3).

The difference between cCMV-positive and cCMV-negative children became more pronounced when the presence of impairment in two or more domains was assessed. The differences between cCMV-positive and cCMV-negative children were statistically significant within the developmental domains (cognitive, motor, and speech-language) and for hearing impairment (Table 4.3).

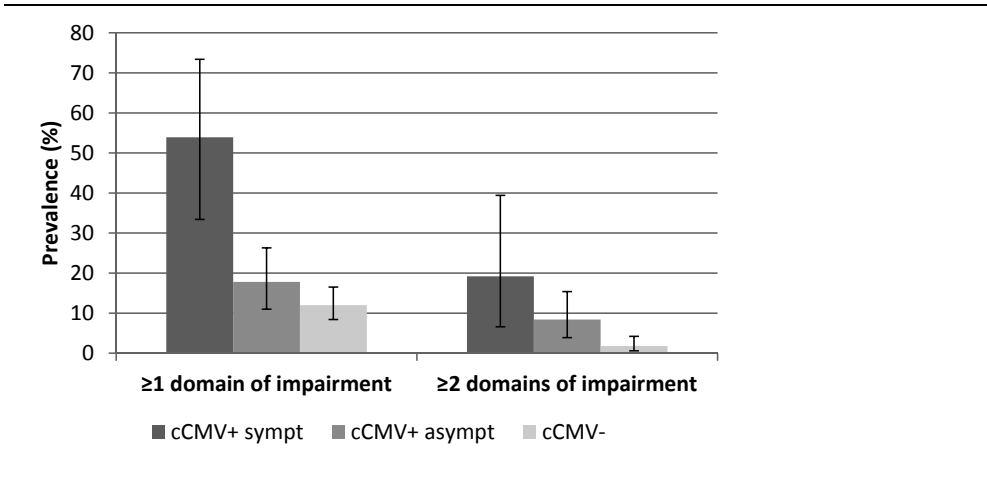


Figure 4.2 - Graphical representation of the prevalence of long-term impairment. The bars represent the prevalence and 95% confidence limits of any long-term impairment (≥ 1) and impairment in multiple domains (≥ 2) for symptomatic (sympt) and asymptomatic (asympt) children with cCMV (cCMV+) and for children without cCMV (cCMV-).

Table 4.3 - Long-term impairment, diagnosed in the first 6 years of life, per domain

	cCMV-positive			cCMV-negative		
	All	Sympt.‡	Asympt.†	All	Sympt.‡	Asympt.†
	n = 133 n (%)	n = 26 n (%)	n = 107 n (%)	n = 274 n (%)	n = 34 n (%)	n = 239 n (%)
Hearing impairment ¹	5 (3.8)	2 (7.7)	3 (2.8)	-	-	-
Visual impairment ²	3 (2.3)	-	3 (2.8)	1 (0.4)	-	1 (0.4)
Neurologic impairment ³	8 (6.0)	5 (19.2)	3 (2.8)	14 (5.1)	1 (2.9)	13 (5.4)
Cognitive impairment ⁴	8 (6.0)	4 (15.4)	4 (3.7)	3 (1.1)	1 (2.9)	2 (0.8)
Motor impairment ⁵	16 (12.0)	5 (19.2)	11 (10.3)	4 (1.5)	1 (2.9)	3 (1.3)
Speech-language impairment ⁶	22 (16.5)	9 (34.6)	13 (12.2)	20 (7.3)	3 (8.8)	17 (7.1)
≥1 domains of impairment ⁷	33 (24.8)	14 (53.8)	19 (17.8)	33 (12.0)	3 (8.8)	30 (12.5)
≥2 domains of impairment ⁸	14 (10.5)	5 (19.2)	9 (8.4)	5 (1.8)	1 (2.9)	4 (1.7)

These figures represent the prevalence of impairment per domain in numbers (n) and percentages.

	cCMV+ vs. cCMV- Risk difference		cCMV+ vs. cCMV- Relative risk	
	%	95% CI	RR	95% CI
Hearing impairment ¹	3.8*	0.5 - 7.0	-	-
Visual impairment ²	1.9	-0.7 - 4.5	6.18	0.65 - 58.9
Neurologic impairment ³	0.9	-3.9 - 5.7	1.18	0.51 - 2.74
Cognitive impairment ⁴	4.9*	0.7 - 9.1	5.49*	1.48 - 20.4
Motor impairment ⁵	10.6*	4.9 - 16.3	8.24*	2.81 - 24.2
Speech-language impairment ⁶	9.2*	2.2 - 16.3	2.27*	1.28 - 4.00
≥1 domains of impairment ⁷	12.8*	4.5 - 21.1	2.06*	1.33 - 3.19
≥2 domains of impairment ⁸	8.7*	3.3 - 14.2	5.77*	2.12 - 15.7

These figures represent risk differences and relative risk (RR) and their 95% confidence intervals (CI).

cCMV-positive (cCMV+): children with cCMV. cCMV-negative (cCMV-): children without cCMV.

‡Symptomatic at birth. †Asymptomatic at birth. *Statistically significant risk difference or relative risk.

1 Sensorineural hearing loss. 2 Optic nerve atrophy, congenital cataract, cortical visual impairment.

3 Cerebral palsy, epilepsy, microcephaly, autism, ADHD. 4 Cognitive impairment based on test or

diagnosis. 5 Motor impairment (fine, gross, or balance) based on test or diagnosis, sensory processing disorder, or developmental coordination disorder. 6 Language impairment based on test or diagnosis, speech impairment, oral motor skill difficulties, or auditory processing disorder.

7 Any long-term impairment, in one or more domains. 8 Impairment in two or more domains.

Discussion

In this nationwide cohort study, based on screening of 31,484 dried blood spots for cCMV, long-term impairment, particularly sensorineural hearing loss and cognitive, motor, and speech-language impairment, was seen in 24.8% of all children with cCMV compared to 12.0% of children without cCMV. Of the symptomatic cCMV-positive children, 53.8% developed one or more long-term impairments, compared with 17.8% of the asymptomatic cCMV-positive children. Long-term impairment in multiple domains was seen in 19.2% and 8.4% of the symptomatic and asymptomatic cCMV positive children, respectively, compared to 1.8% in the total cCMV-negative group.

Including a large matched cCMV-negative control group in this study provided insight into the long-term impairment attributable to cCMV. In other studies, [13-15] control groups were often small or absent, and frequently the prevalence of long-term impairment was described in detail in only the cCMV-positive group. The retrospective design of this study ensured that the vast majority of collected data were not affected by awareness of the diagnosis, therefore limiting information bias. This approach also gives a realistic perspective of the disease burden, as outcomes were based on the actual healthcare that was sought and provided. Furthermore, by collecting a broad range of medical data, this study illustrates in detail the specific medical problems faced by children with and without cCMV during the first 6 years of life.

It is important to note that the retrospective design of this study, while limiting information bias, entails a lack of uniformity in examinations and registration. In particular, only 20% of children had data available on physical examination after birth. On the other hand, it is unlikely that major signs or symptoms were missed, as all newborns are regularly checked in the first weeks of life by a physician or midwife and referred to a pediatrician in the case of abnormalities. Still, some data could be missing or care may not have been sought for specific problems. This could have led to an underestimation of symptoms at birth and long-term impairment in both cCMV-positive and cCMV-negative children. Moreover, it is not unlikely that, especially in some children with cCMV, more long-term impairment, such as sensorineural hearing loss, may become apparent at a later age, which could increase the risk difference between the two groups. A prospective follow-up of this cohort is therefore essential.

Selection bias could have occurred in this study, although it is difficult to assess the potential effect of this bias.[3] Parents of children with an impairment could have been more willing to participate, because they tried to find an explanation for their child's problems, or less willing because they had their hands full in caring for their child. In addition, with a sensitivity of 84% for DBS testing, some children with cCMV remain undetected, most likely those with low viral loads. This may have led to an overestimation of the burden of disease because viral load and outcome are possibly related.

The percentage of children with impairments in the group without cCMV seems relatively high, however the prevalence of, for example, speech and language problems and attention deficit-hyperactivity disorder, which were the most prevalent conditions in this group, are comparable to children of a similar age in the general population[16-18]. This supports our

assumption that few relevant problems were missed and confirms the value of our control group.

Sensorineural hearing loss occurred in 5 children with cCMV (3.8%), which is lower than previously reported in other studies. [19-21] A systematic review concluded that hearing loss occurred in 12.6% of all children with cCMV, in 32.8% of symptomatic children and 9.9% of asymptomatic children. [22] Underreporting of hearing loss could have occurred in our study, for example due to a diagnosis being made after the collection of medical data. Unilateral sensorineural hearing loss especially can be difficult to recognize, and the average age at diagnosis is 5.5 years. [23] Longer follow-up of this cohort could reveal a higher prevalence of sensorineural hearing loss.

In children with cCMV, the occurrence of impairments affecting various developmental domains (cognitive, motor, and speech-language) was frequently seen. In particular, fine and gross motor impairment was 8 times more frequent in both symptomatic and asymptomatic children with cCMV (12.0%) compared to cCMV-negative children (1.5%). Furthermore, cognitive impairment was seen in both symptomatic (15.4%) and asymptomatic (3.7%) children with cCMV, while very few children in the control group had cognitive problems (1.1%). Although speech and language problems are also frequently seen in the general population, [24] these problems occurred twice as often in the cCMV-positive group (16.5%) compared to the cCMV-negative controls (7.3%).

These observed cCMV attributable impairments indicate that, in addition to audiological follow-up and support, it is also necessary to be aware of the risk of speech and language problems and motor impairment.

The broad range of collected data provides insight into the prevalence of specific conditions. This information can be used to confirm or generate new hypotheses. For example, this study supports the potential link between cCMV and balance problems [25-27] and suggests a conceivable link between cCMV and autism spectrum disorder [28-32], conditions which are not commonly recognized as being associated with cCMV. It may also be relevant to evaluate the presence of sensory processing disorder and developmental coordination disorder in other cCMV cohorts.

In conclusion, this study reaffirms the significant long-term disease burden of congenital cytomegalovirus infection. [33, 34] More specifically, by including a control group, it demonstrated which kinds of impairment are attributable to cCMV. Consequently, in addition to frequent audiological follow-up, extra awareness of motor, cognitive, and speech-language problems is indicated for children with cCMV. Moreover, this study demonstrates the current lack of awareness of cCMV as, at the age of 5 years, only 3 of the 133 cCMV-positive children had previously been diagnosed. It is clear that much more needs to be done to increase awareness and prevent cCMV. These findings can serve as a basis for modelling scenarios for preventive measures, such as hygienic advice [35], maternal vaccination [36-38], neonatal screening programs [39] or anti-viral therapy [40].

Acknowledgement

We thank all the parents and children who participated in this study. This study would not have been possible without the cooperation of all the general practitioners, medical specialists, healthcare providers, school teachers, and Regional Public Health Services. We also thank Marlies Weltevrede for her work collecting and processing data. We acknowledge the work of Jeroen Cremer, Lisette Rusman, Hein Boot,[†] and Rob van Binnendijk in testing the dried blood spots. We thank Robine Donken for reviewing the analysis syntax. English-language editing support was provided Elsevier Language Editing, who received financial compensation from the National Institute for Public Health and the Environment.

Authors' contributions

Funding was obtained by De Melker. The study was conceptualized and designed by all authors. Data acquisition, analysis and interpretation were performed by Korndewal, Oudesluys-Murphy, De Melker and Vossen. De Melker, Oudesluys-Murphy, Kroes, Vossen supervised the study.

The manuscript was drafted and statistical analysis was performed by Korndewal. All other authors critically revised the manuscript. De Melker and Vossen contributed equally to this work.

Declaration of interests

The authors declare no conflict of interest.

Role of funding source

This research was funded by Strategic Research of the National Institute of Public Health and the Environment.

The funding source had no part in the data collection, analysis, interpretation, trial design, patient recruitment, or any aspect pertinent to the study. The funding source had also no part in the writing of the manuscript or the decision to submit it for publication.

The corresponding author declares she had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Ethics committee approval

This study was approved by the Medical Ethics Committee of the Leiden University Medical Center.

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Supplement 4.1 - Prevalence of long-term impairment of children with and without cCMV

n (%)	Congenital CMV infection			No congenital CMV infection		
	All n = 133	Sympt. n = 26	Asympt. n = 107	All n = 274	Sympt. n = 34	Asympt. n = 240
Hearing impairment						
<i>Hearing loss (HL)</i>						
Conductive, unilateral	5 (3,8)	2 (7,7)	3 (2,8)	1 (0,4)	1 (2,9)	0
Conductive, bilateral	1 (0,8)	0	1 (0,9)	4 (1,5)	2 (5,9)	2 (0,8)
Sensorineural, unilateral	3 (2,3)	1 (3,9)	2 (1,9)	0	0	0
Sensorineural, bilateral	2 (1,5)	1 (3,9)	1 (0,9)	0	0	0
<i>Right ear (sensorineural HL)</i>						
Moderate (40-70 decibel)	1 (0,8)	0	1 (0,9)	0	0	0
Severe (71-90 decibel)	1 (0,8)	1 (3,9)	0	0	0	0
Profound (> 90 decibel)	2 (1,5)	0	2 (1,9)	0	0	0
<i>Left ear (sensorineural HL)</i>						
Moderate (40-70 decibel)	1 (0,8)	1 (3,9)	0	0	0	0
Profound (> 90 decibel)	2 (1,5)	1 (3,9)	1 (0,9)	0	0	0
Sensorineural hearing loss	5 (3,8)	2 (7,7)	3 (2,8)	0	0	0
Visual impairment						
Optic nerve atrophy	1 (0,8)	0	1 (0,9)	0	0	0
Congenital cataract	0	0	0	1 (0,4)	0	1 (0,4)
Cortical visual impairment	2 (1,5)	0	2 (1,9)	0	0	0
Refractive error	13 (9,8)	4 (15,4)	9 (8,4)	42 (15,3)	8 (23,5)	34 (14,2)
Strabismus	2 (1,5)	0	2 (1,9)	9 (3,3)	4 (11,8)	5 (2,1)
Amblyopia	3 (2,3)	0	3 (2,8)	10 (3,7)	2 (5,9)	8 (3,3)
<i>Visual acuity - n</i>	<i>19</i>	<i>5</i>	<i>14</i>	<i>45</i>	<i>6</i>	<i>39</i>
Right eye <0.3 (visual impaired)	0	0	0	0	0	0
Left eye <0.3 (visual impaired)	1 (0,8)	0	1 (0,9)	1 (0,4)	0	1 (0,4)
<i>Refractive error - n</i>	<i>13</i>	<i>4</i>	<i>9</i>	<i>42</i>	<i>8</i>	<i>34</i>
Right eye: mean ± SD	3,4 ± 2,9	4,5 ± 3,8	2,9 ± 2,5	2,1 ± 2,4	4,0 ± 1,8	1,6 ± 2,4
Left eye: mean ± SD	3,7 ± 3,2	4,5 ± 4,2	3,4 ± 2,9	2,1 ± 2,6	4,2 ± 1,9	1,6 ± 2,5
Any visual impairment	1 (0,8)	0	1 (0,9)	1 (0,4)	0	1 (0,4)
Neurological impairment						
Cerebral Palsy	2 (1,5)	1 (3,9)	1 (0,9)	0	0	0
Epilepsy	2 (1,5)	2 (7,7)	0	3 (1,1)	0	3 (1,3)
Microcephaly (last visit)	2 (1,5)	2 (7,7)	0	3 (1,1)	0	3 (1,3)
Autism (specialist diagnosis)	0	0	0	1 (0,4)	1 (2,9)	0
Autism (DSM-IV diagnosis)	4 (3,0)	2 (7,7)	2 (1,9)	4 (1,5)	0	4 (1,7)
ADHD ¹ (specialist diagnosis)	0	0	0	2 (0,7)	0	2 (0,8)
ADHD ¹ (DMS-IV diagnosis)	1 (0,8)	1 (3,9)	0	5 (1,8)	0	5 (2,1)
Any neurological impairment	8 (6,0)	5 (19,2)	3 (2,8)	14 (5,1)	1 (2,9)	13 (5,4)
Cognitive impairment						
<i>Diagnosis by specialist</i>						
Cognitive delay - mild	3 (2,3)	1 (3,9)	2 (1,9)	0	0	0
Cognitive delay	7 (5,3)	3 (11,5)	4 (3,7)	3 (1,1)	1 (2,9)	2 (0,8)
<i>IQ-test (SON / WPPSI) - n</i>	<i>6</i>	<i>2</i>	<i>4</i>	<i>6</i>	<i>1</i>	<i>5</i>
Mild delay (IQ 71 - 85)	3 (2,3)	1 (3,9)	2 (1,9)	0	0	0
Impaired (IQ ≤ 70)	1 (0,8)	1 (3,9)	0	1 (0,4)	0	1 (0,4)
Any cognitive impairment	8 (6,0)	4 (15,4)	4 (3,7)	3 (1,1)	1 (2,9)	2 (0,8)

Supplement 4.1 - Prevalence of long-term impairment of children with and without cCMV

n (%)	Congenital CMV infection			No congenital CMV infection		
	All n = 133	Sympt. n = 26	Asympt. n = 107	All n = 274	Sympt. n = 34	Asympt. n = 240
Motor impairment						
<i>Diagnosed by specialist</i>						
Fine motor - mild	2 (1,5)	1 (3,9)	1 (0,9)	5 (1,8)	0	5 (2,1)
Fine motor impairment	13 (9,8)	5 (19,2)	8 (7,5)	2 (0,7)	0	2 (0,8)
Gross motor - mild	3 (2,3)	1 (3,9)	2 (1,9)	6 (2,2)	0	6 (2,5)
Gross motor impairment	13 (9,8)	4 (15,4)	9 (8,4)	3 (1,1)	1 (2,9)	2 (0,8)
Balance - mild	2 (1,5)	1 (3,9)	1 (0,9)	2 (0,7)	0	2 (0,8)
Balance impairment	8 (6,0)	1 (3,9)	7 (6,5)	1 (0,4)	1 (2,9)	0
DCD or SPD ²	4 (3,0)	2 (7,7)	2 (1,9)	1 (0,4)	0	1 (0,4)
<i>Test (Movement-ABC) - n</i>	7	3	5	1	0	1
General – mild (< p16)	2 (1,5)	1 (3,9)	1 (0,9)	0	0	0
General impairment (< p5)	4 (3,0)	1 (3,9)	3 (2,8)	0	0	0
Fine motor – mild (< p16)	2 (1,5)	1 (3,9)	1 (0,9)	0	0	0
Fine motor impairment (< p5)	3 (2,3)	1 (3,9)	2 (1,9)	0	0	0
Gross motor impairment (< p5)	3 (2,3)	1 (3,9)	2 (1,9)	0	0	0
Balance – mild (< p16)	1 (0,8)	0	1 (0,9)	0	0	0
Balance impairment (< p5)	3 (2,3)	1 (3,9)	2 (1,9)	0	0	0
Any motor impairment	16 (12,0)	5 (19,2)	11 (10,3)	4 (1,5)	1 (2,9)	3 (1,3)
Speech-language impairment						
<i>Diagnosis by specialist</i>						
Language problem - mild	3 (2,3)	1 (3,9)	2 (1,9)	5 (1,8)	1 (2,9)	4 (1,7)
Language problem - moderate	12 (9,0)	5 (19,2)	7 (6,5)	8 (2,9)	1 (2,9)	7 (2,9)
Language problem - severe	2 (1,5)	1 (3,9)	1 (0,9)	2 (0,7)	0	2 (0,8)
Speech problem - mild	6 (4,5)	1 (3,9)	5 (4,7)	17 (6,2)	2 (5,9)	15 (6,3)
Speech impairment	16 (12,0)	6 (23,1)	10 (9,4)	15 (5,5)	3 (8,8)	12 (5,0)
Mouth motor skills problem	6 (4,5)	2 (7,7)	4 (3,7)	9 (3,3)	1 (2,9)	8 (3,3)
Auditory processing disorders	2 (1,5)	2 (7,7)	0	1 (0,4)	0	1 (0,4)
<i>Test (Reynell) - n</i>	6	3	3	4	0	4
Score <-1 SD - ≥-1,5 SD	3 (2,3)	2 (7,7)	1 (0,9)	0	0	0
Score <-1,5 SD - ≥-2,0 SD	1 (0,8)	1 (3,9)	0	1 (0,4)	0	1 (0,4)
Score <-2,0 SD	0	0	0	1 (0,4)	0	1 (0,4)
<i>Test (Schlichting) - n</i>	13	5	8	7	1	6
Score <-1 SD - ≥-1,5 SD	3 (2,3)	2 (7,7)	1 (0,9)	3 (1,1)	1 (2,9)	2 (0,8)
Score <-1,5 SD - ≥-2,0 SD	1 (0,8)	1 (3,9)	0	1 (0,4)	0	1 (0,4)
Score <-2,0 SD	1 (0,8)	0	1 (0,9)	1 (0,4)	0	1 (0,4)
Any language impairment	22 (16,5)	9 (34,6)	13 (12,2)	20 (7,3)	3 (8,8)	17 (7,1)

1 attention deficit-hyperactivity disorder, 2 development coordination disorder or sensory processing disorder

Chapter 5

Congenital cytomegalovirus infection: child development, quality of life and impact on daily life

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Submitted

Abstract

Background

Congenital cytomegalovirus infection (cCMV) is the most common congenital infection worldwide and can lead to long-term impairments such as developmental delay. It is currently unknown how this affects the daily life of children and their parents.

Methods

Children with cCMV were identified by testing stored dried blood spots of 31.484 five-year-old children born in 2008 in the Netherlands. Parents of 133 children with cCMV and 274 children without cCMV participated and filled in questionnaires on the child's development, the child's and parents' quality of life, care provided for the children, and consequences of cCMV on daily life. School performance reports at six years of age were also investigated.

Results

Children with cCMV had delays in general and expressive language development more often and they attended physical therapists more frequently than children without cCMV. School performance of children with cCMV and symptoms at birth was poorer than that of cCMV-negative children with similar symptoms at birth. The quality of life of children with long-term impairment was lower in children with cCMV than those without cCMV. Parents of children with cCMV and long-term impairments reported more physical and concentration problems than parents of children without cCMV.

Conclusions

These findings indicate that cCMV has a considerable impact, not only on the child's development and school performance, but also on the daily life of children and their parents. The care for children with cCMV should therefore include support for motor and speech-language development as well as family-centered care.

Introduction

A congenital cytomegalovirus infection (cCMV) ensues when cytomegalovirus (CMV) is transmitted from the mother to the fetus during pregnancy. cCMV is the most common congenital infection, with an overall birth prevalence of 0.6 to 0.7% in industrialized countries. [1, 2] In 2007, a key review on sequelae of cCMV estimated that approximately 10 to 15% of children born with cCMV were symptomatic at birth, showing clinical features such as hepatosplenomegaly, thrombocytopenia, petechiae, microcephaly and intracranial calcifications. [1] About half of these symptomatic children develop long term sequelae, including hearing loss, motor deficits and mental retardation. Moreover, about 10 to 15% of children who are asymptomatic at birth develop long term sequelae. [1]

Knowledge concerning the full range of long-term consequences of cCMV is still incomplete. [3] Besides, most studies on long-term consequences of cCMV included no, or only small control groups, and they often had a prospective study design, which may have led to information bias. Some studies were based on a population of referred patients, rather than on a screening program, and thus included a selected population.

We performed a nationwide retrospective screening based cohort study in the Netherlands on the disease burden of cCMV up to the age of six years among congenitally infected children and a cCMV-negative control group. [4] The birth prevalence of 0.5% in this study was comparable to that in other European countries. [5] It is noteworthy that only 2.6% of the cCMV positive children had been previously diagnosed. [4] We found that long-term impairments were twice as prevalent in children with cCMV (24.8%) compared to the cCMV-negative control group (12.0%). [6]

We expected that these impairments will affect the quality of life and daily life activities of these children and their parents. To our knowledge, these consequences have been given no, or only limited attention, in previous studies.

This paper presents the outcomes of the CROCUS-study (**C**onsequences and **R**isk factors **O**f congenital **C**ytomegalovir**U**S infection) concerning the child's development, school achievements, the quality of life of the child and parents and the impact on daily life. These data can be used for assessing the full impact of cCMV and can provide input for optimization of care.

Patients and Methods

Study design

The design of the CROCUS-study has been previously described. [4] In short, the first part of this study consisted of identification of children with and without cCMV at the age of five years. Subsequently data of participating children with cCMV and a selected cCMV-negative control group were collected. Children born in the Netherlands between January 1 and September 30 2008 were eligible for this study. Children with cCMV were identified by testing stored neonatal dried blood spots for CMV DNA using two separate polymerase chain reaction tests, with an assumed sensitivity of 84.4%. [7] A twice as large group of cCMV-negative children, matched to the cCMV-positive children for age, sex and postal-code region, was selected as a control group. All parents gave written informed consent and the study was approved by the medical ethics committee of the Leiden University Medical Center.

Data collection

Parents of children with cCMV were informed of the diagnosis of cCMV shortly after this was made. When the parents of the cCMV-positive children and the selected cCMV-negative control group were willing to participate in the second part of the study, they gave informed consent to collect data from the school and from healthcare providers who were involved in the care of their child. Additionally, four questionnaires were sent to the parents. The Child Development Inventory (CDI) [8, 9] translated into Dutch [10] was used to assess the development of the children. The parental proxy report (age 5-7) of the Pediatric Quality of Life Inventory™ (PedsQL™) [11-15] was used to assess the quality of life of the children. The 12-Item Short Form Health Survey (SF-12®) [16, 17] was used to assess the quality of life of the parents. In addition, a questionnaire was included concerning the medical history of the child, the family composition and characteristics, the amount of care needed by the child and the influence of the child's problems on the life of parents. These parental questionnaires were sent when the child was five years of age, a few months after the diagnosis of cCMV was known.

Definitions

The cCMV-positive and cCMV-negative children were divided into groups of children with symptoms at birth (symptomatic) and those without symptoms at birth (asymptomatic). Additionally they were grouped according to the presence or absence of long term impairments.

Being symptomatic at birth was defined as having one or more of the following signs or symptoms in the first four weeks of life: preterm birth (<37 weeks gestational age), being small for gestational age (birthweight <-2 standard deviations (SD) for gestational age), microcephaly (head circumference <-2 SD for gestational age), abnormal physical examination (hepato- or splenomegaly, petechiae or purpura, hypotonia or seizures), abnormal laboratory findings (thrombocytopenia $<100 \times 10^9/L$, neutropenia $<0.5 \times 10^9/L$, alanine aminotransferase >80 IU/L, conjugated hyperbilirubinemia >2 mg/dL), cranial ultrasound abnormalities, ophthalmological abnormalities or neonatal sensorineural hearing loss (≥ 40 decibel).

Long-term impairment was defined as any moderate-to-severe impairment in one or more of the following domains: hearing (sensorineural hearing loss), visual (optic atrophy, congenital cataract, cortical visual impairment), neurologic (cerebral palsy, epilepsy, microcephaly, autism spectrum disorder, attention deficit-hyperactivity disorder), cognitive (mental retardation), motor (fine or gross motor skill or balance problems, development coordination disorder or sensory processing disorder) and speech-language (speech, language or oral motor skill problems or auditory processing disorder).

The Child Development Inventory gives an estimation of developmental age per domain. In this study, a developmental age of -2 standard deviations below calendar age or lower was classified as developmental delay.

The school performance results were based on a widely used pupil tracking system in the Netherlands. During the first two years of education, from the age of four years, a child is generally tested four times. The test results are divided into either quintiles (I, II, III, IV or V) or quartiles (A, B, C or D/E). A separate category is used for children with the lowest 10% score (V minus or E). When a child was in the lowest 10% scoring range in more than 50% of the tests or if it received special needs education this was considered a poor school performance.

The PedsQL™ consists of a Psychosocial and Physical Health Summary Score and a Total Scale Score. The scores can range from 0 to 100, where higher scores indicate better functioning. The SF-12® results in a Mental and Physical Health Composite Score. These composite scores are standardized to a mean of 50 with a standard deviation of 10 using QualityMetric Scoring Software 4.5 (QualityMetric Inc., Lincoln, RI, USA). Higher composite scores indicate a better health status.

Statistical analysis

The prevalence of developmental delay and poor school results were given as percentages. The health related quality of life scores were presented as means. The proportion of children receiving care and support and the amount of parents experiencing negative consequences in daily life were shown as percentages. The cCMV-positive children were compared to the cCMV-negative children. Mean differences or risk differences between these groups and their 95% confidence intervals (95% CI) were calculated.

For the child development inventory and school results, data were presented primarily for the children with or without symptoms at birth, as these developmental outcomes were considered a potential consequence of cCMV itself. For the quality of life, the amount of provided care and the consequences for the daily life of parents, data were presented for the groups with and without long-term impairment, in order to evaluate the effect of cCMV-associated impairments on these different outcome measures.

Subgroup analyses were performed comparing symptomatic children with and without cCMV and comparing cCMV-positive and cCMV-negative children with long-term impairment.

Additionally, for the children who had cCMV and long-term impairment, the groups with and without symptoms at birth were compared.

All statistical analyses were performed in SAS 9.3 (SAS Institute, Cary, NC, USA).

Results

Baseline data (Table 5.1)

A total of 31,484 dried blood spots were retrospectively tested and 156 children with cCMV were identified. Parents of 407 children, including 133 cCMV-positive and 274 cCMV-negative children, consented to data collection for this part of the study. Only three of the participating children with cCMV had been diagnosed with cCMV prior to this study. The baseline data of the children in this study are displayed in Table 5.1. The parent questionnaire revealed no significant differences in family characteristics such as family size and educational level of the parents, between the cCMV-positive and cCMV-negative children.

Table 5.1 - Patient and family characteristics and response rates of children with and without cCMV

	cCMV+ n = 133	cCMV- n = 274	p-value (chi-square)
Patient characteristic			
Mean age on 1 January, 2014 (years)	5.6 ± 0.2	5.6 ± 0.2	0.799
Male sex	75 (56.4)	149 (54.4)	0.702
Symptomatic at birth [‡]	26 (19.6)	34 (12.4)	0.059
Long-term impairment [‡]	33 (24.8)	33 (12.0)	0.001
Family characteristics			
	n = 119	n = 242	
Number of family members [‡]			
Total	4.3 ± 0.8	4.3 ± 0.9	0.799
Siblings	1.5 ± 0.7	1.5 ± 0.7	0.776
Older siblings	1.3 ± 0.6	1.4 ± 0.7	0.216
Educational level mother [‡]			
Low	10 (8.5)	17 (7.2)	0.935
Middle	42 (35.9)	89 (37.9)	
High	65 (55.6)	129 (54.9)	
Origin mother [‡]			
the Netherlands	115 (94.3)	225 (90.4)	0.202
Foreign	7 (5.7)	24 (9.6)	
Educational level father [‡]			
Low	17 (14.5)	34 (14.2)	0.784
Middle	36 (30.8)	86 (35.8)	
High	64 (54.7)	120 (50.0)	
Origin father [‡]			
the Netherlands	111 (91.0)	226 (90.8)	0.945
Foreign	11 (9.0)	23 (9.2)	

Table 5.1 - Patient and family characteristics and response rates of children with and without cCMV

	cCMV+ n = 133	cCMV- n = 274	p-value (chi-square)
Response rate	n = 133	n = 274	
Child Development Inventory	123 (92.5)	242 (88.3)	0.196
PedsQL™	123 (92.5)	241 (88.0)	0.164
SF-12®	122 (91.7)	242 (88.3)	0.294
Parental questionnaire	119 (89.5)	242 (88.3)	0.731
Test results from schools	117 (88.0)	243 (88.7)	0.832

The figures represent mean ± standard deviation (SD) or number (n) and percentage (between brackets) for children with (cCMV+) and without (cCMV-) congenital CMV infection.

¥ including children with preterm birth, small for gestational age, microcephaly, cranial ultrasound abnormalities and neonatal sensorineural hearing loss. ¥ including any moderate-to-severe impairment in one or more of the following domains: hearing, visual, neurologic, cognitive, motor and speech-language. ‡ based on parental questionnaire

Child development and school results

Child Development Inventory (Table 5.2, Figure 5.1)

On most domains of the Child Development Inventory, the development of children with cCMV was comparable to children without cCMV. However, a delay in expressive language and general development was reported more often in children with cCMV compared to those without cCMV, with risk differences of 8.0% (95% CI 1.3-14.8) for expressive language and 6.9% (95% CI 0.9-12.8) for general development. (Table 5.2)

In children with symptoms at birth, problems in self-help were seen more often in cCMV positive than in cCMV negative children, with a risk difference of 22.9% (95% CI 3.9-41.8). In children with cCMV who had long-term impairment, a delay in general development was more common than in children with long-term impairment without cCMV: 44.4% versus 16.0%, risk difference: 28.4%, 95% CI: 4.8-52.1. (Figure 5.1)

There was no difference in the prevalence of developmental delay between the symptomatic (n = 14) and asymptomatic (n = 19) children with cCMV who developed long-term impairment (data not shown).

Table 5.2 - Prevalence (%) of developmental delay for various domains of the Child Development Inventory for children with and without cCMV.

	cCMV-positive			cCMV-negative		
	Overall (n = 123)	Sympt. (n = 23)	Asympt. (n = 100)	Overall (n = 242)	Sympt. (n = 31)	Asympt. (n = 211)
Expressive language	13.8*	26.1	11.0	5.8*	12.9	4.7
Language comprehension	12.2	21.7	10.0	6.6	12.9	5.7
Fine motor	6.5	17.4	4.0	3.7	12.9	2.4
Gross motor	21.1	26.1	20.0	13.2	22.6	11.8
Self help	13.8	26.1*	11.0	14.5	3.2*	16.1
Social	41.5	47.8	40.0	42.1	41.9	42.2
Letters	6.5	13.0	5.0	5.8	6.5	5.7
Numbers	4.1	4.3	4.0	4.1	6.5	3.8
General development	10.6*	21.7	8.0	3.7*	6.5	3.3

Percentage of children with developmental delay (developmental age below -2 standard deviations for calendar age) for cCMV-positive and cCMV-negative children who were symptomatic (sympt.) or asymptomatic (asympt.) at birth. * statistically significant difference between cCMV-positive and cCMV-negative children (p < 0.05)

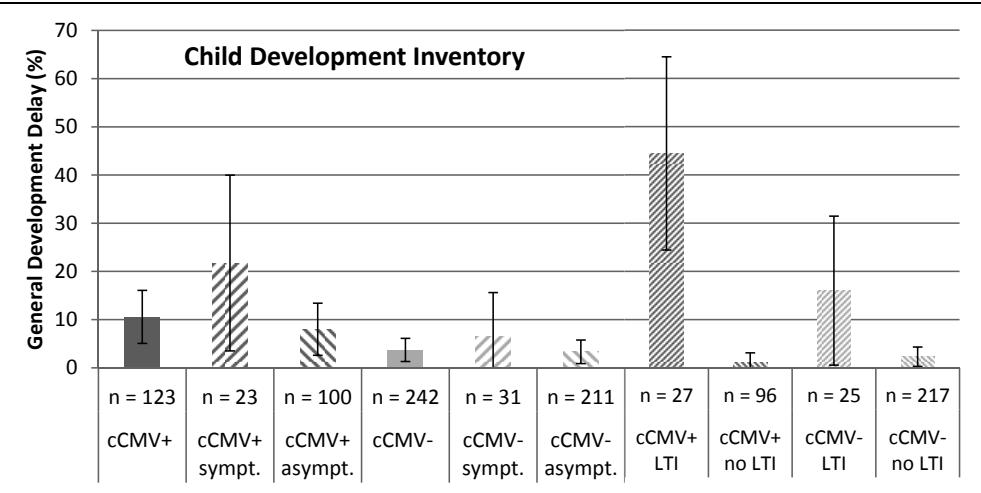


Figure 5.1 - Percentage and 95% CI of children with general developmental delay based on the Child Development Inventory for cCMV-positive (cCMV+) and cCMV-negative (cCMV-) children who were symptomatic (sympt.) or asymptomatic (asympt.) at birth or those with (LTI) or without long-term impairment(no LTI).

School performance (Figure 5.2)

Six children (5.3%) in the cCMV-positive group and seven children (2.9%) in the cCMV-negative group attended special needs education.

The percentage of children with a poor school performance was higher in children with cCMV and symptoms at birth (26.1%) compared to cCMV-negative children with symptoms at birth (3.3%), giving a risk difference of 22.8% (95% CI: 3.7-41.8).

The difference in poor school performance between cCMV-positive (26.9%) and cCMV-negative (17.9%) children with long-term impairment was much smaller and statistically not significant (risk difference: 9.1%, 95% CI: -13.1-31.3). Children with cCMV and long-term impairment who were symptomatic at birth had poor school results more often (41.7%) compared to those without symptoms at birth (14.3%), however the risk difference (27.4%, 95% CI: -6.0-60.8) was not statistically significant (data not shown).

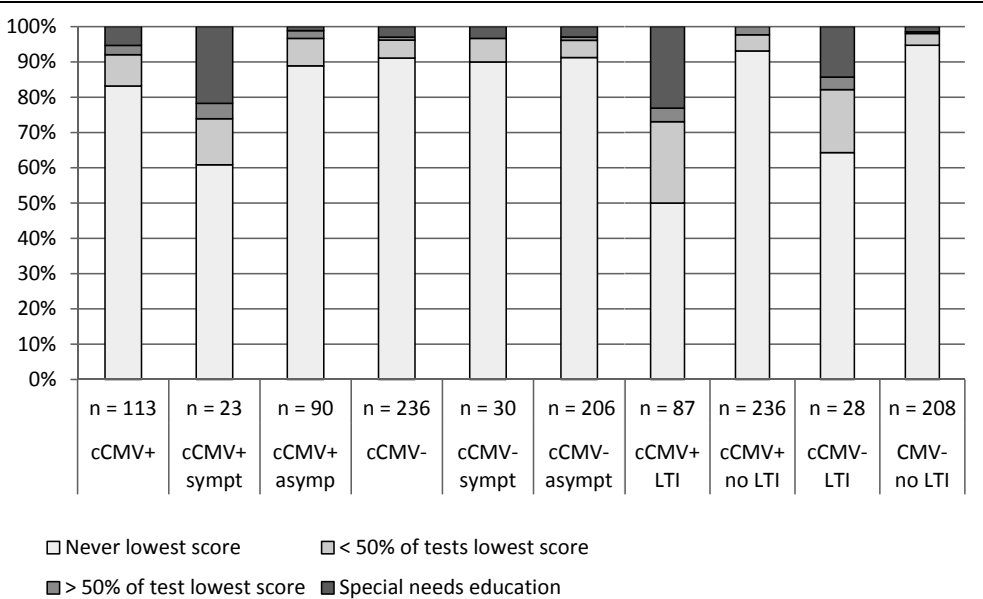


Figure 5.2 - School performance of children with and without cCMV, during the first two years of primary regular education.

Frequency of mathematic and language tests in the lowest score range (under the tenth percentile) and the prevalence of children attending special needs education. For children with (cCMV+) and without cCMV (cCMV-) and the symptomatic (sympt) and asymptomatic (asymp) subgroups as well as for the children with (LTI) and without long-term impairment (no LTI).

Consequences for children

Care and support (Table 5.3)

Children with cCMV attended a physical therapist more often (12.6%) than the cCMV-negative (3.3%) with a risk difference of 9.3% (95% CI: 2.9-15.7). This difference between children with and without cCMV was higher for children with long-term impairment, with a risk difference of 36.0% (95% CI: 15.3-56.7). There was no difference between symptomatic and asymptomatic children with cCMV who developed long-term impairment (data not shown).

Between children with and without cCMV who were symptomatic at birth a difference of 21.8% (95% CI: 1.8-41.8) was seen for physical therapy attendance (data not shown). There were no statistically significant differences between children with and without cCMV for consultations by medical specialists or other healthcare providers.

Table 5.3 - The percentage of children who received care and support, for the children with and without cCMV who did or did not develop long-term impairment (LTI).

	cCMV-positive			cCMV-negative		
	Overall (n = 119)	LTI (n = 25)	no LTI (n = 94)	Overall (n = 242)	LTI (n = 25)	no LTI (n = 217)
Medical day care	1.7	8.0	0.0	0.8	8.0	0.0
Medical specialist ^A	16.8	44.0	9.6	12.8	24.0	11.5
Physical therapist	12.6*	40.0*	5.3	3.3*	4.0*	3.2
Speech therapist	11.8	52.0	1.1	6.6	32.0	3.7
Occupational therapist	3.4	12.0	1.1	0.4	4.0	0.0
Support at school	8.4	32.0	2.1	3.7	12.0	2.8
Additional support ^B	3.4	12.0	1.1	1.7	4.0	1.4
Psychological support	2.5	8.0	1.1	2.1	8.0	1.4
Any support	28.6	76.0	16.0	24.4	52.0	21.2

A including pediatrician, otorhinolaryngologist, ophthalmologist and other medical specialist

B including welfare support worker, remedial educationalist, pedagogical staff member and play therapist * statistically significant difference between cCMV+ and cCMV- children (p <0.05)

Quality of life: PedsQL™ (Figure 5.3)

The average of the total scale score and physical and psychosocial health summary scores were generally comparable between children with and without cCMV. However, children with cCMV and long-term impairment had lower scores than their cCMV-negative counterparts, with a mean difference of 8.6 (95% CI: 1.3-15.8) for the total scale score and 11.9 (95% CI: 2.1 - 21.8) for the physical health summary score. For children who had cCMV and long-term impairment no differences were seen between those with and those without symptoms at birth (data not shown).

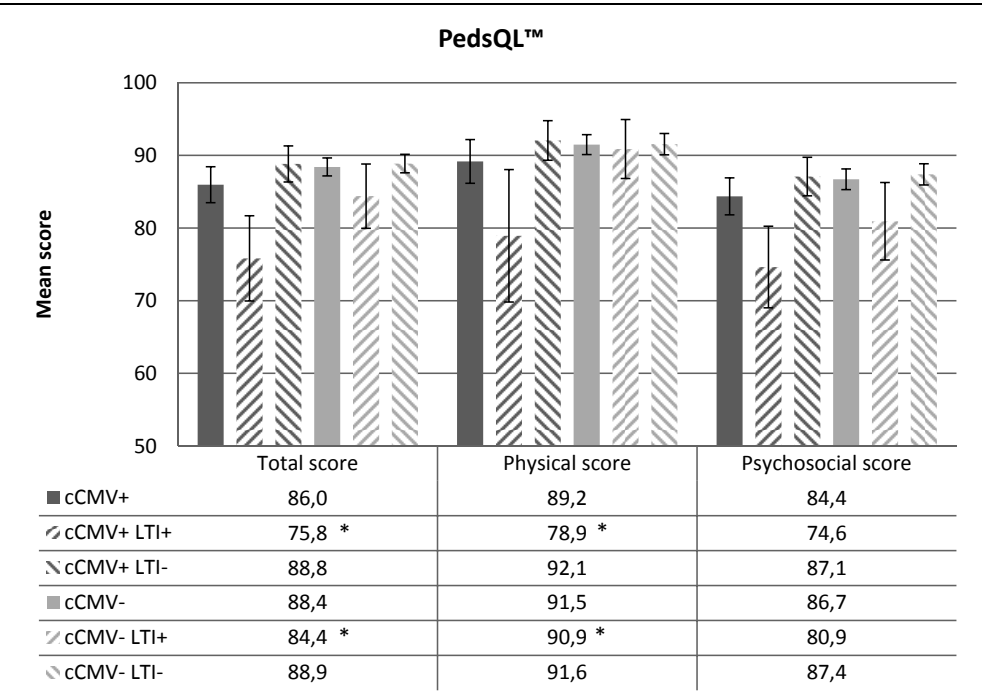


Figure 5.3 - Quality of life of children with and without congenital CMV infection. The quality of life is measured using the PedsQL™ parent proxy report (age 5-7) and presented as mean scores for cCMV-positive (cCMV+) and cCMV-negative (cCMV-) children with (LTI+) or without (LTI-) any long-term impairment. * Statistically significant difference between cCMV+ and cCMV- (p < 0.05). Total score: Total scale score; Physical score: Physical health summary score; Psychosocial score: Psychosocial health summary score

Consequences for parents

Daily life consequences (Table 5.4)

In the parent questionnaire, we asked about the consequences of their child's problems for various aspects of the parents' life. Parents of children with cCMV and long-term impairment reported having more physical and concentration problems than parents of children with long-term impairment without cCMV. The risk difference between cCMV-positive and cCMV-negative was 28.0% (95% CI 3.8-52.2) for physical problems and 24% (95% CI 0.1-48.0) for concentration problems. Even though the differences between cCMV-positive and cCMV-negative children with long-term impairment were not statistically significant for the other aspects of daily life, they were consistently more common in the parents of cCMV-positive children.

Similar results were found for cCMV-positive children with long-term impairment who were symptomatic at birth and those who were asymptomatic at birth (data not shown).

Table 5.4 - Impact on various aspects of daily life of parents, due to their child's problems, shown for parents of children with and without cCMV.

Aspects of daily life	cCMV-positive			cCMV-negative		
	Overall (n = 119)	LTI (n = 25)	no LTI (n = 94)	Overall (n = 242)	LTI (n = 25)	no LTI (n = 217)
Physical	13.4	44.0*	5.3	7.4	16.0*	6.5
Sleep	10.1	24.0	6.4	7.4	16.0	6.5
Concentration	11.8	40.0*	4.3	5.4	16.0*	4.1
Work / daily activities	5.9	28.0	0.0	5.8	16.0	4.6
Housekeeping	5.9	24.0	1.1	3.3	12.0	2.3
Leisure time	7.6	32.0	1.1	5.0	24.0	2.8
Social	8.4	32.0	2.1	7.4	28.0	5.1
Financial	5.0	24.0	0.0	3.3	16.0	1.8
Emotional	16.8	44.0	9.6	12.8	32.0	10.6
Any consequence	25.2	68.0	13.8	21.5	44.0	18.9
Any severe consequence	7.6	24.0	3.2	6.6	24.0	4.6

Data are presented as number and percentage for children with (cCMV+) and without cCMV (cCMV-) and the subgroups with (LTI+) and without long-term impairments (LTI-).

Quality of life: SF-12® (Figure 5.4)

In general, there were no differences in mean health-related quality of life scores between parents of children with and without cCMV. The largest difference in quality of life was seen between parents of children with cCMV and long-term impairment and parents of children with long-term impairment without cCMV. The mean difference between these groups was 4.1, however this difference was not statistically significant (95% CI: -1.8-9.9). No differences were seen between parents of symptomatic and asymptomatic children with cCMV who developed long-term impairment (data not shown).

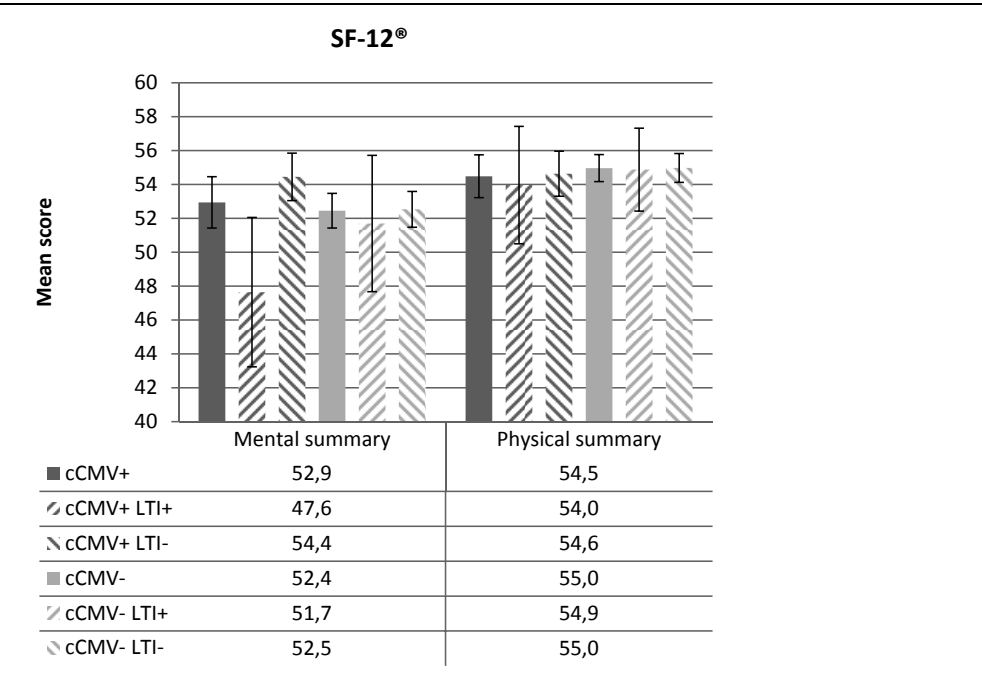


Figure 5.4 - Mean mental and physical component summary scores, measured using the SF-12®, for parents of cCMV-positive (cCMV+) and cCMV-negative (cCMV-) children, with (LTI+) or without (LTI-) any long-term impairment.

Discussion

This study demonstrated that a delay in general development and in expressive language is more common in children with cCMV than in children without cCMV. Children with cCMV and symptoms at birth have poorer school performances compared to cCMV-negative children with symptoms at birth. These consequences of cCMV are reflected in the overall and physical quality of life, which is lower for cCMV-positive children with long-term impairment compared to their cCMV-negative counterparts. Moreover, the parents of children with cCMV and long-term impairment reported significantly more physical and concentration problems than parents of cCMV-negative children with long-term impairment.

Although it is known that congenital CMV infection is related to developmental delay, especially in those with symptoms at birth, [1, 5, 18] this study reveals new insights by looking at various domains of development. The delay in expressive language in children with cCMV is interesting since only 5 cCMV-positive children in this study (3 in the asymptomatic group) had been diagnosed with sensorineural hearing loss before the age of five or six years. This means that language development is impaired in cCMV-positive children even in the absence of hearing loss. Only a few studies looked at language development in asymptomatic children with cCMV of whom only a few had hearing loss. [5, 19] They found language impairment in 6.8 to 31.6% of these children.

In line with the problems in expressive language reported in the child development inventory, speech therapy was more frequently needed by children with cCMV, than by cCMV-negative children, although this difference was not statistically significant. The need of children with cCMV for physical therapy, both in the symptomatic and asymptomatic groups, was significantly higher than in children without cCMV. Although other authors reported that motor developmental delay occurs in children with cCMV, [6, 20] this area has received little attention up to now.

Furthermore, this is the first study to assess the quality of life of children and parents and the consequences for the daily life of parents in relation to their child's cCMV. The slightly, but statistically significant, lower quality of life in the cCMV-positive children in comparison to the cCMV-negative children with long-term impairment is certainly relevant, especially considering that the quality of life is usually subject to adaptation. [21, 22] Patients experiencing a disability generally give themselves higher quality of life ratings, presumably due to physical and psychological adaptation, compared to people imagining having such a disability. [23] Moreover, in another study parents of children with disabilities rated the quality of life of their children significantly higher than the physicians did. [24]

In addition to physical and concentration problems, parents of children with cCMV and long-term impairment reported a markedly lower mental quality of life compared to parents of cCMV-negative children with long-term impairment. The lack of statistical significance of this difference is probably due the relatively small number. However, in view of the abovementioned suggestion that the quality of life in studies is often overestimated due to adaptation, [21, 22] this difference is a relevant finding.

It is interesting to see that there is no difference within the group of children with cCMV and long-term impairment between those who were symptomatic at birth and those who were asymptomatic. This suggests that if children develop long-term impairment the developmental and daily life problems in symptomatic and asymptomatic children are equally severe. It is important to realize that, although the risk of long-term impairment is higher in the symptomatic children, the majority of children with cCMV and long-term impairment are asymptomatic at birth.

The design of this study has both advantages and disadvantages. Due to the inclusion of a cCMV-negative control group, this study can estimate the consequences attributable to cCMV. The retrospective design of this study enabled evaluation of the actual care that was provided, mostly without previous knowledge of the cCMV diagnosis, since only three parents were aware of the diagnosis prior to this study. On the other hand, there is a risk of recall and information bias, because the parental questionnaires were administered after the diagnosis of cCMV was communicated. This may have led to an overestimation of negative consequences in the cCMV-positive group and subsequently to an overestimation of the risk difference between the groups.

Conclusion

Children with congenital CMV face general developmental delay and expressive language delay more often than children without cCMV. Children with cCMV and long-term impairment, comprising 25% of all children with cCMV, have a lower quality of life. The parents of cCMV-positive children with long-term impairment experience more physical and concentration problems and their mental quality of life is lower, than parents of cCMV-negative children.

Overall, these findings indicate that a congenital CMV infection leads to a considerable burden of care for children and has an impact on the quality of life of both children and their parents. This study demonstrates that, besides the current regular medical care for children with cCMV, support for language and motor development is required and that family-centered care is essential. Moreover, these data can be used in discussions on the necessity of measures to attempt to prevent cCMV.

Acknowledgement

We thank all children and their parents for their participation in this study. We thank the schools for their cooperation. Furthermore, we thank persons who have contributed to this study including Jeroen Cremer and Lisette Rusman for testing the dried blood spots. We thank Marlies Weltevrede for her help in collecting and organizing the data.

Funding

This study was funded by Strategic Research of the National Institute of Public Health and the Environment.

Conflict of interest

All authors reported having no disclosures.

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Chapter 6

Healthcare costs attributable to congenital cytomegalovirus infection

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Submitted

Abstract

Importance

Congenital cytomegalovirus infection (cCMV) is the most common congenital infection worldwide. It can cause symptoms at birth as well as long-term impairment, including sensorineural hearing loss and developmental delay.

Objective

This study estimates cCMV-related healthcare costs in the Netherlands in the first six years of life.

Design, Setting, and Participants

In a nation-wide retrospective cohort study 156 children with cCMV were identified by testing 31,484 stored neonatal dried blood spots using polymerase chain reaction. Use of healthcare resources in the first six years of life by children with cCMV and a matched cCMV-negative control group was analyzed. Mean costs per child were calculated from a healthcare perspective, by multiplying healthcare resource use by its reference prices.

Exposure

Children with cCMV (exposed) were compared with children without cCMV (unexposed).

Main Outcome and Measures

The average total healthcare costs per child were calculated. The total costs were based on the average costs for hospital admissions and consultations by medical specialists, general practitioners and other healthcare providers.

Results

Medical data of 133 cCMV-positive and 274 cCMV-negative children were analyzed. Mean healthcare costs of children with cCMV (€6,113) were higher than those of cCMV-negative children (€3,570). The additional healthcare costs of cCMV were €2,544 (95% CI -€451, €5,538) per child in the first six years of life. When cCMV-positive children had symptoms at birth (€15,922), costs were more than four times higher than in asymptomatic cCMV-positive children (€3,730). In the Netherlands a total of €2.3 million could be attributed to cCMV for the whole birth cohort (184,634 children) of 2008 in the first six years of life.

Conclusions and Relevance

Children with cCMV, especially those symptomatic at birth, accrue higher healthcare costs than cCMV negative children in the first six years of life, although this is not statistically significant. Costs of special needs education, psychological healthcare and non-healthcare costs such as productivity losses of parents will further increase costs. In addition to the health impact, the economic impact needs to be taken into account in preventive measures aiming to reduce the prevalence and burden of cCMV.

Trial Registration

Dutch Trial Register: Identifier: NTR 3582.

Introduction

Cytomegalovirus (CMV) infections are endemic worldwide and generally cause no harm in immunocompetent individuals. [1] Congenital CMV infection (cCMV) is caused by vertical transmission of CMV from mother to fetus. About 10 to 15% of children with cCMV have symptoms at birth including hepatosplenomegaly, microcephaly and intracranial calcifications. [2, 3] Children with cCMV, both with and without symptoms at birth, can develop long-term impairments such as sensorineural hearing loss [4, 5], cognitive and motor developmental delay [2, 6] and visual impairment [7, 8]. The birth prevalence of cCMV varies between 0.2% and 6% globally [3, 9] and is 0.5% in the Netherlands. [10, 11]

The economic impact of cCMV and its impact on the healthcare system may be substantial. The cCMV-related symptoms at birth and long-term impairment are expected to result in a significant demand on the healthcare system, both during childhood and later in life. Moreover, severe and permanent cCMV-related health problems may lead to reduced lifetime productivity. It has been estimated that four billion dollars of healthcare costs, for 40,000 children with cCMV and 2800 transplant patients, could be saved annually in the United States of America if a 100% effective vaccine against CMV would be available with a 100% uptake. [12]

Previously, we assessed the clinical consequences of cCMV in the Netherlands. [13] In this nation-wide retrospective cohort study, medical data of the first six years of life of children with cCMV and without cCMV were compared. We found that 19.6% of children with cCMV and 12.4% of children without cCMV had symptoms at birth. Long-term impairment was seen in 24.8% of children with cCMV compared to 12.0% in those without cCMV. [13]

This nation-wide cohort was used to investigate cCMV-related healthcare use and related costs in the first six years of life. This is the first study that directly estimates the costs of healthcare for children with cCMV. The retrospective design of this study allowed us to evaluate the care that was actually sought and provided, while generally parents and healthcare providers were unaware of the cCMV-status of the child. The information provided by this study can be used to estimate the impact of cCMV on the healthcare system and to support policy-makers in their ongoing discussions on cost-effectiveness of preventive strategies.

Methods

Study design and participants

The CROCUS-study is a nation-wide retrospective cohort study aimed to assess the prevalence of cCMV and its long-term consequences in the first six years of life in the Netherlands. The study design has been published elsewhere [11]. In short, parents of 73,693 children, born between January 1 and September 30 2008, living in the Netherlands were invited to participate in the study between October 2012 and January 2013. In the first part of this study neonatal dried blood spots of 31,484 children were tested retrospectively by polymerase chain reaction for CMV DNA and 156 children with cCMV were detected. Of these, only four children had been diagnosed with cCMV prior to this study. From the cCMV-negative children a control group was selected, matched for sex, month of birth and postal code region. Parents of 133 cCMV-positive children and 274 cCMV-negative children gave informed consent for collection of medical data from general practitioners, medical specialists and other healthcare providers. Parents were asked to list all healthcare providers who had seen their child. Between May 2014 and January 2015 requests for medical records or information concerning hospital admissions and outpatient clinic visits, sent to these healthcare providers and the requested information was received.

Collection of data on healthcare resource use

All collected medical data were entered in the study database without knowing whether the child in question had cCMV or not. The number of consultations with the various healthcare providers were recorded. Additionally, the number of inpatients days and number of specific diagnostic procedures and interventions during hospital admissions or at the outpatient clinic were registered. Afterwards the cCMV-status and categorization of patients as being symptomatic or asymptomatic at birth and as having long-term impairments or not, were added to the database for analysis. Being symptomatic at birth was defined as having one or more specific signs or symptoms in the first four weeks of life. These were preterm birth, being small for gestational age, microcephaly, hepatomegaly or splenomegaly, generalized petechiae or purpura, seizures and hypotonia, thrombocytopenia, neutropenia, elevated alanine transaminase, conjugated hyperbilirubinemia, neonatal sensorineural hearing loss, cranial ultrasound abnormalities and ophthalmological abnormalities. Long-term impairments included sensorineural hearing loss, permanent visual impairment, neurological impairment (epilepsy, cerebral palsy, microcephaly, attention deficit-hyperactivity disorder and autism spectrum disorder) and moderate to severe motor, cognitive or speech-language developmental delay.

Healthcare reference prices

This study was carried out from a healthcare perspective. Reference prices for consultations with healthcare providers, hospital admissions and outpatient visits were obtained from the "Dutch Manual for Costing: Methods and Reference Prices for Economic Evaluations in Healthcare" (from here on referred to as the manual) [14]. These reference prices from 2009 were converted into 2013 Euros according to the consumer price index [15]. Medication costs were not included in our analyses because no expensive medicines had been prescribed and medication costs in this study were generally low. In those cases where the manual did not provide reference prices, other references were used, the reference prices of

similar activities were extrapolated, or prices were obtained from the previous version of the manual [16] or from the financial department of a University Medical Center. Reference prices of outpatient clinic visits and inpatient days, as provided by the manual [14], included costs of medical personnel, materials, building maintenance, overheads and equipment and also nutrition and medication in the case of hospital admissions. However, diagnostic procedures and interventions were not included in these reference prices. Separate reference prices of diagnostic procedures, as determined for January 2013, were obtained from the website of The Dutch Healthcare Authority [17]. Simple laboratory tests, such as hematology and biochemistry tests, were not taken into account because costs were generally low. Reference prices for individual healthcare providers were added up to calculate the costs of multidisciplinary consultations. Further details on the reference healthcare prices are displayed in Supplement 6.1.

Data analysis and missing data handling

Healthcare costs were calculated per child by multiplying the healthcare resource use by their reference prices.

It was assumed that all children who needed healthcare were first seen by the general practitioner, since referral by a general practitioner is required for appointments with medical specialists or other healthcare providers in the Netherlands. If the exact number of consultations with the physical therapist or speech therapist was not clear from the medical data, this was estimated on the basis of the condition and severity of the disorder. These estimates were provided by a healthcare provider working in this specific field and are shown in supplement 6.2. When no data were available concerning visits to the general practitioner or other healthcare providers mentioned by parents, multiple imputation (25 imputations) was used to estimate the number of visits. This was based on sex, date of birth, cCMV-status and the recorded number of visits to healthcare providers, number of days of hospital admissions and costs of diagnostic procedures and interventions.

The mean healthcare costs in the first six life years of the cCMV-positive and cCMV-negative children were compared using a t-test, which is an appropriate statistical method for comparing cost data [18]. A pooled t-test analysis was performed on the data estimated by multiple imputation. Furthermore, analyses were performed to investigate the healthcare costs for children who were symptomatic or asymptomatic at birth and those with and without long-term impairments.

To assess the impact of cCMV on the Dutch healthcare system, the additional healthcare costs per child attributable to cCMV were extrapolated to the Dutch birth cohort of 2008, comprising 184,634 live-born children [19]. All statistical analyses were performed using SAS version 9.3.

Ethical and legal issues

This study is registered in the “Dutch Trial Register” (NTR 3582). It was approved by the medical ethics committee of the Leiden University Medical Center in Leiden. The authors declare compliance with the Helsinki Declaration of 1975, as revised in 2008, regarding all procedures during this study.

Results

Baseline data and collected data

Parents of 133 children with cCMV and 274 children without cCMV gave informed consent for collection of their children's medical data. Baseline data of these children are presented in Table 6.1.

Detailed clinical consequences were described elsewhere. [13] The collected medical data included general practitioner files of 95% of the participating children. Data were available for 100% of children whose parents indicated that they had attended a medical specialist. Data were obtained of 96% of children attending a speech therapist or a physical therapist. Data were available of 89% of children whose parents noted that they had attended a speech and hearing center. The amount of missing data did not differ between the cCMV-positive and cCMV-negative groups.

Table 6.1 - Baseline characteristics of children with and without cCMV

	cCMV-positive (n = 133)	cCMV-negative (n = 274)
Age on 1-1-2014 (years)	5.6 ± 0.2	5.6 ± 0.2
Female sex	58 (43.6%)	125 (45.6%)
Symptomatic at birth*	26 (19.5%)	34 (12.4%)
- With long-term impairment	14 (53.8%)	3 (8.8%)
- Without long-term impairment	12 (46.2%)	31 (93.4%)
Long-term impairment**	33 (24.8%)	33 (12.0%)
- With symptoms at birth	14 (42.4%)	3 (9.1%)
- Without symptoms at birth	19 (57.6%)	30 (90.9%)

Data are presented as number (%) or mean ± standard deviation

* Any of the following in the first four weeks of life:

preterm birth, being small for gestational age, microcephaly, hepatomegaly or splenomegaly, generalized petechiae or purpura, seizures and hypotonia, thrombocytopenia, neutropenia, elevated alanine transaminase, conjugated hyperbilirubinemia, neonatal sensorineural hearing loss, cranial ultrasound abnormalities or ophthalmological abnormalities.

** Any of the following impairments in the first six years of life:

sensorineural hearing loss, permanent visual impairment, neurological impairment (epilepsy, cerebral palsy, microcephaly, attention deficit-hyperactivity disorder, and autism spectrum disorder) and moderate to severe motor, cognitive or speech-language developmental delay

Healthcare costs

Mean healthcare costs during the first six years of life of children with and without cCMV are presented in Table 6.2. Mean healthcare costs per child were €6,113 for children with cCMV compared to €3,546 for cCMV-negative children, a difference of €2,568 with a 95% confidence interval (CI) from -€418 to €5,553. Especially physical therapists and speech therapists were attended more often by cCMV-positive children, and the mean healthcare costs for these types of care were statistically significantly higher in the cCMV-positive group compared to the cCMV-negative children.

Table 6.2a - Overview of healthcare costs of cCMV-positive and cCMV-negative children in the first six years of life (in 2013 €)

	cCMV-positive (n = 133)				cCMV-negative (n = 274)			
	% children ^a	mean visits/days ^b	mean costs/child ^c	SD mean costs	% children ^a	mean visits/days ^b	mean costs/child ^c	SD mean costs
Hospital admissions								
- Pediatric admissions ¹	45,9%	8,1 d	€1,901	€5183	36,5%	6,8 d	€1,283	€4295
- Neonatal admissions ²	13,5%	9,1 d	€1,693	€10298	16,8%	8,5 d	€941	€3742
Medical specialists								
- Outpatient clinic visits ³	75,2%	9,7	€701	€1300	71,9%	7,8	€492	€1093
General practitioner								
- All GP-visits ⁴	100%	15,9	€575	€504	100%	16,4	€606	€573
Healthcare providers								
- Physical therapy	22,6%	31,2	€275	€942	11,3%	12,3	€55	€177
- Speech therapy	24,8%	55,9	€498	€1887	16,1%	21,3	€123	€453
Healthcare facilities								
- Speech/hearing center	12,8%	7,5	€82	€338	3,3%	9,8	€45	€362
- Rehabilitation center	3,0%	346,8	€391	€3091	0,7%	4,5	€2	€28
Total costs	100%		€6,113	16678	100%		€3,546	€7376

1) hospital admission to a pediatric ward; 2) hospital admission to a neonatal ward or neonatal intensive care unit; 3) visits to outpatient clinic and hospital emergency room, mean costs also include diagnostic procedures and medical interventions; 4) visits to general practitioner (GP) including out-of-hours visits to a general practitioner clinic.

a) % children: percentage of children of the whole group visiting a specific healthcare provider; b) mean visits or days: average number of visits by the children visiting the specific healthcare provider or the average number of days (d) of hospital admission; c) mean cost per child: average cost of specific healthcare based on the total costs divided by the total number of children in the whole group (cCMV+ or cCMV-); SD: standard deviation

Table 6.2b - Overview of healthcare costs of cCMV-positive and cCMV-negative children in the first six years of life (in 2013 €)

	cCMV+ vs cCMV- Mean difference	95% CI
Pediatric admissions	€618	-€408, €1644
Neonatal admissions	€752	-€1075, €2578
Outpatient clinic visits	€209	-€49, €466
All GP-visits	-€32	-€148, €85
Physical therapy	€220*	€56, €384
Speech therapy	€375*	€48, €703
Speech/hearing center	€37	-€37, €111
Rehabilitation center	€389	-€143, €921
Total costs	€2,568	-€418, €5553

95% CI: 95% confidence interval; * Statistically significant ($p < 0.05$)

Children with and without symptoms at birth

In both the cCMV-positive and cCMV-negative children, the healthcare costs were much higher in children with symptoms at birth compared to children who were asymptomatic at birth (Figure 6.1, Table 6.3, Supplement 6.3). As to be expected, a large part of the average total healthcare costs, more than €5,000, in these children with symptoms at birth was due to hospital admissions for neonatal care. In cCMV-positive children with symptoms at birth costs for physical therapy, speech therapy and rehabilitation center visits were noticeably higher compared to children without cCMV who had symptoms at birth. However, these differences were not statistically significant. In children without symptoms at birth, the costs for physical therapy were significantly higher for children with cCMV than for their cCMV-negative counterparts (mean difference: €110, 95% CI €8-€212).

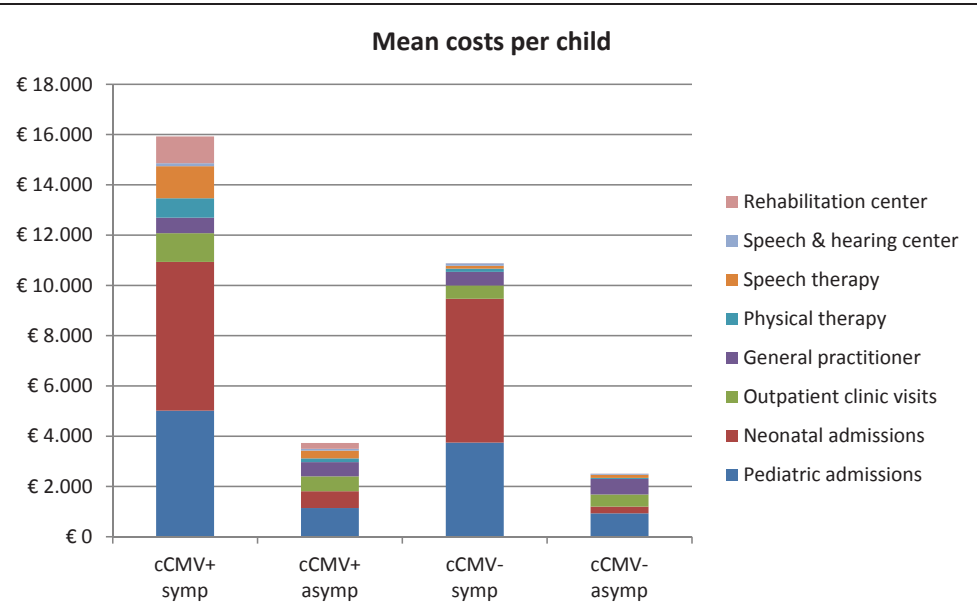


Figure 6.1 - Mean costs per child (in 2013 €) for children with (cCMV+) and without cCMV (cCMV-) who were symptomatic (symp) or symptomatic (asymp) at birth.

About half of the total costs of children with cCMV were attributable to the small group (20%) of children with symptoms at birth, while the other half was accounted for by the 80% asymptomatic children. In children without cCMV the 12% symptomatic children incurred almost 40% of the total costs (Supplement 6.5).

Children with and without long-term impairment

The average healthcare costs of children with long-term impairment were seven times higher in cCMV-positive children and three times higher in cCMV-negative children compared to children without long-term impairments (Figure 6.2, Table 6.3, Supplement 6.4). Children with long-term impairment and cCMV had higher costs for physical therapy compared to children with long-term impairment without cCMV (mean difference: €801, 95% CI €178-€1423). Also the costs for neonatal hospital admissions and attending a rehabilitation center and speech therapy were higher in these cCMV-positive children, but this was not statistically significant.

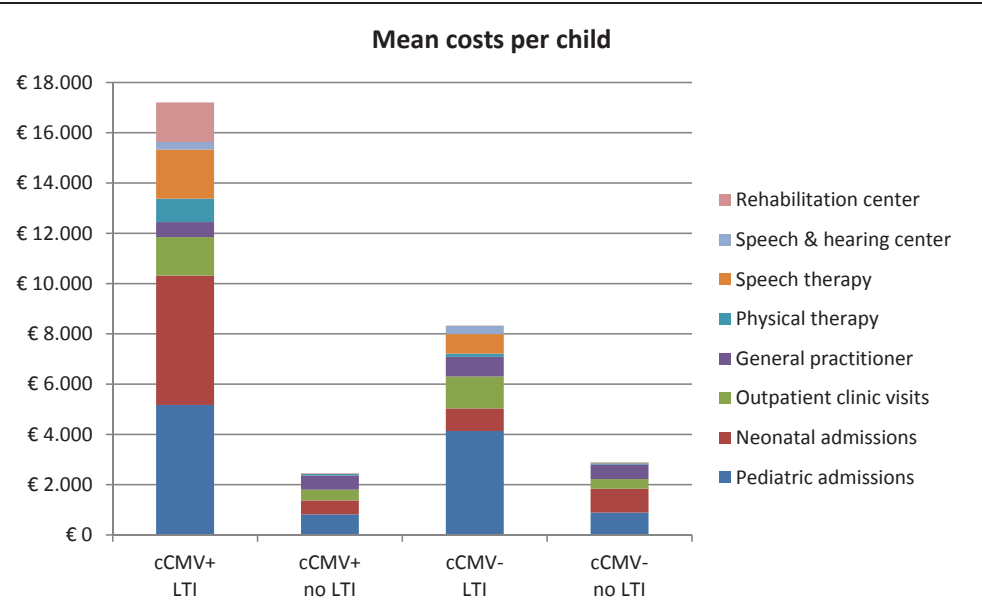


Figure 6.2 - Mean costs per child (in 2013 €) for children with (cCMV+) and without cCMV (cCMV-) who developed long-term impairment (LTI) or did not develop long-term impairment (no LTI).

In cCMV-positive children, 70% of the total costs were incurred by the 25% of children with long-term impairment in this group. In children without cCMV, the 12% children with long-term impairment accounted for approximately 30% of the total costs. An important part (43%) of the costs of cCMV-positive children were incurred by the 11% of children who were symptomatic at birth and had long-term impairments (Supplement 6.5).

Table 6.3 - Mean difference in mean total costs per child in 2013 € for children with and without cCMV

	cCMV-positive (n = 133)		cCMV-negative (n=274)		Mean difference	95% CI
	Mean	SD	Mean	SD		
Symptomatic at birth	€15,922	€32,530	€10,878	€16,697	€5,044	-9,213, 19,302
Asymptomatic at birth	€3,730	€7,739	€2,507	€3,736	€1,223	-330, 2,777
Long-term impairment	€17,205	€30,034	€8,332	€14,652	€5,044	-2,924, 20,671
No long-term impairment	€2,453	€4,307	€2,890	€5,378	-€437	-1,529, 655

SD: standard deviation, 95% CI: 95% confidence interval

Costs of congenital cytomegalovirus infection

Based on the birth rate of 184,634 live-born children in 2008 and the birth prevalence of cCMV of 0.5% in the Netherlands, an estimated 915 children were born with cCMV in that year in the Netherlands. The total healthcare costs of children with cCMV during the first six years of life would add up to approximately 5.6 million euro (3.0 million euro/100,000 children). Approximately half of these costs would be attributable to the approximately 80% children with cCMV who are asymptomatic at birth. The additional national healthcare costs during the first six years of life, attributable to cCMV, for this year cohort were estimated at 2.3 million euro (1.3 million euro/100,000 children).

Discussion

This is the first cost-of-illness study on cCMV using real-life data from a cCMV-positive cohort and a matched cCMV-negative control group to estimate the attributable costs of cCMV. It demonstrated that children with cCMV have higher average healthcare costs in the first six years of life than cCMV-negative children. The difference in total healthcare costs between these groups, although statistically not significant, is more than €2,500 per child. When extrapolated to the Dutch birth cohort of 2008 this means that 2.3 million euro healthcare costs in the first six years of life could be attributed to cCMV in the Netherlands. The total costs of children with cCMV in this birth cohort would add up to 5.6 million euro for the first six years of life. Although the costs in cCMV-positive children are highest in children with long-term impairment (70% of the total costs), especially in those who also had symptoms at birth (43% of the total costs), the large and usually unrecognized group of children with cCMV who are asymptomatic at birth are responsible for half of the costs

Estimates of costs of cCMV are scarce and up to now these were all based on assumptions of the prevalence of impairments and the costs associated with these impairments. For example the estimated costs of cCMV and CMV-related morbidity of 4 billion United States dollars per year, calculated on an expected 40,000 cCMV-positive newborn infants (assuming a 1% birth prevalence) and 2800 transplant patients per year in the United States were based on general assumptions concerning hospitalization, outpatient visits and special schooling expenses. [12] The few other studies that have been conducted on the costs of cCMV, were mostly cost-effectiveness studies on the effect of preventive measures such as targeted neonatal screening [20, 21] and vaccination [22]. These studies did not aim to estimate the total costs of cCMV and most of these cost-effectiveness studies focused primarily on the costs of hearing loss, since sensorineural hearing loss is frequently seen in children with cCMV. [4]

Costs of illness studies are difficult to compare, due to differences in design and calculation methods. However, a rough indication of the costs of different illnesses in the Netherlands can be obtained from the Cost of illness tool. [23] To put our results into perspective, the results of this study were compared to the costs for congenital disorders of the central nervous system in children 0 to 6 years in the Netherlands in 2011. These costs are estimated to be around 3.5 million euro (in 2011 euros), which is noticeably lower than the estimated costs of cCMV. [24]

It is important to realize that costs are now estimated for the first six years of life, in one birth cohort. These figures will generally be applicable to the other birth cohorts as well. The total healthcare cost of cCMV for children up to six years of age are therefore assumed to be around 5.6 million euro every year and the costs are presumably even higher when older children and adults are taken into account.

Even though the difference in average healthcare costs between the cCMV-positive and cCMV-negative children is quite large (€2,500), it is statistically not significant. This is due to the large variance in costs, which is probably the result of differences in disease severity and the relatively small number of children availing of specific care types (e.g. rehabilitation center).

Moreover, there are several reasons to presume that the actual difference in healthcare costs between the cCMV-positive and cCMV-negative children is larger than found in this study. First of all, children who died of cCMV in the first five years of life were not included in this study as inclusion started at five years of age. The healthcare costs of these children before they died could have been substantial.

Second, this study estimated the healthcare costs only up to six years of age. When this cohort will be followed-up for longer the difference between cCMV- positive and cCMV-negative children might become even larger. The onset of some cCMV-related sequelae, such as sensorineural hearing loss can occur up to, or even after, the age of six years. [25] Consequently, more healthcare costs can be incurred later in these children's lives. For example, the cost of a pediatric cochlear implant is considerable (estimated lifetime cost: €82,000 - €108,000). [26]

Importantly, cCMV-related sequelae are often permanent, leading to ongoing healthcare costs, while in the general population most healthcare costs in children are usually made in the first five years of life. [27]

Moreover, specific data about visits to, for example, dieticians and psychosocial care centers, were often not available because they were not mentioned by the parents and consequently no medical data was collected, even though it was clear from other medical records that this type of care had been provided. As cCMV is associated with poorer cognitive development [2] and potentially with neuro-behavioral problems such as autism, [28, 29] lack of detailed data on psychological healthcare in this study means that the overall health costs, specifically of cCMV-positive children, would probably be higher. All in all, it is likely that the total healthcare costs of children with cCMV are underestimated in this study.

Finally, it should be noted that this study only looked at healthcare costs. Other costs related to the impairment of children with cCMV, such as special needs education, [30] future reduced productivity and potential productivity loss of parents, were not taken into account.

Conclusion

This study shows that healthcare costs are higher in children with cCMV, albeit not statistically significantly, compared to cCMV-negative children. In cCMV-positive children, the highest costs were incurred by the 11% of children with long-term impairment and symptoms at birth, who are responsible for 43% of the total costs. However, half of the total costs in children with cCMV are caused by children who are asymptomatic at birth. Moreover, the real healthcare and other costs are probably even higher in the cCMV-positive group, because costs for psychosocial healthcare and special needs education were not taken into account in this study. This study provides crucial information essential for discussions on the necessity of programs to reduce or prevent the burden of cCMV.

Acknowledgment

We thank all the parents and children who participated in this study. We appreciate the cooperation of the general practitioners, medical specialists, healthcare providers, school teachers, and Regional Public Health Services. We acknowledge the work of Lisette Rusman and Jeroen Cremer in testing the dried blood spots.

Conflict of Interest Disclosures:

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Funding/Support:

This study was funded by Strategic Research of the National Institute of Public Health and the Environment.

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Supplement 6.1 - Reference prices of Consultations per Healthcare Provider (in 2013 €)

Reference prices of consultations with healthcare professionals	Reference price	Reference
Reference prices of General Practitioners consultations		
General Practitioner's visit (10 minutes)	30.48	[14]
General Practitioner's house call	46.81	[14]
General Practitioner's consultation by telephone	15.24	[14]
Out-of-hours GP visit	151.97	a
Out-of-hours GP consultation by telephone	75.90	a
Reference prices of outpatient-clinic visits		
Outpatient clinic visit (general hospital)	69.67	[14]
Outpatient clinic visit (university hospital)	140.42	[14]
Outpatient clinic visit (unknown type of hospital)	78.37	[14]
Outpatient clinic telephone consultation (general hospital)	34.84	b
Outpatient clinic telephone consultation (university hospital)	70.21	b
Outpatient clinic telephone consultation (unknown type hospital)	39.69	b
Outpatient treatment (one-day admission)	320.18	[14] c
Emergency room visit	164.37	[14]
Emergency room telephone consultation	82.19	b
Specialized Skeletal dysplasia team (university hospital)	460.45	d
Reference prices of hospital admissions – per day		
Inpatient day (general hospital)	473.51	[14]
Inpatient day (university hospital)	625.91	[14]
Inpatient day (unknown type of hospital)	497.46	[14]
Inpatient day (Burn Center)	971.81	[31]
Neonatal or pediatric Intensive Care Unit (university hospital)	2376.28	[14]
Reference prices per visit to an allied health professional		
Physiotherapist (30 minutes)	39.19	[14] e
Speech therapist (30 minutes)	35.92	[14] e
Remedial therapist (30 minutes)	38.10	[14] e
Occupational therapist (30 minutes)	11.98	[14]
Orthoptist (30 minutes)	27.98	f
Reference prices per discipline in speech and hearing center		
Audiologist (15 minutes)	78.37	g
Clinical linguist (15 minutes)	78.37	g
Speech-language pathologist (15 minutes)	78.37	g
Ear, Nose and Throat specialist (15 minutes)	78.37	[14]
Cochlear Implant coordinator/physician (15 minutes)	78.37	[14]
Speech therapist (30 minutes)	35.92	[14]
Acoupedist (30 minutes)	35.92	h
Rehabilitation therapist (30 minutes)	35.92	h
Physiotherapist (30 minutes)	39.19	[14]
Occupational therapist (30 minutes)	11.98	[14]
Audiology assistant (30 minutes)	11.98	i
Reference prices rehabilitation center		
Visit to a rehabilitation specialist	69.67	[14]
Visit to rehabilitation center therapists. such as physiotherapist, occupational therapist. speech therapist (60 minutes)	119.74	[14]

a No reference prices were available for out-of-hours GP consultations. The Dutch Healthcare Authority states tariffs of €8.67 and of €43.15, for standard GP consultations and out-of-hours consultation [32], respectively, indicating that out-of-hour consultations are 4.98 times higher than standard GP consultations. Therefore reference prices for GP consultations as stated in Hakkaart et al. [14] were multiplied by 4.98, resulting in an assumed reference price of €151.97 for out-of-hours GP visits and of €75.90 for out-of-hours GP consultation by telephone.

b In line with GP reference prices of general practitioners, telephone consultations by the outpatient clinic and emergency room were assumed to cost half of the reference price of outpatient clinic and emergency room visits.

c The reference price for outpatients' treatment is based on the reference price as provided by the Dutch Manual for Costing in Healthcare Economic Evaluations. This reference price does not include fees for physicians, therefore additional weighted reference prices for time spent by the medical specialist and residents are added.

d One child had visited a specialized team in a University hospital because of skeletal dysplasia. This team consists of an orthopedic surgeon, a medical revalidation specialist, a physiotherapist and a pediatrician. The reference price of per consultation was calculated by adding reference prices of these medical specialists and the physical therapist, resulting in a reference price of $(3 \times 140.42) + (1 \times 39.19) = 460.45$ per consultation.

e Reference prices were stated in the manual per session for physical therapist, speech therapists, and remedial therapists. After contacting the Institute for Medical Technology Assessment, who had developed the Manual, we were informed that duration of an average session with these therapists is half an hour.

f No reference prices were available for consultations with an orthoptist. Since the level of education is equal to other allied health professionals, we assumed the mean reference prices per half hour for physical therapist, speech therapist, remedial therapist, occupation therapist and dietician. In addition, we assumed the duration of consultations with an orthoptists to be half an hour.

g No reference prices were available for consultations with audiologists, clinical linguists and speech-language pathologists. Since the education level of these professions is similar to the education level of medical specialists, the same rate has been assumed for these professions. Following personal communication with a speech and hearing center the duration of an audiologist's consultation was calculated as lasting fifteen minutes, hearing diagnostics as one hour and diagnostics by a speech therapist as two and a half hours.

h No reference price was available for consultations of an acoupedist and rehabilitation therapist, however, these professionals are all specialized speech therapists and therefore the reference price of a speech therapist is applied here.

i No reference price was available for a consultation with an audiologist assistant. Since this profession has a lower education level than the professions of the allied health professionals, we assumed the lowest reference price of allied health professionals, the reference price of an occupational therapist, which is €11.98 per half an hour.

Supplement 6.2 - Assumed numbers of consultations per diagnosis for speech therapy and physical therapy

Physical therapist	Assumed number consultations		
Behavioral and movement disorders (e.g. developmental coordination disorder, attention deficit-hyperactivity disorder, autism spectrum disorder)	20		
Balance disorders	20		
Hypermobility	20		
Hypertonicity	20		
Urine or fecal Incontinence	10		
KISS Syndrome (Kinematic Imbalances due to Suboccipital Strain)	9		
Mild restraint of abduction upper arm	18		
Hypertonicity (newborn)	12		
Proprioceptive problems	18		
Scoliosis	18		
Sensory processing disorder - not otherwise specified	18		
Developmental delay - fine motor skills	18		
Developmental delay - gross motor skills	18		
Urge incontinence	10		
Positional plagiocephaly	10		
Speech therapist	Assumed number consultations		
	Mild	Moderate	Severe
Articulation disorder - not otherwise specified	8	20	40
Lisping	8	16	24
Stuttering or speech dysfluency	12	20	40
Auditory processing disorder	12	16	24
Expressive language disorder	12	24	40
Hearing impairment		16	
Hyper salivation		8	

A disorder was classified as mild, moderate or severe based on the medical information from the speech therapist; when no statements were made about disease severity it was assumed to be moderate. All medical data were classified by one author (MK) and checked by a second author (AO).

Supplement 6.3 - Overview of healthcare costs (in 2013 €) in the first six years of life of cCMV-positive and cCMV-negative children with or without symptoms at birth

cCMV-positive (n = 133)								
	Symptomatic at birth (n = 26)				Asymptomatic at birth (n = 107)			
	% ^a	mean visits ^b	mean costs ^c	SD ^d	% ^a	mean visits ^b	mean costs ^c	SD ^d
Hospital admission								
- Pediatric admissions 1	65,4	14,8 d	€ 5,016	9,956	41,1	5,5 d	€ 1,144	2,531
- Neonatal admissions 2	30,8	11,8 d	€ 5,918	20,792	9,3	7,0 d	€ 666	4,638
Medical specialists								
- Outpatient clinic visits 3	84,6	14,1	€ 1,140	2,175	72,9	8,4	€ 594	946
General practitioner								
- All GP visits 4	100	16,6	€ 619	521	100	15,8	€ 564	500
Healthcare providers								
- Physical therapy	42,3	46,6	€ 772	1,769	17,8	22,2	€ 154	519
- Speech therapy	42,3	84,0	€ 1,277	3,607	20,6	41,8	€ 309	1,043
Healthcare facilities								
- Speech & hearing center	19,2	7,6	€ 120	372	11,2	7,4	€ 72	328
- Rehabilitation center	3,8	737,0	€ 1,060	5,306	2,8	216,7	€ 228	2,215
Total costs			€ 15,922	32,530			€ 3,730	7,739
cCMV-negative (n = 274)								
	Symptomatic at birth (n = 34)				Symptomatic at birth (n = 34)			
	% ^a	mean visits ^b	mean costs ^c	SD ^d	% ^a	mean visits ^b	mean costs ^c	SD ^d
Hospital admission								
- Pediatric admissions 1	44,1	18,5 d	€ 3,942	10,850	35,4	4,9 d	€ 934	2,524
- Neonatal admissions 2	52,9	16,3 d	€ 5,719	8,824	11,7	3,4 d	€ 264	1,129
Medical specialists								
- Outpatient clinic visits 3	85,3	8,5	€ 534	733	70,0	7,7	€ 486	1,135
General practitioner								
- All GP visits 4	100	14,1	€ 537	423	100	16,7	€ 616	591
Healthcare providers								
- Physical therapy	26,5	12,3	€ 128	255	9,2	12,3	€ 44	160
- Speech therapy	17,6	18,2	€ 115	313	15,8	21,7	€ 124	470
Healthcare facilities								
- Speech & hearing center	5,9	17,5	€ 95	513	2,9	7,5	€ 38	335
- Rehabilitation center	2,9	1,0	€ 2	13	0,4	8,0	€ 2	30
Total costs			€ 10,878	16,697			€ 2,507	3,736

1) hospital admission to a pediatric ward; 2) hospital admission to a neonatal ward or neonatal intensive care unit; 3) visits to outpatient clinic and hospital emergency room, mean costs also include diagnostic procedures and medical interventions, 4) visits to general practitioner including out-of-hours general practitioner clinic visits. a) %: percentage of children of the whole group visiting a specific healthcare provider; b) mean visits: average number of visits by the children visiting the specific healthcare provider or the average number of days (d) of hospital admission; c) mean costs: average costs of specific healthcare based on the total costs divided by the total number of children in the whole group; d) SD: standard deviation of average cost of specific type of healthcare.

Supplement 6.4 - Overview of healthcare costs (in 2013 €) in the first six years of life of cCMV-positive and cCMV-negative children with or without long-term impairment

cCMV-positive (n = 133)								
	Any long-term impairment (33)				No long-term impairment (100)			
	% ^a	mean visits ^b	mean costs ^c	SD ^d	% ^a	mean visits ^b	mean costs ^c	SD ^d
Hospital admission								
- Pediatric admissions	66,7	15,5d	€ 5.173	€ 8.905	39,0	3,9d	€ 821	€ 2.211
- Neonatal admission	21,2	14,3d	€ 5.143	€ 19.515	11,0	5,8d	€ 554	€ 3.203
Medical specialists								
- Outpatient clinic visits	87,9	17,8	€ 1.536	€ 2.169	71,0	6,3	€ 425	€ 625
General practitioner								
- All GP visit	100	15,8	€ 595	€ 568	100	16,0	€ 568	€ 481
Healthcare providers								
- Physical therapy	51,5	46,1	€ 928	€ 1,712	13,0	11,6	€ 59	€ 166
- Speech therapy	72,7	74,8	€ 1,954	€ 3,392	9,0	5,4	€ 17	€ 111
Healthcare facilities								
- Speech & hearing center	39,4	8,9	€ 301	€ 615	4,0	2,8	€ 9	€ 76
- Rehabilitation center	12,1	346,8	€ 1,575	€ 6,056	0		€ 0	€ 0
Total costs			€ 17,205	€ 30,034			€ 2,453	€ 4,307
cCMV-negative (n = 274)								
	Any long-term impairment (33)				Any long-term impairment (33)			
	% ^a	mean visits ^b	mean costs ^c	SD ^d	% ^a	mean visits ^b	mean costs ^c	SD ^d
Hospital admission								
- Pediatric admissions	63,6	63,6	63,6	63,6	63,6	63,6	63,6	63,6
- Neonatal admission	12,1	12,1	12,1	12,1	12,1	12,1	12,1	12,1
Medical specialists								
- Outpatient clinic visits	78,8	78,8	78,8	78,8	78,8	78,8	78,8	78,8
General practitioner								
- All GP visit	100	100	100	100	100	100	100	100
Healthcare providers								
- Physical therapy	30,3	30,3	30,3	30,3	30,3	30,3	30,3	30,3
- Speech therapy	63,6	63,6	63,6	63,6	63,6	63,6	63,6	63,6
Healthcare facilities								
- Speech & hearing center	15,2	14,0	€ 325	€ 975	1,7	4,4	€ 6	€ 84
- Rehabilitation center	6,1	4,5	€ 16	€ 80	0		€ 0	€ 0
Total costs			€ 8.332	€ 14.652			€ 2,890	€ 5,378

1) hospital admission to a pediatric ward; 2) hospital admission to a neonatal ward or neonatal intensive care unit; 3) visits to outpatient clinic and hospital emergency room, mean costs also include diagnostic procedures and medical interventions, 4) visits to general practitioner including out-of-hours general practitioner clinic visits. a) %: percentage of children of the whole group visiting a specific healthcare provider; b) mean visits: average number of visits by the children visiting the specific healthcare provider or the average number of days (d) of hospital admission; c) mean costs: average costs of specific healthcare based on the total costs divided by the total number of children in the whole group; d) SD: standard deviation of average cost of specific type of healthcare.

Supplement 6.5 -Total healthcare costs for children with and without cCMV and the relative contribution of the different subgroups

		cCMV-positive (n = 133)					
		number children	% of total number	total costs	% of total costs	mean cost	standard deviation
Symptomatic	Total	26	20%	€ 413,967	51%	€ 15,922	€ 32,530
Asymptomatic	Total	107	80%	€ 399,109	49%	€ 3,730	€ 7,739
Long-term imp.	Total	33	25%	€ 567,759	70%	€ 17,205	€ 30,034
No long-term imp.	Total	100	75%	€ 245,317	30%	€ 2,453	€ 4,307
Long-term imp.	Sympt.	14	11%	€ 347,927	43%	€ 24,852	€ 41,550
	Asympt.	19	14%	€ 219,833	27%	€ 11,570	€ 14,898
No long-term imp.	Sympt.	12	9%	€ 66,040	8%	€ 5,503	€ 8,913
	Asympt.	88	66%	€ 179,277	22%	€ 2,037	€ 2,972
Total		133		€ 813,076			
		cCMV-negative (n = 274)					
		number children	% of total number	total costs	% of total costs	mean cost	standard deviation
Symptomatic	Total	34	12%	€ 369,843	38%	€ 10,878	€ 16,697
Asymptomatic	Total	240	88%	€ 601,634	62%	€ 2,507	€ 3,736
Long-term imp.	Total	33	12%	€ 274,942	28%	€ 8,332	€ 14,652
No long-term imp.	Total	241	88%	€ 696,536	72%	€ 2,890	€ 5,378
Long-term imp.	Sympt.	3	1%	€ 97,069	10%	€ 32,356	€ 34,448
	Asympt.	30	11%	€ 177,873	18%	€ 5,929	€ 7,440
No long-term imp.	Sympt.	31	11%	€ 272,774	28%	€ 8,799	€ 11,972
	Asympt.	210	77%	€ 423,761	44%	€ 2,018	€ 2,478
Total		274		€ 971,478			

The total number and percentage of children, the total costs and percentage of total cost and the mean costs and standard deviations are displayed for the subgroups with and without symptoms at birth, the children with and without long-term impairment and for the subgroups of children with or without symptoms at birth who develop long-term impairment or those who do not develop long-term impairment. Sympt: symptomatic, Asympt: asymptomatic, imp.: impairment

Chapter 7

General Discussion

General Discussion

In this chapter, first the main findings of the CROCUS (Consequences and Risk factors Of congenital Cytomegalovirus infection) study are summarized. Then, the methodological challenges and advantages of this study are considered. This is followed by a reflection on the potential preventive measures against congenital cytomegalovirus infections (cCMV), which have been described in the introduction chapter of this thesis. Finally, the implications of this study for the care of children with cCMV are discussed and suggestions are given for future studies.

Consequences of congenital cytomegalovirus infection

The results of the CROCUS study show that cCMV leads to a considerable disease burden. cCMV is relatively common and it can have substantial clinical consequences. It can influence the child's development and school performance and it can also affect the quality of life. Additionally, cCMV has an economic impact on the healthcare system.

Prevalence of cCMV and morbidity (Figure 7.1)

Stored dried blood spots of 31,484 children, born between January and September 2008, were tested for cCMV for the CROCUS-study. This is 17% of the entire birth cohort of 2008 in the Netherlands, which consisted of 184,634 children. The birth prevalence of cCMV in the Netherlands as estimated in this study was 0.5%. When this is extrapolated to the entire birth cohort of 2008 this means that around 915 children would have had cCMV in the Netherlands. [Chapter 3] In the next paragraphs this group of children is described in more detail.

Based on the results of this study, of these 915 cCMV-positive children, almost 20% (179) will be symptomatic at birth and about 80% (736) will have no symptoms at birth. However, it is important to keep in mind that the same or similar symptoms at birth are also commonly found in children without cCMV (12.4%). Based on the risk difference between these groups it is expected that approximately 66 children with cCMV have symptoms at birth that are attributable to cCMV. Nonetheless, it is in general impossible to determine which children with cCMV and symptoms at birth have these symptoms as a result of cCMV. [Chapter 4] It is expected that about 25% of children with cCMV (227) will develop some moderate-to-severe long-term impairment in the first six years of life. Of these children, 96 (42%) will have been symptomatic at birth and 131 (58%) will have been asymptomatic at birth. However, only a proportion of this long-term impairment is actually attributable to cCMV given that 12% of the cCMV-negative children have similar problems. Approximately 75 (41.8%) of the 179 symptomatic children and 43 (5.8%) of the 736 asymptomatic children will have long-term impairment attributable to cCMV. [Chapter 4]

About one-third of symptomatic children with long-term impairments will have impairments in multiple domains (19.2% of all symptomatic children with cCMV), which indicates more severe disease. In the group of children who were asymptomatic at birth, almost half of the children with long-term impairment will have problems in multiple domains (8.4% of all asymptomatic), while only 1.8% of the children without cCMV will have impairments in multiple domains.

It is clear from these data (Figure 7.1) that children with cCMV who have symptoms at birth have a much higher risk of long-term impairment compared to those without symptoms at birth. However, because there are many more children without symptoms at birth (80% of the total) than there are children with symptoms at birth, the majority of children with long-term impairment, and with impairments in multiple domains, will be asymptomatic at birth. Overall, in the Netherlands about 80 children will have severe impairments (impairments in multiple domains) attributable to cCMV every year (43/100.000 live births/year), of whom almost two-thirds are asymptomatic at birth.

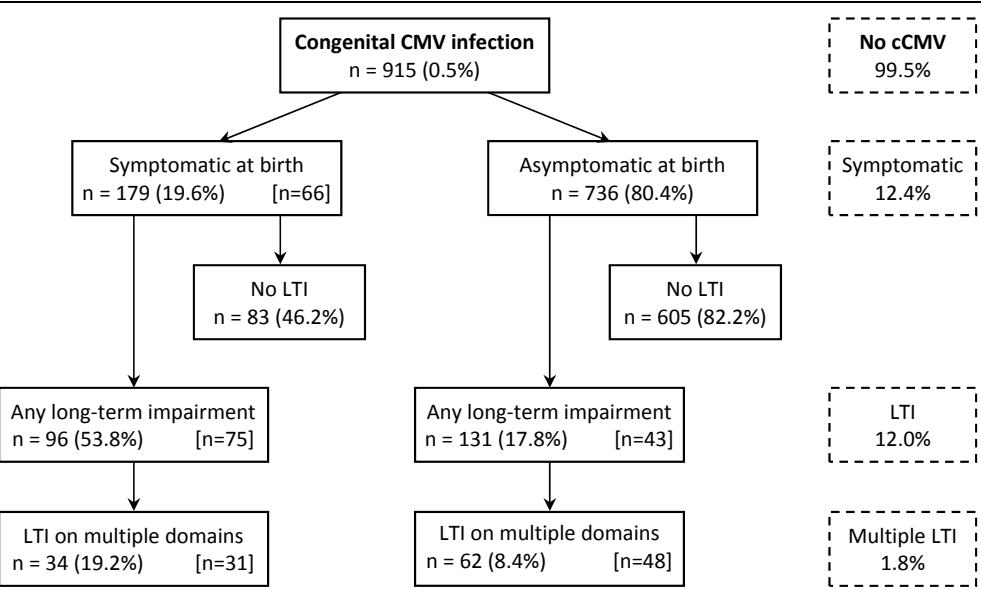
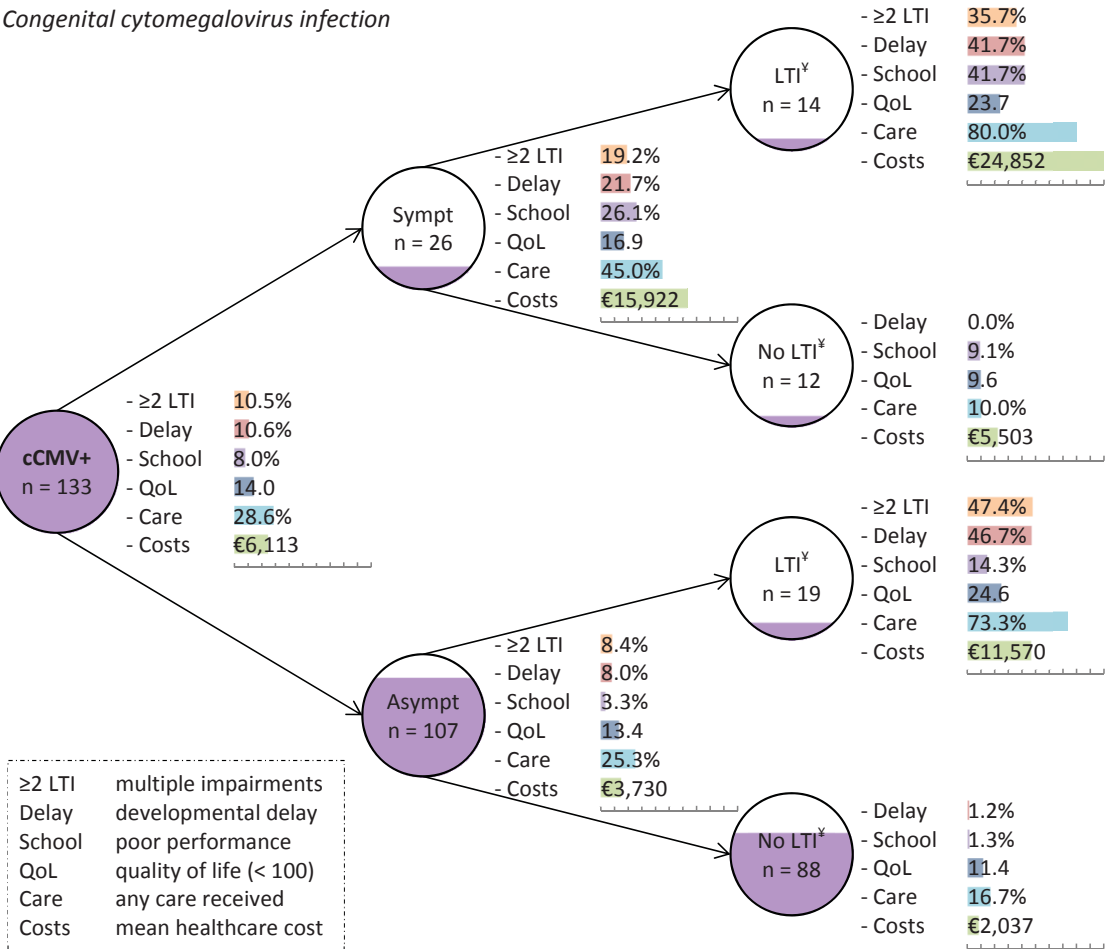


Figure 7.1 - Results of the CROCUS-study extrapolated to the entire birth cohort of 2008 (n=184,634). The figures represent number and percentages (%).The figures between square brackets represent the number of children with symptoms or impairment attributable to cCMV. The numbers in the dashed text boxes represent the percentages of cCMV-negative children. cCMV: congenital cytomegalovirus infection, LTI: long-term impairment.

Clinical, daily life and economic consequences of cCMV (Figure 7.2)

The CROCUS-study demonstrated that cCMV can lead to a broad array of long-term impairments. Besides sensorineural hearing loss, which is the most well-known and a much studied consequence of cCMV, we found other less-studied impairments to be much more common in children with cCMV than in the control group. The largest risk differences between children with and without cCMV were seen for motor, cognitive and speech-language impairment. [Chapter 4] Some of these problems, such as balance problems [1-3], have recently been described in other studies. Others, such as sensory processing disorders and developmental coordination disorders, have not been reported previously and require further investigation.

Congenital cytomegalovirus infection



No congenital cytomegalovirus infection

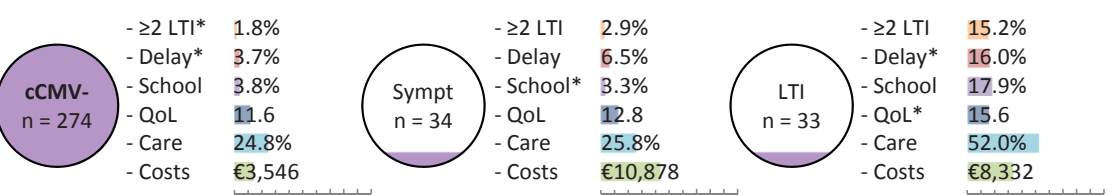


Figure 7.2 - Overview of the consequences on different aspects of daily life and on the average costs of healthcare. ≥2 LTI: long-term impairment at multiple domains, **Delay**: general development delay on child development inventory, **School**: poor school performance (> 50% of test lowest score or special needs education), **QoL**: Quality of life, mean score below the maximum score (100) on PedsQL™, **Care**: percentage of children who received any care, **Costs**: average healthcare costs per child in 2013€. cCMV: congenital CMV infection, LTI: long-term impairment. *Statistically significant difference between cCMV+ and cCMV- (p < 0.05). No significant differences were found in children with cCMV and LTI (¥) or in children with cCMV without LTI (¥) between symptomatic and asymptomatic were found. The colored part of the circles represents the percentage of children compared to the total group.

Besides the clinical consequences, we found that children with cCMV had poorer school performances (in symptomatic children), had more frequently general development delay (all children), and had a lower quality of life (in children with long-term impairment) than children without cCMV. Children with cCMV and long-term impairment had a lower overall, and physical quality of life and they had a higher healthcare consumption than children with cCMV without long-term impairment. (Figure 7.2) [Chapter 5]

The parents of children with cCMV and long-term impairment also had a lower mental quality of life, although not statistically significant, and they experienced more negative consequences in their daily life than parents of children with cCMV without long-term impairment. Compared to parents of children without cCMV with long-term impairment, the parents of children with cCMV and long-term impairments had a lower mental quality of life score, although this difference was not significant. [Chapter 5]

Children with cCMV were expected to incur higher healthcare costs because they had higher healthcare consumption than children without cCMV. Indeed, the mean healthcare costs for children with cCMV were more than € 2,500 higher than for children without cCMV in the first six years of life. Especially the costs for physical and speech therapy and visits to a rehabilitation center were higher. The difference in average healthcare costs between children with and without cCMV is not statistically significant, due to the large range of costs. Moreover, the costs for children with cCMV are probably underestimated, as explained in chapter 6.

When the total costs were extrapolated to the entire birth cohort of 2008, the total healthcare costs for children with cCMV were estimated at 5.6 million euros, of which 2.3 million euros are expected to be attributable to cCMV. Almost 50% of the total costs of children with cCMV could be accounted for by the 20% of children with symptoms at birth.

Overall burden of congenital CMV infection

In summary, cCMV leads to long-term impairments, in different domains. Especially motor, cognitive and speech-language impairments are frequently seen in children with cCMV and these children consume more care for these problems and consequently have higher healthcare costs. Children with these problems are also affected in the course of their daily life; they have poorer school performances and a lower quality of life than children with cCMV without long-term impairment. Moreover, the parents of these children experience negative consequences in their daily life and have a lower mental quality of life. Currently the care for children with cCMV is still mainly focused on audiological follow-up and care for major neurological impairment. This research shows that these children and their families deserve more attention, care and support in other areas including motor, cognitive and speech-language development and daily life consequences.

It is important to realize that in the group of children with cCMV and LTI no significant difference, in multiple impairments, child development, quality of life and healthcare costs, was found between children with and without symptoms at birth. In combination with the fact mentioned before that most of the children with LTI were asymptomatic at birth, this means that the problems in this large group of asymptomatic children are as severe as in children with symptoms at birth and occur twice as frequently. It will be a challenge to give

these children the care and support they need, given that they will probably not be identified, as they are asymptomatic at birth.

In the previous sections, the results of the CROCUS-study have been extrapolated to the birth cohort of 2008. However, cCMV is endemic, meaning that each year roughly the same number of patients is expected to be born with the same amount of clinical consequences, daily life consequences and economic consequences.

Finally, another very important finding in our study which has not yet been mentioned is the underdiagnosis of cCMV. Of the whole cohort of 156 children identified by the CROCUS study, only 4 (2.6%) children had been diagnosed prior to this study. In virtually the whole cohort of children, of whom 25% had moderate to severe impairment, cCMV had not been diagnosed by healthcare professionals. This is a clear indication of the low awareness of cCMV among healthcare professionals, at least between 2008 and 2014 in the Netherlands. When comparing this to other congenital infections, such as Zika virus for instance, which is virtually non-existent in the Netherlands, it is remarkable to realize that probably many more people have heard of Zika virus than of CMV.

Methodological considerations

Why design another study on long-term consequences?

As described in the introduction quite a few studies on the long-term clinical consequences of cCMV had been performed before we started our project. [Chapter 1] As all studies do, these studies had some limitations. First of all, many of these studies were relatively small, which is not surprising considering the low birth prevalence. A small study size can decrease the reliability of the estimated outcome. Often no, or only a small control group of children without cCMV was included in the studies. A control group can be used as a representation of the general population. In addition, most studies had relatively short follow-up periods, which could lead to an underestimation of the outcome, because some impairments can become manifest later in life and some conditions cannot be reliably assessed at a young age. About half of the studies that are mentioned in the introduction, were screening based and represent an unselected population of children with cCMV. However, the other half was mostly based on a referred population of children with cCMV, which can lead to an overestimation of the clinical consequences, because symptoms at birth are associated with a higher risk of long-term impairment. Moreover, virtually all studies used a prospective design. This enables uniform and detailed data collection, but on the other hand it can lead to information bias as parents and doctors are aware of the condition, which may lead to an overestimation of the long-term impairments. Finally, most articles focused primarily or solely on hearing loss as the outcome measure, paying less attention to other problems that might occur.

Our aim was to investigate a broad range of clinical and other consequences of cCMV. In order to try to avoid most of the abovementioned limitations we decided to design a retrospective cohort study. [Chapter 3] This study design had a number of advantages. First of all, it is a screening based study, meaning that there is no selection based on clinical signs or symptoms at birth. The screening based selection of children resulted in the inclusion of the whole range of children with cCMV in the study, from completely asymptomatic to overtly symptomatic at birth. Second, this study is quite large compared to many of the other studies, consisting of 17% of the total annual birth cohort of the Netherlands, which strengthens the reliability of the outcomes. Additionally, the study included a twice-as-large control group. This allowed us to compare the children with cCMV to a population which is expected to have about the same risk of impairment as the general population.

The retrospective nature of this study also had some advantages. Because cCMV was first diagnosed in 97.4% of the children when they were five years old, we could look at the health problems that occurred in the first five years of life without information bias due to doctors or parents being aware of the diagnosis. This gave us a picture of the healthcare that was actually sought by and provided for these children. The design also enabled us to obtain data on a relatively long-term follow-up period in a short amount of time. Finally, by looking at a broad range of medical data, from general practitioners, medical specialists and other healthcare providers, we could estimate the prevalence of various sensory, neurological and developmental outcomes, without being restricted to one specific outcome. In addition, the medical data could be used to assess other outcomes such as cost of illness, and by inclusion of parental questionnaires we could look at aspects such as quality of life, which has not been done before.

Methodological limitations and challenges

Estimation of the birth prevalence

- Participation rate of Regional Public Health Services

Before we started this study we sought cooperation with the Regional Public Health Services. Only children who were living in a region of a cooperating Regional Public Health Service were invited to participate in this study. (Figure 7.3) The public health services in the east of the Netherlands participated less in this study, often because they were already involved in other studies or were busy with changes within the organization. In the regions that did not participate in the study the level of urbanization, the number of births and the proportion of Non-Western migrants is somewhat lower, although the socio-economic status is not clearly different compared to the rest of the Netherlands. [4-8] This may have resulted in a slightly higher proportion of non-Western migrants being invited to our study. In the PIENTER study we found that non-Western migrants have a higher seroprevalence of CMV. Because a high CMV seroprevalence is related to a higher birth prevalence cCMV, the birth prevalence in our study might have been slightly overestimated due to this selection of regions.



Figure 7.3 - Geographical representation of the Netherlands, with the different Regional Public Health Services displayed as dots. The grey dots represent the regions that participated in the CROCUS-study. The open dots represent the regions that did not participate.

- Participation rate of parents in the first part of the study

Parents of more than 73.000 children were invited to participate in the CROCUS study, and 44% accepted the invitation and gave informed consent. There were some differences between responders and non-responders based on the 4-digit postal code, including a higher percentage of non-Western migrants and a lower income level in the non-responder group. [Chapter 3] As persons of non-Western origin and those with a lower socio-economic status have a higher seroprevalence and are therefore at a greater risk of having a child with cCMV [Chapter 2], this could have led to a small underestimation of the birth prevalence of cCMV. However, if the proportion of non-Western migrants was slightly higher in the population that was invited for this study, as suggested in the previous paragraph, these effects on the estimated birth prevalence might have cancelled each other out.

- Testing of dried blood spots

Testing more than 31.000 dried blood spots was a major logistic challenge. The initial screening was performed at the National Institute for Public Health and the Environment (RIVM) using a real-time multiplex polymerase chain reaction, incorporating two independent CMV target genes (UL55 and UL 123) [9], with a detection limit of 850 copies/ml. In the Leiden University Medical Center (LUMC) the dried blood spots were tested for confirmation using the QIAamp DNA minikit [10] for DNA extraction from one full spot and using an internally controlled quantitative real-time polymerase chain reaction in triplicate, against a different target within the immediate-early gene (UL 123), to test for CMV DNA. [11, 12]

It is important to critically assess the test characteristics of the tests that were used. The estimated specificity of the polymerase chain reaction on dried blood spots for CMV is very high (99.9%). [13] The specificity indicates the percentage true negatives that are correctly identified by the test. The sensitivity of this test is assumed to be 84.4%, [13] which means that approximately 84% of the children who have cCMV are correctly classified as such by the test.

When the estimated sensitivity and specificity are applied to our study population this would mean that 154 children were correctly tested positive (true positive) and that 2 children had a false positive test result. The sensitivity of roughly 84% means that 29 children had a false negative test result and were consequently missed in our study. (Table 7.1)

Table 7.1 - Implications of the test characteristics for the CROCUS-cohort.

		true condition		
		cCMV+	cCMV-	total
test result	positive	154 ^A	2 ^C	156 ^G
	negative	29 ^B	31299 ^D	31328 ^H
	total	183 ^E	31301 ^F	31484 ^I
true prevalence (total positive ^E / total tested ^I)				0,58%
sensitivity (correctly tested positive ^A / total positive ^E)				84,2%
specificity (correctly tested negative ^D / total negative ^F)				99,99%*
positive predictive value (correctly tested positive ^A / total tested positive ^G)				98,7%
negative predictive value (correctly tested negative ^D / total tested negative ^H)				99,9%

* The specificity is estimated to be 99,99% because two separate tests were used.

This means that the actual birth prevalence of cCMV would be 0.58% instead of 0.5%, thus the birth prevalence is presumably slightly underestimated in our study. However, with a chance of 29/31328 (0.1%), it is very unlikely that cCMV-positive children ended up in the cCMV-negative control group ($n = 274$). Because the population tested was very large, and the great majority of children had a negative test-result both the positive predictive value and the negative predictive value would be very good, being 98.7 and 99.9% respectively. (Table 7.1)

Furthermore, it is known that the sensitivity of this test depends on the viral load. In the case of a very low viral load the chance of missing a child with cCMV becomes higher. Missing some children with a low viral load could mean that the children identified as having cCMV are those with a somewhat higher viral load. Because a higher viral load is possibly related to a worse outcome [14-16] this could lead to an overestimation of the proportion of children with long-term consequences of cCMV.

Estimation of the burden of disease of cCMV

- Participation in the first part of the study

The potential effect of the difference in response rate (a lower proportion of non-Western migrants and persons with a low socio-economic status in the responder group) on the estimation of long-term impairment is difficult to assess. Assuming that the long-term consequences are the same for children with cCMV due to primary and recurrent maternal infection, it is unlikely that the children who were identified with cCMV in this study would have had a different outcome than the children with cCMV in the non-responder group. However, it is possible that parents of children with medical problems were more willing to participate in this study, for example because they wanted to find an explanation for their child's problems. This might have led to the inclusion of a higher proportion of children with long-term impairment, especially in the control group, and possibly an underestimation of the disease burden of cCMV. In the end we did not find an indication for this underestimation in our study as the prevalence of a number of conditions we found in the control group were similar to that in the general population. [17-20]

- Participation in the second part of the study

After identifying children with cCMV and selecting the control group, parents were invited to take part in the second part of the study. About 85% (133/156) of parents in the cCMV-positive group and 75% (274/365) of parents in the cCMV-negative group were willing to participate in this part of the study. Since not all parents were willing to take part in this study, it is likely that some selection bias has occurred. It is possible that in the group of children with cCMV, parents of children with long-term impairment were more likely to participate, while in the group of children without cCMV the opposite could possibly happen. In this situation the long-term consequences due to cCMV might be overestimated. However, some parents of children with long-term impairment were unwilling to participate because they already had their hands full with caring for their child. Because impairment is more frequent in the cCMV group this might have led to an underestimation of the results. Overall it is difficult to predict the effect due to selection bias on the estimation of the consequences of cCMV.

Definitions of symptoms at birth and long-term impairment

Another problem, which has been mentioned in the introduction, is the lack of uniform and generally accepted definitions in discussing cCMV. For example, it is unclear which definition should be used to classify symptoms at birth. In our study we included preterm birth in the definition. In many other studies preterm birth is not included in the definition of symptomatic cCMV, even though most studies report a high prevalence (around 35%) of preterm births in children with symptomatic cCMV. [16, 21, 22] If we exclude preterm birth from our definition of being symptomatic at birth the estimated prevalence of symptomatic children would be almost halved in the group of children with cCMV (from 19.6% to 10.5%). Even though the number of children classified as being symptomatic would also be lower in the group without cCMV (from 12.4% to 6.9%), the risk difference between these groups would consequently be less (from 7.2% to 3.6%).

A similar problem occurs with the estimation of long-term impairment, because it is difficult to assess which of the, often late-onset or late-diagnosed, impairments are related to cCMV. Moreover, some problems that might be related to cCMV have not been described or studied before which makes the definition of long-term impairments even more challenging. Additionally, when studying the prevalence of moderate and severe impairments, the assessment of the severity of some conditions can be quite subjective. Even when using a standardized test, for example for measuring intelligence or development, the cut-off values used in the different studies were not uniform.

To enhance comparability between studies it would be useful to reach international consensus among researchers in this field on at least the definition of symptoms at birth and to develop a classification system for identifying mild, moderate and severe long-term impairments related to cCMV.

Missing data

Even though the retrospective design of this study has advantages, it also has a number of limitations. For instance, the collected data were not systematically recorded. Although we can assume that essentially all newborns in the Netherlands will be examined after birth, this was not always systematically documented or available for this study. It is very likely that clinically visible abnormalities will have been reported, but laboratory and ultrasound investigations were rarely carried out, meaning that abnormalities which could only be detected by these means would not have been discovered. This could have led to an underestimation of the children classified as symptomatic at birth in the current study. However, it is not clear if the percentage of symptomatic children would then increase in only the children with cCMV, or that more abnormalities would also be found in the children without cCMV. It is unknown how many healthy children have cranial ultrasound or laboratory abnormalities for example, because these investigations are generally not performed in these children. Therefore it is difficult to estimate the impact of a more complete examination after birth in this study.

Furthermore, it is also possible that some long-term impairments were not recorded in our study. This could be due to the late onset of the condition or because it had not yet been diagnosed at the time the data were collected. This might be an explanation for the relatively low prevalence of hearing loss we found in this cohort. Further follow-up of this cohort could clarify whether this is the case. This problem could have led to an underestimation of the prevalence of long-term impairments in this study.

Information bias

It was not possible to collect all the data before the diagnosis of cCMV was made. We considered it unethical to collect the parental questionnaires before giving the dried blood spot test results to the parents. This meant that all parental questionnaires could possibly be influenced by recall bias and diagnostic suspicion bias. Parents' answers could have been influenced because of knowing that their child was cCMV-positive or cCMV-negative. This could also be true for doctors' appointments after the diagnosis was made.

Implications of the limitations

It is difficult to say what the overall effect is of these limitations and biases.

It is likely that the birth prevalence has been underestimated. Taking the sensitivity of the test into account, the estimated birth prevalence of cCMV in the Netherlands is probably 0.58%.

Overall, for the estimation of long-term outcome, it is unclear whether all above-mentioned limitations and biases lead to over- or underestimation of the long-term consequences.

Presumably, the overestimation possibly resulting from recall bias and diagnostic suspicion bias would be balanced by the underestimation due to the missing data or onset of disease after the data collection.

What should we do about cCMV?

Now that it is clear that cCMV has considerable clinical, personal and economic consequences, the next question is: “What can and should we do about it?” In this section the potential preventive measures against cCMV that have been mentioned in the introduction will be considered.

Awareness

As discussed in the introduction current awareness of cCMV, in the Netherlands and many other countries, is generally low, among healthcare professionals as well as among women of reproductive age. Awareness is the first step; people need to know about cCMV before they can act upon it. Pregnant women need to know about the risk of cCMV and the measures they can take to minimize this risk. Doctors and midwives need to be knowledgeable about cCMV in order to give advice to women who want to become pregnant or who are already pregnant. Healthcare providers also need to be aware of the consequences of cCMV in order to recognize this condition.

The low level of awareness of cCMV is even more frustrating when it is realized that cCMV-related morbidity in children is at least as common as several better-known childhood disorders such as Down syndrome, fetal alcohol syndrome and spina bifida. [23, 24] In addition, the prevalence of cCMV is much higher than that of several disorders currently included in newborn screening in Europe. [25] Furthermore, due to the media attention, probably many more women of reproductive age currently know about congenital Zika virus infection than about cCMV.

How can we increase awareness of cCMV?

There are two important groups of people who should know about cCMV, the healthcare providers and women who are pregnant or who want to become pregnant.

Education is the key for the first group. Not only should they be aware of cCMV, they also need information about horizontal and vertical transmission risks and about ways to prevent transmission and they should be able to give adequate information to women of reproductive age. This means that medical students should learn about cCMV and that it should be a standard subject in their curriculum. A start has already been made to increase the knowledge of midwives about cCMV. An e-learning program has been developed by students of the Midwifery Academy Amsterdam Groningen. If this e-learning program would be incorporated in the curriculum and continuing education, it could increase the knowledge of all midwives concerning cCMV.

A first step has also been taken for the second group, i.e. women of reproductive age. In the brochure “Pregnant!”, a paragraph has recently been added on cCMV. This brochure is produced by the Dutch Association for Obstetrics and Gynaecology (INVOG), Royal Dutch Organization of Midwives (KNOV), Dutch College of General Practitioners (NHG), Association of Doctors Practising Obstetrics (VVAH), National Information Center for Heredity, Pregnancy, and Medical Biotechnology (Erfocentrum), Royal Dutch Organization for promoting Pharmacy (KNMP), Children in Hospital Foundation (K&Z), and National Institute of Public Health and the Environment (RIVM). It is routinely handed to pregnant women by midwives and gynecologists. However, cCMV is usually not mentioned in other generally available resources (books, internet, etc.) women use to find information about pregnancy.

A minimum goal to aim for is that women of reproductive age should know as much about preventive measures against cCMV as against toxoplasmosis and listeriosis. These disorders are routinely discussed during antenatal care.

In the Netherlands parents of children with cCMV are starting to play a more active role in creating awareness. The CMV Foundation has recently been established and a charity run has been organized by parents of children with cCMV. In other countries parent organizations play an important role in creating awareness with, for example, the 'Stop CMV' campaigns, in the USA (National CMV foundation), the UK (CMV action UK) and Australia (cCMV Association Australia).

It may be an option to join in the "success" that other patient organizations have achieved. For example, the ice-bucket challenge probably greatly increased awareness of Amyotrofische Laterale Sclerose (ALS). It could also be an option to try to share in the current media-attention that has been created by the Zika outbreak.

Primary prevention

Vaccination

Although no vaccine against CMV is currently registered or available this would probably be the most convenient preventive measure. It has been estimated that a vaccine which protects 60% of the population could lead to eradication of CMV [26] and that a vaccine with an efficacy of 60% would be cost-effective [27]. A vaccine which was 50% effective in preventing primary CMV infection has already been developed and tested in a phase II trial. [28] Moreover, if only a portion of CMV infections could be prevented this would already reduce the overall disease burden of cCMV. Further development and investigation of the already existing vaccines could accelerate the prevention of cCMV.

Hygienic measures

Several studies have demonstrated the effect of hygienic measures in the prevention of cCMV during pregnancy. [29-32] Michael Cannon suggests that if you could only reach 1 in 3 pregnant women and reduce the risk of transmission in these women by one third this would already prevent a number of children from obtaining cCMV. [33, 34] Subsequently this would reduce at least a part of the clinical, personal and economic burden of cCMV. Education about cCMV and giving hygienic advice to pregnant women or women who want to become pregnant has no adverse effects and these steps could be started right now. This will also increase awareness of CMV. While it is important that women know about cCMV, it is even more important that they know what they can do to prevent becoming infected with this virus during pregnancy as much as possible.

Secondary prevention

Screening pregnant women

Screening of pregnant women for CMV IgG has some advantages and disadvantages. First of all, screening pregnant women would lead to increased awareness. For example, in France the awareness among women of reproductive age is much higher (60%) [35] than in other countries and this might be related to the relatively common screening of pregnant women. [36]

If screening would be introduced the reason for screening and the results of the test would need to be discussed during the consultation with the midwife or gynecologist. Advice about hygienic measures should then be given at the same time, to both seronegative and seropositive women.

Yet, screening might lead to a false sense of security in women who are seropositive. It is important that gynecologists and midwives are made aware of the fact that antibodies against CMV are not fully protective against cCMV. Moreover, women with a reactivation or reinfection cannot be diagnosed by IgM or IgG testing. This means that about half of the cases of cCMV would still be missed by this screening method.

Furthermore, besides follow-up, little can currently be done to influence the transmission of cCMV from mother to fetus when a primary maternal infection is detected, as hyper-immune globulin was reported to be ineffective [37]. Yet, some studies found encouraging effects of hyper-immune globulin in improving the outcome in cases of fetal CMV infection (tertiary prevention), but this requires further investigation.

Because no proven effective therapeutic option is available and screening would not lead to a different approach towards seropositive or seronegative women, screening cannot be justified, unless as part of a research project. Women who are diagnosed with a primary CMV infection during pregnancy, in such a research project, could participate in trials for evaluation of promising treatment options. In addition, these research projects could provide valuable data about potential predictive factors.

Tertiary prevention

Universal neonatal screening

According to the criteria of Wilson and Jungner for screening, the evaluated condition should be an important health problem, which cCMV most certainly is. However there should also be a generally accepted treatment for the condition. It is debatable whether this is true for cCMV.

There is general consensus on the benefit of treatment of children with cCMV who have neurologic signs and symptoms at birth. These children should be treated with (val)ganciclovir as this has been demonstrated to be effective in preventing deterioration of hearing loss. [38, 39] However, there is no evidence for antiviral treatment in children with only mild, reversible or solitary symptoms or in children without symptomatic disease at birth.

On the other hand, screening children for cCMV can have some benefits. The most important benefit is the early identification of children with cCMV enabling early treatment of potential impairment. A clear example of this is late-onset hearing loss, which commonly occurs in children with cCMV and which is missed by neonatal hearing screening in probably one third to half of the cases.[40] Early detection and initiation of intervention for hearing loss, such as hearing aids or cochlear implants, [41, 42] can lead to improved speech- and language development. [43, 44] Repeated audiological follow-up should be standard care in children with cCMV, because of the unstable nature of the hearing loss. [45-48] Even though this might not prevent disease, the audiological follow-up could be considered as an intervention and it can prevent or limit harm such as impaired language development. However, universal neonatal CMV screening also has some disadvantages. At present there are no highly predictive prognostic markers available. Even though we know that half of the symptomatic children will develop long-term impairment, we cannot predict who will

develop these problems. The same is true for the even larger group of asymptomatic children. It would be difficult to tell a parent that his or her child has cCMV without knowing the prognosis for that specific child other than a 50% or 20% chance of impairment. Another potential disadvantage of screening is the detection of children who have no clinically apparent disease at birth, but who could be classified as symptomatic at birth. These include children who have isolated cranial ultrasound abnormalities, transient thrombocytopenia and neutropenia, or isolated sensorineural hearing loss. Even though these children could be classified as symptomatic at birth, it is not yet clear whether they would benefit from treatment. This problem is a point of discussion at international conferences.

The combination of screening with research could provide an answer for this problem. The children who would be identified by a research related screening program would receive the best possible care, including audiological follow-up, and promising interventions could be evaluated in randomized controlled trials. Furthermore this screening based research could provide valuable information about prognostic markers. This approach could also give more insight in the effects of treatment of mildly symptomatic children as has been mentioned above.

Targeted screening

Screening of children with sensorineural hearing loss at birth is more efficient than universal screening because children with hearing loss have a much higher risk of cCMV. [49] The benefit of screening these children is availability of a treatment option for children with neurologic symptoms when they are diagnosed shortly after birth. Furthermore, for many parents it can be satisfying to have an explanation for the hearing loss.

Recommendations for the care of children with cCMV

Some recommendations based on current evidence are available when a child has been diagnosed with cCMV. Firstly a child diagnosed at birth with cCMV and having neurological signs and symptoms should be treated with (val)ganciclovir, since antiviral therapy can influence the course of hearing loss in some symptomatic cCMV children. [38, 39, 50] Secondly, audiological follow-up until the age of six years is recommended for children with cCMV, because of potential late-onset hearing loss and possible progression and fluctuation of hearing problems. [45-47]

All available current advice is focused on hearing loss. However, we also recommend care and support for other consequences of cCMV based on the results of our study.

We advise regular evaluation of motor and speech and language development, preferably by a physical therapist and speech therapist. These evaluations could be scheduled around various developmental milestones which the child is expected to reach.

Furthermore, it would be convenient if one central professional would be the first port of call for a child and its family. This would preferably be a pediatrician who could manage and assess the care for this child. This case manager could also keep an eye on the quality of life and potential problems in the daily life of the child and the parents.

Ideally a register of all children with known cCMV should be kept, for instance by a pediatrician. This would facilitate the detection and evaluation of specific problems, for example behavioral problems, that are possibly related to cCMV. Furthermore, such a registry could be used to evaluate the long-term effects of various interventions.

Recommendations for future research

Clear definitions of symptoms and sequelae of cCMV

There is a need for a universal definition of being symptomatic at birth. In order to achieve this, the first step is to decide which signs and symptoms should be included in the definition. Subsequently the definitions of the separate signs and symptoms, such as microcephaly and conjugated hyperbilirubinemia, should be agreed on by researchers in this field. The same is true for the overall long-term impairment due to cCMV which needs to be defined in more detail. In addition, the various impairments need to be more clearly defined and the cut-off values for diverse conditions should be used more consistently.

Universal definitions will improve the comparability of different studies, create the possibility of performing meta-analyses in order to get more reliable estimates, and are essential for international registries on cCMV. It may even be an option to define different categories of symptomatic children, for example using a scoring system for clinically apparent abnormalities, neurological abnormalities, and abnormal laboratory parameters or abnormal imaging findings. Registration of these findings could provide valuable information for making clearer prognoses and choosing an individual treatment program.

Research on recurrent infections

In chapter 2 we identified risk factors for a high seroprevalence of CMV. In women of reproductive age with a high seroprevalence the chance of a primary infection during pregnancy is relatively low, whereas the risk of reactivation or reinfection is fairly high. The risk of a child with cCMV in this group is relatively high as there is a positive correlation between maternal CMV seroprevalence and birth prevalence of cCMV. However, it is not known what proportion of recurrent infections is due to reactivation and what proportion to reinfection. The differentiation between reactivation and reinfection is important for preventive strategies.

Primary infection and presumably reinfection can be partially prevented by hygienic measures. Therefore, if most recurrent infections would be reinfections then preventive hygienic measures should be pursued in seropositive women. However, if most of the recurrent infections would be due to reactivation of the virus, then prevention of cCMV by hygienic measures would be of little help to seropositive women. Also the specifications of a future vaccine, whether a vaccine only needs to prevent CMV infections arising from external sources or if it also needs to prevent CMV reactivation, depends on this outcome. One way to study the relative contribution of reinfections and reactivations is by using the information collected in the PIENTER-study on CMV-seroprevalence in modelling studies.

Evaluation of potential vaccination strategies

The CROCUS-study provided estimates of the prevalence of cCMV and the prevalence of the long-term consequences of cCMV. These detailed data, together with complete data of the prevalence of CMV provided by the PIENTER-study, can provide input for mathematical models. In this way the potential effects of various vaccination strategies against CMV in the Netherlands can be evaluated. Together with the cost data available from the CROCUS-study the cost-effectiveness of these strategies could also be calculated.

Research on prognostic factors

Even though some prognostic factors for long-term impairment have been found, including cranial ultrasound findings and viral load, it is still very difficult to predict the prognosis of an individual child. This could be very important, especially when neonatal screening becomes a more realistic option.

Identification of specific markers, in for example dried blood spots, would be very valuable. Data and material from the CROCUS-study will be used to investigate prognostic markers, as it contains a large cohort of children with cCMV and controls whose long-term consequences are already known and whose dried blood spot material is still available. Within the VACTRAIN consortium this investigation is currently being undertaken.

Follow-up of the CROCUS-study

The CROCUS-study cohort is a valuable study population and it is important that they are followed-up for a longer period. In this study we have used data from parents and their children up to the age of six years, mainly before the diagnosis of cCMV was made. A more systematic collection of data from the children could be possible by beginning a prospective follow-up study of these children within the coming years. It would be important to collect and especially analyze the data as blinded as possible, as the major drawback of a prospective study is awareness of the diagnosis. Because the parents and most of the involved healthcare providers now know the diagnosis the collection of the medical history is no longer blinded. However, systematic investigations of the children would provide relevant information on specific topics and this could be performed by a blinded researcher and analyst. The children could also be seen by an independent healthcare provider (e.g. audiometrist or ophthalmologist) for this study. In view of the relatively limited information that is available on long-term consequences at an older age (school age and adolescence) this study could also be used to evaluate whether some problems are only temporary or if these problems are permanent. That would provide valuable information for parents, children and healthcare providers. If some problems are temporary they deserve attention and care, but than parents can be reassured that the problems will resolve over time.

Final conclusion

The long-term consequences of cCMV include clinical impairment, impact on the quality of life and increased healthcare costs compared to children without cCMV. Altogether, it has a considerable effect on the life of children and parents.

This justifies taking action with preventive measures that are readily available. The first step is creating awareness along with implementing existing preventive measures. Women who are, or want to become, pregnant should learn about cCMV. Increased knowledge and awareness among healthcare professionals would enable the education of pregnant women or women who want to become pregnant. The education should consist of advice on hygienic measures, in order to prevent primary infections and reinfections. Of course, development of a vaccine against CMV should continue to have a high priority, but in the meanwhile the focus should remain on education of women and healthcare providers. Screening pregnant women for CMV and universal neonatal screening for cCMV could provide benefits, but they also have disadvantages. Further research, on potential treatment options and prognostic factors is essential for the implementation of these programs. In order to optimize and take advantage of the benefits of screening programs, they should be combined with research into treatment options and prognostic factors.

Furthermore, the availability of information about the broad range of impairments justifies an update of guidelines on the care of children with cCMV, with more attention for their motor, cognitive and speech-language development, as well as for the consequences in the daily life of the children and their families.

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Summary

**Consequences of congenital cytomegalovirus
infection in the first years of life.**

Cytomegalovirus (CMV) belongs to the Herpesviridae family. Like other herpesviruses, it can establish life-long latency after a primary infection. Besides reactivation of the latent virus, reinfection with a different strain of CMV can occur. The term 'recurrent infection' is often used to cover both reactivation and reinfection. An infection with CMV is usually asymptomatic, although it can cause a mononucleosis-like syndrome with fever and malaise in some persons. It can cause severe illness in immunocompromised or immunosuppressed patients.

The virus can be transmitted via body fluids (e.g. urine, saliva, breastmilk) through intimate contact. In addition, the virus can be transmitted vertically in the case of a primary or recurrent infection of the mother during pregnancy. The transmission rate is higher with a primary maternal infection (32%) compared to a recurrent maternal infection (1.4%) and the transmission rate increases with the duration of pregnancy.

A **congenital cytomegalovirus infection (cCMV)** occurs if the virus is transmitted from the mother to the unborn child through the placenta. cCMV is the most common congenital infection worldwide, with a birth prevalence of around 0.6-0.7% in industrialized countries. The birth prevalence increases with the ambient maternal seroprevalence and is higher in developing countries. cCMV can lead to symptoms and signs at birth, such as being small for gestational age, microcephaly, intracranial calcifications, hepatosplenomegaly, petechiae or purpura, chorioretinitis and neurological findings. These findings occur in approximately 10-15% of children born with cCMV. Long-term impairment occurs in about 50% of children with symptoms at birth and in 10-15% of children who are asymptomatic at birth. Long-term consequences related to cCMV include sensorineural hearing loss, cerebral palsy, epilepsy, chorioretinitis, optic atrophy, delayed psychomotor development and mental retardation. Despite the fact that the prevalence of cCMV is relatively high and that the consequences are considerable, awareness of cCMV among the general population as well as healthcare workers, in the Netherlands and in many other countries, is low. There are some **preventive measures** that could potentially reduce the prevalence and consequences of cCMV. With primary prevention, transmission of the virus from mother to fetus is prevented. This could be achieved by hygienic measures such as avoiding contact with urine and saliva of young children. Women who are or want to become pregnant should not kiss a child on the mouth, share foods or utensils or put anything that has been in the mouth of a young child in her mouth and she should wash her hands after changing diapers. A vaccine would be an alternative way to prevent CMV infections in pregnant women, however there is currently no vaccine available against CMV. Secondary prevention is the prevention or limitation of disease due to cCMV. This could include treating the mother with antiviral drugs or CMV-specific antibodies. Currently there is no evidence that these treatments are beneficial. Prevention of deterioration of disease (tertiary prevention) has been demonstrated in children with neurologic symptoms at birth who were treated with (val)ganciclovir. It is unknown if this treatment would also be beneficial for other groups of children with cCMV.

The **CROCUS study** (Consequences and Risk factors of Congenital cytomegalovirus infection) was designed in order to obtain an estimation of the burden of cCMV. This study aimed to evaluate a broad range of consequences of cCMV in children up to six years of age in the Netherlands. Not only medical conditions, but also personal and financial consequences of cCMV were assessed using this nation-wide retrospective cohort study, which included a large group of children with cCMV and a twice as large control group.

In **chapter 2** the **seroprevalence of CMV** was discussed. This article was based on the PIENTER2 study, which is a cross-sectional population-based serum bank established in 2006-2007 in the Netherlands. Blood samples from 6249 persons, aged 6 months to 79 years, were tested for CMV-specific IgG antibodies and questionnaires were obtained from these participants.

The overall seroprevalence of CMV in the Netherlands was 45.6% and it increased steadily with age. Because the seroprevalence was much higher in non-Western migrants (76.7%) compared to Western migrants (57.3%) and native Dutch persons (40.1%) the analyses were stratified for origin. In the Dutch and Western populations, the seroprevalence was higher in women, persons with a low educational level and those who had contact with young children from outside their own household. In the non-Western population, the risk factors for seropositivity were a lower educational level and being a first-generation migrant. Risk factors for higher antibody levels, which may be related to CMV reactivation or reinfection, could be identified by looking at the geometric mean concentration among seropositive individuals. The geometric mean concentration increased with age and was higher in women than in men and in non-Western migrants compared to native Dutch and Western migrants. It was also higher among Dutch and Western individuals in persons who lived in a household with young children.

The most prominent independent risk factors for CMV seropositivity were age and origin. The high seroprevalence among non-Western migrants, especially among first generation migrants, could be related to a higher infection rate in the country of origin and to differences in lifestyle. The increase in CMV seroprevalence with age could be related to cumulative exposure throughout life, although it could also reflect a higher infection rate during the childhood of persons who are now elderly.

Chapter 3 describes the **design of the CROCUS study**. This study was designed to estimate the long-term consequences of congenital CMV infection up to the age of six years in the Netherlands. Many studies on the long-term consequences of cCMV are prospective, have a relatively short follow-up, and include small or no control groups. The CROCUS study is a nationwide cohort study in which retrospectively diagnosed children with cCMV and a cCMV-negative control group are included. The study looks at a broad range of consequences of cCMV. The CROCUS study consists of two parts. First, parents of more than 73.000 children were invited to allow their children to be included in this study. Children with a cCMV infection were identified by testing stored dried bloods spots of 31.484 children for CMV using a polymerase chain reaction. In total 156 children (0.5%) with cCMV were diagnosed. Surprisingly only four of these children (2.6%) had been diagnosed prior to this study, which indicates that cCMV is currently being underdiagnosed in the Netherlands. Second, medical data and school results of children with cCMV and a twice as large control group were collected. In addition four parental questionnaires; on child development, the quality of life of the children and their parents, and a general questionnaire on medical history, everyday life consequences and demographic features, were sent to participating parents. Parents of 133 cCMV-positive and 274 cCMV-negative children gave informed consent for participation.

This study design limits information bias due to its retrospective nature because parents and professionals looking after the children did not know of the CMV status of the child until the diagnosis was made by the CROCUS study. However, this design can also lead to some

limitations because data was not collected prospectively in a systematic manner and was sometimes incomplete. In addition, selection bias can be caused by for example response rate differences between parents of children with and without health problems, and by a lower sensitivity of the test for cCMV when the viral load is low.

The **clinical consequences** of congenital CMV infection are presented in **chapter 4**. In the CROCUS study we found that 19.6% of children with cCMV could retrospectively be classified as having been symptomatic at birth compared to 12.4% of children without cCMV. The risk of developing any moderate-to-severe long-term impairment, including hearing, visual, neurologic, cognitive, motor and speech-language impairment, was higher in children with cCMV (24.8%) compared to children without cCMV (12.5%). Moreover, this risk was higher in cCMV-positive children with symptoms at birth (53.8%), compared to children with cCMV who were asymptomatic at birth (17.8%). In the cCMV-negative group, there was no significant difference in prevalence of long-term impairments between the symptomatic (8.8%) and asymptomatic (12.5%) children. In addition, impairment in multiple domains was seen much more often in children with cCMV (10.5%) than in children without cCMV (1.8%). Again this was more frequent in symptomatic (19.2%) than in asymptomatic (8.8%) children with cCMV.

Even though it is clear that children with cCMV who are symptomatic at birth have the highest risk of long-term impairments, this study demonstrates that asymptomatic children with cCMV have more long-term impairments than children without cCMV. The impairments in these children with cCMV include not only hearing loss, but also cognitive, motor and speech-language impairment. This study justifies increased awareness and prompt initiation of preventive measures against cCMV. Additionally, it shows that more attention and care is needed for the cognitive, motor and speech-language development of children with cCMV.

In **chapter 5** other consequences of cCMV are investigated. In the CROCUS study child development, **quality of life** and daily life consequences for children with and without cCMV and their parents were assessed using parental questionnaires and data from schools. Assessment of child development showed that delays in the general and expressive language development were more frequent in children with cCMV (13.8% and 10.6%) compared to those without cCMV (5.8% and 3.7% respectively). Additionally, more children with cCMV and symptoms at birth attended special needs education or had poorer school performances compared to their cCMV-negative counterparts.

Physical therapy was needed by 10.9% of all children with cCMV and by 40% of those with long-term impairment, but only by 2.9% of children without cCMV. In addition, the overall and physical health-related quality of life of children with cCMV and long-term impairments was lower than children without cCMV even in those with long-term impairment.

Parents of children with cCMV and long-term impairment reported having physical problems (44%) and concentration problems (40%) due to the problems of their child more often than parents of children with long-term impairments without cCMV (16% and 16% respectively). Parents of children with cCMV and long-term impairment also had lower mental health scores for quality of life compared to parents of cCMV-negative children with long-term impairment, although this difference was not statistically significant.

Even though the differences in quality of life between the cCMV-positive and cCMV-negative groups with long-term impairment, for both children and parents, are relatively small it is still a relevant finding. This is especially so when one considers that perception of quality of

life is usually subject to physical and psychological adaptation to disabilities. Furthermore, this study confirms the findings in chapter 4 that children with cCMV have more problems than only hearing loss and neurological disability.

The **financial consequences** of congenital CMV infection are discussed in **chapter 6**. This chapter was also based on the CROCUS study, using the healthcare resources use by children with cCMV and the matched control group and their reference prices. The mean healthcare costs per child in the first six years of life were higher in the cCMV-positive children (€6,113) than in the cCMV-negative children (€3,546). If this mean difference in costs (€2,568) is extrapolated to the whole birth cohort of 2008, then the additional national healthcare costs attributable to cCMV in the Netherlands would be 2.4 million euro. The total healthcare costs of children with cCMV would add up to approximately 5.6 million euro in the first six years of life (3.0 million euro/100,000 children).

Children with symptoms at birth and cCMV had higher average healthcare costs (€15,922) in the first six years of life than children with cCMV who are asymptomatic at birth (€3,730) and than children without cCMV (€10,878 in symptomatic and €2,507 in asymptomatic). A similar picture arises when looking at children with and without long-term impairment. The average costs in children with cCMV and long-term impairment (€17,205) were twice as high as in children with long-term impairment without cCMV (€8,332) and six to seven times higher than children without long-term impairment. The largest differences in costs between children with and without cCMV were seen in costs for physical and speech therapy and visits to a rehabilitation center.

Even though the cost difference between cCMV-positive and cCMV-negative children is not statistically significantly different, the costs attributable to cCMV are substantial. The lack of statistical significance is probably due to the large variance in costs. In addition, if other costs, such as costs for psychosocial care, future healthcare costs, special needs education and decreased productivity would be taken into account, the difference between children with and without cCMV would probably increase further. These considerable costs of cCMV should also be included in the equation when considering preventive measures against cCMV.

Discussion and conclusion

The reason to design the CROCUS study was to overcome some limitations that were observed in other studies. These included prospective study designs, small study populations, very small or absent control groups and focus on only a few specific conditions. Although the CROCUS study overcame most of these problems, it had some limitations of its own. For example, differences in response rates in various parts of the study could have led to selection bias. Differences between parents who responded and those who did not respond might have led to an underestimation of the birth prevalence. Due to the test characteristics of the polymerase chain reaction, with an assumed sensitivity of 84.4%, it is likely that the birth prevalence of cCMV has been underestimated in this study. Furthermore, the retrospective design of the study has led to a less systematic manner of recording the data generated during the first years of life. This caused a relatively large amount of missing data for some of the outcomes. However, as many of these biases can both lead to either over- or underestimation of the disease burden, it is impossible to assess the true impact of the limitations in our study design

If the results from the CROCUS study are **extrapolated to the annual birth cohort** of 2008 in the Netherlands, it is estimated that 915 children with cCMV were born that year. Of these children 179 (19.6%) would have been symptomatic and 736 (80.4%) would have been asymptomatic at birth. Long-term impairment in the first six years of life would occur in 227 children, of whom the majority would have been asymptomatic at birth ($n = 131$).

In addition to these medical consequences, these children will also experience a lower quality of life and their condition will have an impact on the daily life of their parents. It is important to realize that approximately the same number of children with these problems will be born each year with the same substantial burden for the children themselves, their parents and society.

When **evaluating the preventive measures** that have been mentioned in the introduction, it is clear that measures that are evidence-based and currently available should be implemented directly. This includes the hygienic advice, both for seropositive and seronegative women who are, or want to become pregnant. In addition, all children with clinically apparent cCMV and neurological symptoms at birth should be treated with (val)ganciclovir, in order to prevent (deterioration of) hearing loss. More evidence is needed before other preventive measures can be advised.

This research leads to some **recommendations** for the care of children with cCMV. Besides the evaluation of hearing loss, the standard care for children with cCMV should include evaluation for motor development and speech-language development and instigation of appropriate care when indicated. Additionally attention should be paid to the quality of life of both children and parents. **Future research** on cCMV should be focused on obtaining consensus for definitions used in this field, evaluation of the relative contribution of reactivations and reinfection in seropositive persons and evaluation of prognostic markers preferably in neonates. Additionally, it is essential that the CROCUS study is continued and that the children in this cohort will be followed in a prospective manner. This valuable cohort can supply much information on the consequences of cCMV at later ages. The cooperation and contribution of these children and their parents will provide valuable information in the fight against cCMV.

Samenvatting

Gevolgen van aangeboren cytomegalovirus infectie in de eerste levensjaren

Cytomegalovirus (CMV) behoort tot de herpes virus familie, net zoals de virussen die waterpokken en gordelroos (varicella zoster virus), de ziekte van Pfeiffer (Epstein-Barr virus) en een koortslip (herpes simplex virus) veroorzaken. Herpes virussen blijven na de eerste besmetting levenslang in het lichaam aanwezig. Ze kunnen later opnieuw actief worden, bijvoorbeeld als het afweersysteem wordt onderdrukt.

Infectie met CMV geeft meestal geen klachten, hoewel sommige mensen last kunnen krijgen van klachten die lijken op de ziekte van Pfeiffer zoals koorts, vermoeidheid en opgezette klieren. Bij mensen met een onderdrukt afweersysteem, zoals na chemotherapie en na orgaantransplantatie, kan CMV wel tot ernstige ziekte leiden.

CMV kan van mens op mens worden overgedragen door intiem contact. Het virus komt voor in alle lichaamsvloeistoffen, zoals speeksel, urine en moedermelk. Het kan ook tijdens de zwangerschap worden overgedragen van moeder op kind via de placenta (moederkoek). Als een moeder voor het eerst een infectie met CMV doormaakt tijdens de zwangerschap is de kans dat het virus wordt overgedragen ongeveer 1 op 3. Voor zwangere vrouwen die al eerder besmet zijn geraakt met CMV geldt het volgende: Als het virus opnieuw actief wordt (reactivatie) of als de moeder opnieuw een infectie met het virus oploopt (herinfectie) tijdens de zwangerschap dan is de kans dat het virus wordt overgedragen ongeveer 1 op 60. Als het ongeboren kind tijdens de zwangerschap met CMV wordt besmet dan spreken we over een aangeboren CMV infectie (congenitale CMV infectie).

Aangeboren CMV infectie (cCMV) is de meest voorkomende aangeboren infectie wereldwijd. In Europa en Amerika komt cCMV voor bij 6 op de 1000 pasgeborenen. In Afrika ligt dit aantal nog hoger.

Kinderen met cCMV kunnen bij de geboorte klachten hebben zoals vroeggeboorte, een te klein hoofd, een te laag geboortegewicht, maar ook een vergrote lever en milt, huidbloedingen, oogafwijkingen en hersenafwijkingen. Ongeveer 1 op de 8 kinderen met cCMV hebben bij de geboorte dit soort problemen. Op lange termijn kunnen deze kinderen ook last krijgen van een ontwikkelingsachterstand, zowel op het gebied van denken (cognitief) als bewegen (motoriek).

Ze kunnen ook epilepsie of spastische verlamming (cerebrale parese) ontwikkelen of doof worden. Bijna de helft van de kinderen die bij de geboorte al klachten heeft, krijgt tijdens de kinderleeftijd met dit soort problemen te maken. Maar ook kinderen die bij de geboorte volledig gezond zijn kunnen tijdens de kinderleeftijd een achterstand oplopen in hun ontwikkeling of slechthorend worden. Dit gebeurt bij ongeveer 1 op de 8 kinderen zonder klachten bij geboorte.

Ondanks dat cCMV redelijk vaak voorkomt en ernstige gevolgen kan hebben is cCMV erg onbekend in Nederland. Dit is onwenselijk, omdat een deel van de aangeboren CMV infecties te voorkómen is. Er zijn een aantal **preventieve maatregelen** die het ontstaan van cCMV kunnen tegengaan. Als tijdens de zwangerschap een (her)infectie met CMV van de moeder kan worden voorkomen dan zal het ongeboren kind het ook niet krijgen.

Met behulp van een aantal hygiënische maatregelen kan het aantal infecties bij toekomstige moeders worden verminderd. Bijvoorbeeld door contact met speeksel en urine van jonge kinderen te vermijden. **Kus een klein kind niet op de mond, stop niets in je mond wat net ook in de mond van een kind is geweest (zoals eten en drinken of een speen) en was je handen na het verschonen van een luier.** Ook een vaccin zou infecties kunnen voorkomen, maar helaas is er op dit moment nog geen vaccin tegen CMV.

Er zijn onderzoeken gedaan naar het voorkómen van cCMV of de gevolgen van cCMV als moeder al is besmet, bijvoorbeeld door behandeling met medicijnen of antistoffen tijdens de zwangerschap. Op dit moment is er nog geen effectief middel hiervoor ontdekt.

Er is wel een medicijn dat verslechtering van gehoorverlies tegengaat bij baby's die cCMV hebben. Dit medicijn is alleen onderzocht bij kinderen met ernstige klachten bij de geboorte. Het is nog niet duidelijk of het ook werkt bij kinderen zonder klachten bij de geboorte.

Dit proefschrift

Tot nu toe zijn de gevolgen van cCMV tijdens de kinderleeftijd nog niet volledig duidelijk. Daarom hebben wij het CROCUS-onderzoek opgezet. Dit onderzoek heeft als doel de gevolgen van aangeboren CMV infectie tot de leeftijd van 6 jaar in Nederland in kaart te brengen. Hierbij wordt niet alleen gekeken naar de medische gevolgen, maar ook naar de gevolgen voor kinderen en ouders in het dagelijks leven en naar de kosten van cCMV. In dit onderzoek gebruiken wij een andere aanpak dan veel andere onderzoeken tot nu toe hebben gedaan. Een belangrijk verschil is dat wij een groep kinderen zonder cCMV meenemen in het onderzoek, zodat wij deze twee groepen kunnen vergelijken.

In **hoofdstuk 2** is het **voórkomen van cytomegalovirus infectie in Nederland bestudeerd**. In 2006 en 2007 is het PIENTER-onderzoek uitgevoerd in Nederland. Daarvoor zijn er bij een steekproef van Nederlanders (van 0 tot 80 jaar) bloed en vragenlijsten afgenomen om te kijken naar verschillende infectieziekten. Dit bloed is onder andere getest op antistoffen tegen CMV. Hieruit bleek dat bijna de helft van de Nederlanders (45.6%) een infectie met CMV heeft doorgemaakt. Het aantal mensen dat een infectie heeft doorgemaakt ligt hoger bij Westerse (57.3%) en niet-Westerse (76.7%) migranten, vooral bij eerste generatie migranten. Ook neemt het aantal geïnfecteerde personen duidelijk toe met de leeftijd. Bij vrouwen, bij mensen met een lager opleidingsniveau en bij personen die contact hebben met jonge kinderen buiten hun gezin worden ook meer doorgemaakte infecties gezien. Er is in dit onderzoek ook gekeken naar de hoeveelheid antistoffen tegen CMV in het bloed. Een grote hoeveelheid antistoffen in het bloed zou het gevolg kunnen zijn van veel reactivaties (opnieuw actief worden van het virus) of van veel herinfecties (opnieuw geïnfecteerd raken met een ander subtype van het virus). Hierbij werd gezien dat ouderen, vrouwen, personen met jonge kinderen in het gezin, en niet-Westerse migranten meer antistoffen in hun bloed hadden.

In **hoofdstuk 3** is de **opzet van het CROCUS-onderzoek** beschreven. CROCUS staat voor Consequenties en RisicOfactoren van aangeboren CytomegalovirUS infectie. De meeste onderzoeken naar de gevolgen van cCMV hebben een vrij korte periode waarin de kinderen gevolgd en onderzocht worden. Vaak zijn er kleine groepen van kinderen met cCMV en zijn er weinig of geen kinderen zonder cCMV in het onderzoek meegenomen. Verder wordt bij de meeste onderzoeken eerst de diagnose gesteld, vaak al kort na de geboorte, en wordt daarna gekeken naar de gevolgen van cCMV. Doordat onderzoekers dan al weten dat een kind cCMV heeft, kunnen de gevolgen soms vertekend worden door persoonlijke meningen en ideeën. Bij het CROCUS-onderzoek is de diagnose cCMV pas gesteld op de leeftijd van vijf jaar. De medische gegevens van de eerste vijf levensjaren zijn daardoor niet beïnvloed doordat de ouders of de artsen en hulpverleners wisten van de diagnose. Verder vond dit onderzoek plaats in heel Nederland waardoor er een grote groep

kinderen met cCMV kon meedoen. Bovendien deed een twee keer zo grote groep van kinderen zonder cCMV mee aan dit onderzoek.

Hierdoor konden we klachten in de groep kinderen met cCMV vergelijken met klachten die in de algemene bevolking altijd al voorkomen en dus los staan van cCMV. Met dit onderzoek konden we in een korte tijd informatie krijgen over een relatief lange periode en ook over een groot aantal verschillende problemen. De vragenlijsten die ouders hebben ingevuld, maakten het mogelijk te achterhalen wat de kwaliteit van leven van kinderen en ouders en de gevolgen voor het dagelijks leven van deze gezinnen waren.

Voor dit onderzoek zijn ruim 73.000 ouders gevraagd om mee te doen en er zijn uiteindelijk 31.000 hielprikkaartjes van kinderen, geboren in 2008, onderzocht op cCMV. Bij 156 kinderen werd cCMV vastgesteld. Opmerkelijk is dat van slechts vier kinderen al bekend was dat ze cCMV hadden. Dit betekent dat 97.4% van de kinderen met cCMV niet eerder herkend zijn. Uiteindelijk deden de ouders van 133 kinderen met cCMV en 274 kinderen zonder cCMV mee aan het onderzoek. Zij gaven toestemming voor het opvragen van medische informatie en schoolgegevens en zij vulden meerdere vragenlijsten in.

In **hoofdstuk 4** zijn de **medische gevolgen** van aangeboren CMV infectie beschreven. Van de 133 kinderen met cCMV hadden 26 kinderen (19.6%) last van klachten bij de geboorte, zoals vroeggeboorte, laag geboortegewicht, een kleine hoofdomtrek. In de groep kinderen zonder cCMV hadden 34 van de 274 kinderen (12.4%) last van vergelijkbare klachten bij geboorte. Ook de kans op lange termijn gevolgen is hoger bij kinderen met cCMV. In deze groep hadden 33 van de 133 kinderen (24.8%) één of meer matige tot ernstige medische problemen in de eerste zes levensjaren. Bij kinderen zonder cCMV waren dit 33 van de 274 kinderen (12.5%). Het gaat hierbij om neurologische problemen en klachten op het gebied van gehoor, het zicht, en de cognitieve (denken), motorische (bewegen) en spraak-taal ontwikkeling.

Kinderen met cCMV die bij geboorte klachten hadden, kregen veel vaker lange termijn gevolgen (53.8%) dan kinderen die bij de geboorte geen klachten hadden (17.8%). Verder hebben kinderen met cCMV veel vaker problemen op meerdere gebieden (10.5%), bijvoorbeeld gehoorverlies en een ontwikkelingsachterstand, dan kinderen zonder cCMV (1.8%).

Vooral problemen op gebied van gehoor en ontwikkeling (denkvermogen, beweging en spraak-taal) kwamen vaker voor bij kinderen met cCMV. Tot nu toe is er bij de behandeling van kinderen met cCMV vooral veel aandacht voor gehoorverlies. Dit onderzoek toont aan dat bewegingsontwikkeling en spraak-taalontwikkeling van deze kinderen meer aandacht en begeleiding verdienen.

De gevolgen van aangeboren CMV infectie op het **dagelijks leven van kinderen en hun ouders** worden beschreven in **hoofdstuk 5**. Via een vragenlijst voor ouders werd de ontwikkeling van kinderen onderzocht. Kinderen met cCMV hadden vaker een achterstand in de algemene ontwikkeling (13.8%) en op gebied van spraak-taal ontwikkeling (10.6%) dan kinderen zonder cCMV (algemene ontwikkeling 5.8% en spraak-taal 3.7%). Op basis van de schoolgegevens zagen wij dat kinderen met cCMV die bij geboorte klachten hadden vaker naar speciaal onderwijs gaan en dat zij vaker een lage score behalen bij Cito-toetsen. Daarnaast gaan kinderen met cCMV vaker naar een fysiotherapeut en logopedist dan kinderen zonder cCMV.

Kinderen met cCMV en medische problemen hadden een lagere kwaliteit van leven dan kinderen zonder cCMV met vergelijkbare medische problemen.

Ook bij de ouders werden er gevolgen gezien door de aandoening van hun kind. Ouders van kinderen met cCMV en lange termijn gevolgen gaven aan door de problemen van hun kind meer lichamelijke en concentratie problemen te hebben. Ook de kwaliteit van leven van deze ouders was iets lager dan bij ouders van kinderen zonder cCMV.

In **hoofdstuk 6** is gekeken naar de **kosten van aangeboren CMV infectie**. Op basis van het aantal bezoeken aan het ziekenhuis, de huisarts en andere zorgverleners kon worden ingeschat hoeveel de kosten voor de zorg ongeveer waren. De gemiddelde zorgkosten voor kinderen met cCMV in de eerste zes levensjaren zijn € 6,113. Dit is bijna twee keer zo veel als de gemiddelde zorgkosten voor kinderen zonder cCMV (€ 3,546). Als je dit verschil doortrekt naar de hele groep kinderen die in 2008 geboren is dan bedragen de extra kosten als gevolg van cCMV in Nederland zo'n 2,4 miljoen euro per jaar. Vooral de kosten voor fysiotherapie, logopedie en bezoeken aan een revalidatiecentrum zijn hoger bij de kinderen met cCMV. Het verschil in kosten tussen beide groepen is relatief groot maar niet wezenlijk verschillend. Niet alle kosten zijn echter in dit onderzoek meegenomen. Als andere kosten zouden worden meegerekend, zoals de kosten van psychosociale zorg, toekomstige kosten en ook kosten door minder kunnen werken van ouders, dan zal het verschil tussen beiden groepen waarschijnlijk veel groter worden en wel substantieel zijn.

Discussie en conclusie

De opzet van het CROCUS-onderzoek was anders dan veel eerdere onderzoeken, om een aantal **problemen in het onderzoek** te vermijden. Zo was er in andere onderzoeken vaak sprake van een klein aantal kinderen met cCMV en geen of weinig kinderen zonder cCMV. Verder richtte deze onderzoeken zich vaak op maar één of twee specifieke medische gevolgen. Veel van deze problemen werden voorkomen in het CROCUS onderzoek, maar in dit onderzoek waren er weer andere problemen die mogelijk nadelig kunnen zijn. Als de groep mensen die meedoet aan het onderzoek heel anders is dan de groep mensen die niet meedoet aan het onderzoek dan kan dit de uitkomst van het onderzoek verstoren. Dit is mogelijk ook het geval bij het CROCUS-onderzoek, maar het effect hiervan op de uitkomst is lastig in te schatten. Het is wel waarschijnlijk dat in dit onderzoek het aantal kinderen met cCMV iets is onderschat. Dit heeft te maken met de test die is gebruikt voor het opsporen van cCMV. Er kunnen enkele kinderen met cCMV gemist zijn door deze test. Verder is een deel van de gegevens van dit onderzoek niet compleet, dit komt omdat we gebruik maken van gegevens die in het verleden zijn vastgelegd. Soms zijn deze gegevens niet volledig en daarom kan dit de uitkomst van het onderzoek verstoren.

Als we de **resultaten van het CROCUS-onderzoek vertalen** naar alle kinderen in Nederland, dan zijn er, in 2008, 915 kinderen met cCMV geboren. Hiervan hebben 179 (19.6%) klachten bij de geboorte en 736 (80.4%) geen klachten bij de geboorte. In totaal hebben 227 van de 915 kinderen met cCMV op lange termijn medische gevolgen, waarvan het merendeel (131 kinderen) bij de geboorte geen klachten had. Daarnaast ondervinden deze kinderen gevolgen in hun dagelijks leven. Bovendien beïnvloedt het hebben van een kind met cCMV het leven van ouders. Dus de gevolgen van cCMV voor de kinderen, hun families en de maatschappij zijn aanzienlijk.

Ons onderzoek maakt duidelijk dat meer aandacht moet uitgaan naar het toepassen van preventieve maatregelen om de ziektelast ten gevolge van cCMV zo veel mogelijk te beperken. Deze maatregelen bestaan vooral uit voorlichting en hygiënische adviezen aan zwangere vrouwen. Ook de behandeling van kinderen met cCMV en neurologische klachten bij de geboorte met antivirale medicijnen is een manier om de ziektelast te verminderen. Dit laatste gebeurt al in Nederland, het eerste (voorlichting en advies) wordt helaas nog te weinig gedaan.

Het **advies** op basis van dit proefschrift is dan ook om zwangere vrouwen beter voor te lichten. Daarnaast zouden kinderen met cCMV vaker gecontroleerd en begeleid moeten worden op hun bewegingsontwikkeling en hun spraak-taal ontwikkeling. Verder is aandacht van de behandelaren voor de gevolgen van cCMV in het dagelijks leven belangrijk.

Toekomstig onderzoek naar cCMV zou gericht moeten zijn op voorspellende factoren, zodat al bij een baby kan worden bekeken wat de gevolgen op lange termijn zullen zijn. Maar ook onderzoek naar preventieve maatregelen en de bijdrage van bijvoorbeeld vaccinaties bij herinfecties en reactivaties van personen die al eerder een infectie met CMV hebben doorgemaakt kan in de toekomst veel voordeel opleveren. Ten slotte is het heel belangrijk dat de kinderen van het CROCUS-onderzoek ook in de toekomst nog gevolgd en onderzocht worden, zodat we straks ook weten wat de effecten zijn van cCMV op de leeftijd van 10 of 15 jaar.

Abbreviations

Abbreviations

ADHD	Attention Deficit Hyperactivity Disorder
cCMV	Congenital Cytomegalovirus infection
CDI	Child Development Inventory
CI	Confidence Interval
CMV	Cytomegalovirus
CROCUS	Consequences and Risk factors Of congenital Cytomegalovirus infection
DBS	Dried Blood Spot
DQ	Development Quotient
ELISA	Enzyme-linked immunosorbent assay
GMC	Geometric Mean Concentration
GP	General Practitioner
IQ	Intelligence Quotient
LTI	Long-Term Impairment
LUMC	Leiden University Medical Center
PCR	Polymerase Chain Reaction
PedsQL™	Pediatric Quality of Life™ (Quality of life questionnaire)
RIVM	National Institute for Public Health and the Environment
SD	Standard Deviation
SF-12®	Short-Form 12® (Quality of life questionnaire)
SNHL	Sensorineural hearing loss

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Curriculum Vitae

Marjolein Korndewal werd geboren op 7 november 1982 te Baarn. Zij volgde het voorbereidend wetenschappelijk onderwijs op het Alberdingk Thijm College te Hilversum, waarvoor zij in 2001 haar diploma ontving.

Na uitloting voor de studie Geneeskunde behaalde zij in 2002 haar propedeuse voor de opleiding ergotherapie aan de Hogeschool van Amsterdam. Tussen 2002 en 2008 volgde zij de studie Geneeskunde aan de Universiteit Utrecht. In 2008 behaalde zij haar diploma cum laude. Zij werkte achtereenvolgens als arts-assistent niet-in-opleiding-tot-specialist op de afdeling Kindergeneeskunde van Gelre Ziekenhuizen te Apeldoorn (2008-2009) en in het Emma Kinderziekenhuis AMC te Amsterdam (2010). In 2011 ontwikkelde zij een tiental zorgpaden voor de afdeling Metabole Ziekten binnen het Emma Kinderziekenhuis AMC in samenwerking met de patiëntenvereniging Volwassenen en Kinderen met Stofwisselingsziekten.

In 2011 begon zij aan haar promotieonderzoek naar congenitale cytomegalovirus infectie binnen een samenwerkingsverband tussen de afdeling Medische Microbiologie van het Leids Universitair Medisch Centrum en het Centrum Epidemiologie en Surveillance van Infectieziekten van het Rijksinstituut van Volksgezondheid en Milieu te Bilthoven. Daarnaast volgde zij gedurende deze periode, onder begeleiding van prof. dr. F.W. Dekker, de opleiding tot epidemioloog B bij de afdeling klinische epidemiologie van het Leids Universitair Medisch Centrum, welke zij in 2016 voltooide.

Sinds september 2016 is zij werkzaam als arts-assistent niet-in-opleiding-tot-specialist op de afdeling Kindergeneeskunde van Tergooi te Blaricum.

Dankwoord

Met veel plezier kijk ik terug op de afgelopen jaren waarin ik dit onderzoek heb mogen uitvoeren. Dankzij twee werkplekken heb ik geprofiteerd van de kennis, ervaring en gezelligheid van vele collega's en slechts een paar honderd woorden om allen te bedanken.

Allereerst wil ik mijn copromotores en promotores hartelijk bedanken. Ann, ik ben je heel dankbaar voor je betrokkenheid, ondersteuning en begeleiding de afgelopen jaren. De manier waarop jij je inzet voor je werk vind ik bewonderenswaardig. Hester, ik heb veel waardering voor de manier waarop je zo goed op de hoogte blijft en persoonlijk betrokken bent, ondanks dat je zo'n grote afdeling onder je hoede hebt. Anne Marie, jouw betrokkenheid zowel op werkgebied als op persoonlijk vlak is heel erg fijn. Een "personal English editor" is iets waarvan menig PhD-student alleen kan dromen. Louis, dank voor je het delen van je brede kennis en je focus op het overkoepelende geheel.

Ten tweede wil ik de honderden ouders, huisartsen en andere zorgverleners bedanken voor hun medewerking, zonder hen zou dit proefschrift er niet zijn geweest. Ook de vele jeugdgezondheidszorg-artsen ben ik zeer dankbaar voor hun inzet, tijd en medewerking.

Voor het harde werken bij het testen van duizenden hielprikkaarten, de praktische ondersteuning en bovenal de fijne samenwerking wil ik Jeroen en Lisette ontzettend bedanken. Daarnaast is de hulp bij het verwerken van alle duizenden brieven en documenten zeer gewaardeerd. Daarvoor veel dank aan Marlies, secretariaat EPI, Anneke, Francoise en Jolanda, heel de EPI-afdeling en alle uitzendkrachten.

Voor de samenwerking, gezelligheid en praktische adviezen wil ik de hele afdeling RVP bedanken en in het bijzonder Mirjam, Anneke, Nicoline, Alies, Liesbeth.

Voor de gezelligheid, inspiratie, hulp, kamer DOD's, het meedenken en meeleven ben ik mijn (buur)kamergenootjes (Loes, Dineke, Robine, Annemarijn, Petra, Renske en Esther) dankbaar. Ilse, Susan en Iris, dank dat jullie altijd voor mij stonden klaar voor praktisch hulp of een praatje. Ten slotte dank aan de 'epi-uurtje' groep voor alle leerzame discussies. Maarten, dank voor het overnemen van dit initiatief.

Binnen het LUMC heb ik genoten van de gezelligheid, lunches en gesprekken met de AIOS medische microbiologie (Liz, Anne, Marjolein K en alle anderen).

Sofie, dank voor je steun en adviezen en de gedeelde interesse in de epidemiologie.

Ik ben vele mensen van de afdeling epidemiologie dankbaar voor de mogelijkheid om in het LUMC mijn opleiding tot epidemioloog B te volgen.

Fleurtje en Roberta, dank voor het delen van het OIO-schap en hotelkamers, jullie gezelschap en het uitwisselen van ideeën. Ik wens jullie beiden veel succes voor de toekomst. Manon, Ien en Sophie dank voor jullie hulp, het was heel fijn dat ik met al mijn vragen bij jullie terecht kon.

Voor alle ervaring en alles wat ik geleerd heb ben ik alle mensen dankbaar die ik heb ontmoet en die mij hebben bijgestaan, bij de kindergeneeskunde in Gelre ziekenhuizen in Apeldoorn, bij de kindergeneeskunde in het AMC, bij de afdeling van de metabole ziekten in het AMC en bij de kindergeneeskunde in Tergooi.

Verder ben ik heel blij met mijn lieve vriendinnen van basisschool, studie en mijn eerste baan (Marijn, Annika, Irene, Naomi, Denise en Marjolein E).

Marieke, dank je wel dat jij mijn grote zus bent, dat je zo goed voor mij zorgt en met mij meeleeft. Onze gesprek-en-wandeltochtjes en hardloop/strompelsessies zijn superfijn. Mama, dank je wel voor je onvoorwaardelijke steun en vertrouwen. Papa, ik ben je dankbaar voor de levenslessen die ik van je heb meegekregen en probeer deze zo veel mogelijk in praktijk te brengen.

François, ik ben zo blij dat wij samen zijn. Ik waardeer je onophoudelijke begrip, aanmoediging en steun ontzettend.

