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## Consequences of congenital cytomegalovirus infection in early childhood

Korndewal, Marjolein

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**Author:** Korndewal, Marjolein

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# Chapter 3

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

### **Authors**

M.J. Korndewal <sup>1,2</sup>

A.C.T.M. Vossen <sup>2</sup>

J. Cremer <sup>3</sup>

R.S. van Binnendijk <sup>3</sup>

A.C.M. Kroes <sup>2</sup>

M.A.B. van der Sande <sup>1,5</sup>

A.M. Oudesluys-Murphy <sup>4</sup>

H.E. de Melker <sup>1</sup>

### **Affiliations**

1. National Institute of Public Health and the Environment (RIVM), Center for Infectious Diseases, Epidemiology and Surveillance, Bilthoven, the Netherlands
2. Leiden University Medical Center (LUMC), Department of Medical Microbiology, Leiden, the Netherlands
3. National Institute of Public Health and the Environment (RIVM), Center for Infectious Disease Research, Diagnostics and Screening, Department for Virology, Bilthoven, the Netherlands
4. Leiden University Medical Center (LUMC), Willem-Alexander Children's Hospital, Leiden, the Netherlands
5. University Medical Center Utrecht (UMCU), Julius Center for Health Sciences and Primary Care, Utrecht, the Netherlands

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## 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

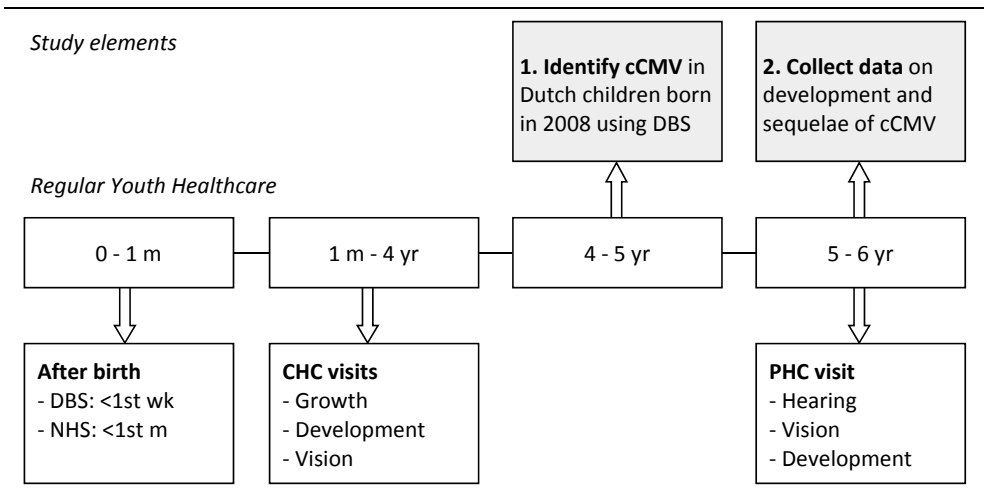
Cytomegalovirus (CMV) infection is endemic worldwide and is usually asymptomatic in healthy individuals. In immunocompromised patients however, it can cause serious complications. [1] Primary infections, reinfections and reactivations with CMV during pregnancy can lead to infection in the fetus. [2]

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 ( $\beta$ ) of 90% and two-sided alpha ( $\alpha$ ) 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

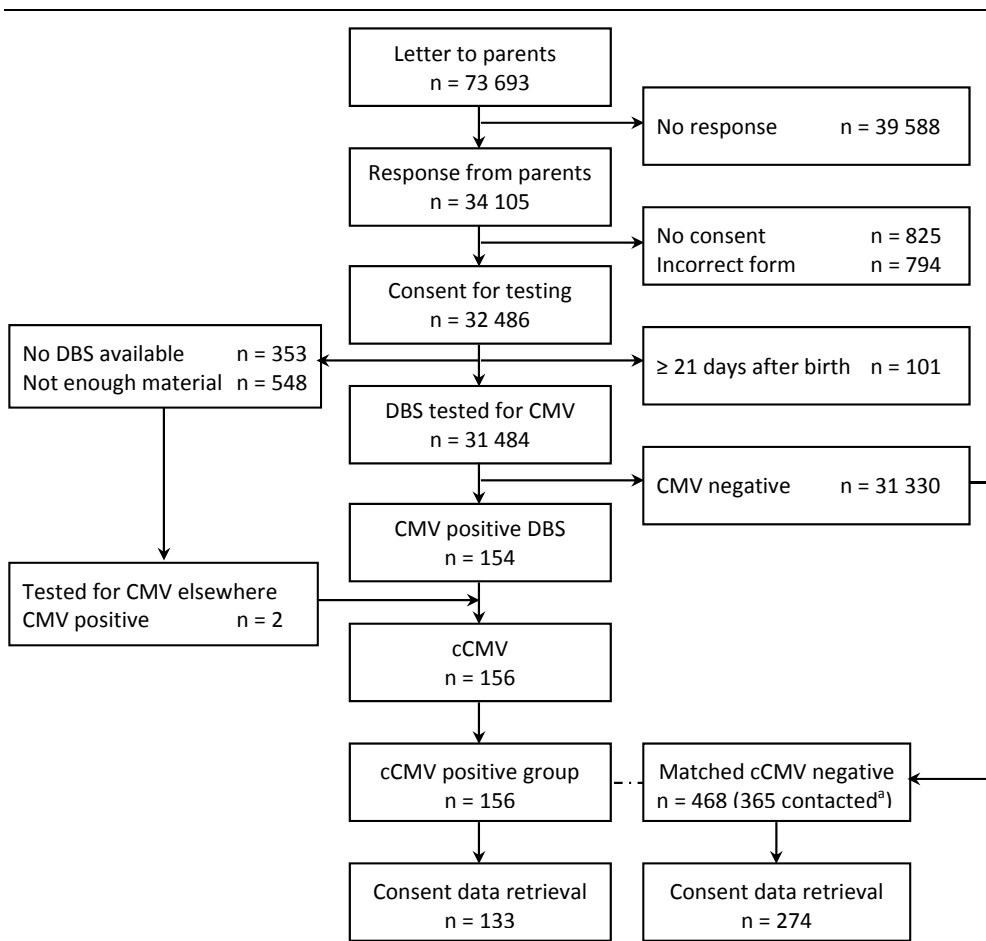
\*\*\* Persons from Europe (except Turkey), North America, Oceania, Japan and Indonesia

\*\*\*\* 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. <sup>a</sup> 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].

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### Conflict of interest

None.

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