

Cover Page



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Chapter 1

General introduction

General introduction

Human cytomegalovirus (CMV) was first isolated in cell culture in 1950 from the salivary gland and kidney of two infants who had died with enlarged or cytomegalic inclusion-bearing cells.¹ About 70 years before the identification of the causative agent, these cellular changes with a typical **owl's eye** appearance observed in affected newborns had led to use of the term cytomegalic inclusion disease (CID)². After initially being called "salivary gland virus", the term "cytomegalovirus" was proposed by Weller et al in 1962.³

CMV

Human CMV or human herpesvirus (HHV) 5 is a member of the family *Herpesviridae*, which also includes the human viruses herpes simplex virus 1 and 2, varicella-zoster virus, Epstein-Barr virus, and HHV 6, 7 and 8. CMV has been sub-classified as a betaherpesvirus, originally based on its growth characteristics in vitro, but nowadays based on genetic sequence homologies.⁴ Other beta-herpesviruses include HHV 6 and 7, the agents associated with the childhood disease exanthema subitum (sudden rash) or roseola infantum (rose rash of infants). CMV is amongst the largest human viral pathogens, measuring about 200 nm in diameter. The CMV virion consists of a capsid containing a large linear double stranded DNA genome encoding more than 200 potential proteins, enclosed by a protein tegument and a lipid envelope.⁵ The tegument contains key regulatory proteins, among which the immunomodulatory protein pp65 (UL83).⁴ This pp65 is the most abundant virion protein, accounting for about 15% of the total virion protein⁴, and is the antigen that is detected in the diagnostic antigenemia assay. The capsid exhibits icosahedral symmetry of triangulation number (T) 16, with 162 capsomere subunits.⁶ The envelope contains several glycoproteins with the most abundant ones being the gM (UL100)/gN (UL73) complex, gB (UL55), and the gH (UL75)/gL (UL115) /gO (UL74) complex.⁷ The envelope renders the virus susceptible to lipid solvents, low pH, heat, and ultraviolet light. Unlike herpes simplex and varicella-zoster viruses, CMV exhibits an exceptionally broad cellular tropism, rendering CMV capable of infecting most cell types including dendritic, endothelial, epithelial, fibroblast, and monocytes/ macrophages.⁵ A large number of cellular receptors for CMV have been proposed, mainly interacting with the CMV envelope glycoproteins gB, gH and gM.⁵ The association of specific CMV glycoprotein genotypes with severity of disease has been addressed, with contradictory results.^{8,9,10,11} There is little genetic homology between human CMV

and CMV of other species, restricting cell entry to human host cells and rendering humans the only reservoir. Primary infection results in lifelong latency with intermittent reactivation and excretion, applying the dictum 'once infected, always infected'. Viral latency is established within myeloid cells including myeloid progenitors, monocytes, macrophages, and dendritic cells. About 1 per 10,000 peripheral blood mononuclear cells of healthy seropositive individuals harbor several copies of the CMV genome¹², which is present in an episomal form.¹³

Transmission of CMV

Transmission of CMV occurs by acquisition of cell free virus at mucosal sites, by close contact with a person shedding the virus in body fluids including urine, saliva, breast milk, cervical and vaginal secretions, and semen.⁷ Particularly urine and saliva of young children may contain high virus titers and are therefore major sources of CMV.⁷ No studies have supported transmission of CMV through respiratory droplets. Blood-borne transmission through blood products and organ allografts can occur, and transplacental transmission results in congenital infection (discussed below).⁷ Perinatal transmission of CMV in the birth canal and during breast-feeding is common. Up to 96% of CMV seropositive mothers shed CMV (DNA) in mature cell free breast milk at some time during lactation, with a peak excretion between 2 weeks and 2 months postpartum.^{7,14} About 40% of the preterm newborns breastfed for at least 1 month by CMV seropositive mothers become infected postnatally.^{7,14,15}

Epidemiology of CMV infection

CMV circulates worldwide and is endemic in the whole human population, without seasonal variation. The seroprevalence of CMV increases with age and varies widely depending on ethnic and socioeconomic background. CMV seroprevalence is high in developing countries, up to 95-100% among preschool children in sub-Saharan Africa, South America, and Asia.⁷ In contrast, CMV seroprevalence of less than 20% has been found in subpopulations in the United Kingdom and the United States.⁷ In women of childbearing age, CMV seroprevalence is above 90% in developing countries, and 40-85% in the United States and Western Europe.^{7,16} In the Netherlands, maternal CMV seroprevalence ranges between 41-73% among various subgroups.^{17,18} High CMV seroprevalence among populations of low socioeconomic status reflects increased exposure to CMV due to factors including large household size, crowding, certain child care practices, and possibly sexual practices.⁷ Day-care centers facilitate transmission of CMV. About half of the infants attending day

care centers with middle- to upper-income background shed CMV in their urine and saliva.¹⁹

The reproductive number (R_0) of CMV has been estimated using mathematical models based on age-specific seroprevalence data.^{20,21} This R_0 is relatively modest, being 1.7-2.4 in Western populations, indicating that an infected person transmits CMV to approximately two susceptible people.^{20,21} The R_0 is somewhat higher in subpopulations of low socioeconomic status, up to 4.1 in non-Hispanic blacks in the United States.^{20,21} Corresponding with this R_0 , the force of infection is relatively low and has been calculated as an average annual seroconversion rate of 1.6-2.3% among pregnant women in the United States and the United Kingdom, and 5-20% in subpopulations of low socioeconomic status (Figure 1).^{20,22,23}

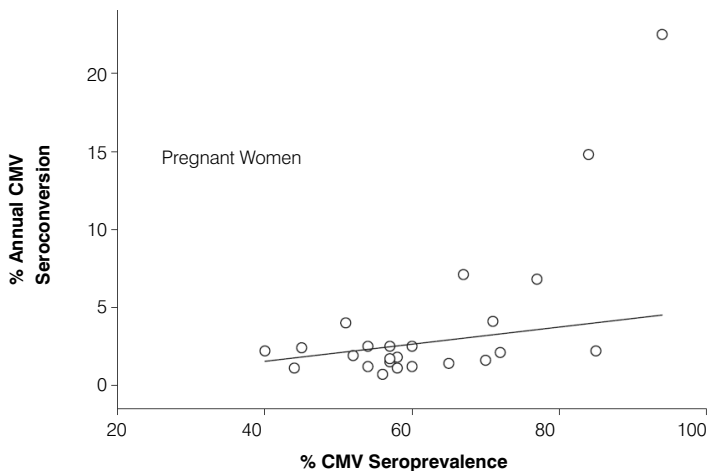


Figure 1 Meta-analysis of studies reporting annual CMV IgG seroconversion rates among pregnant women, as a function of CMV seroprevalence in the underlying population. (Adapted from Hyde et al.²²)

Clinical manifestations in adults

Primary CMV infection in the immunocompetent child or adult has been described as being usually asymptomatic⁴, however there are no data on the exact proportion of symptomatic primary infections. Uncommonly, primary infection in the immunocompetent host results in a mononucleosis syndrome clinically similar to the syndrome associated with Epstein-Barr virus infection.^{6,24} CMV mononucleosis may account for 8-20% of cases with mononucleosis syndrome presentations.^{6,24}

Infrequent complications of CMV mononucleosis include pneumonia, hepatitis, central nervous system involvement (Guillain-Barré syndrome), aseptic meningitis, encephalitis, pericarditis, and myocarditis.^{4,6} Most postnatally infected newborns do not develop symptoms^{4,25}, although occasional cases of severe disease including pneumonitis, hepatosplenomegaly, lymphadenopathy, and aseptic meningitis within the first 3 months of life have been reported.^{4,25} Low birth weight (<1500 g) has been described as a risk factor for symptomatic postnatal infection.^{4,25} No association of postnatal CMV infection with hearing loss or neurological developmental impairment has been found, though data on the long-term follow-up of postnatally infected (premature) infants are limited.²⁵ In immunocompromised patients, CMV can cause significant morbidity and mortality due to CMV colitis, hepatitis, encephalitis, pneumonitis and retinitis.

Epidemiology of congenital CMV infection

Intrauterine infection with CMV is thought to result from maternal viremia and associated placental infection.^{4,7} In a meta-analysis, intrauterine transmission was estimated to occur in approximately 32% (95%CI 30-35%, range 14-52%) of the pregnant women with primary infection.¹⁶ The maternal-to-fetal transmission risk after primary infection increases with gestational age and has recently been reported to be up to 64-73% in the third trimester.^{26,27,28} In contrast, the highest risk of fetal damage (including hearing loss) exists around conception and in the first two trimesters of pregnancy.^{28,29,30,31} In contrast to congenital rubella and toxoplasmosis, where intrauterine transmission occurs principally as a result of primary maternal infection, intrauterine transmission of CMV can occur as a consequence of non-primary or recurrent infection, i.e. reactivation of latent virus or re-infection with a new strain. In a meta-analysis of data on the birth prevalence of congenital CMV among the offspring of seropositive women, the pooled risk of maternal-to-fetal transmission following recurrent infection was 1.4% (95%CI 1.1-1.7%) (Figure 2).¹⁶

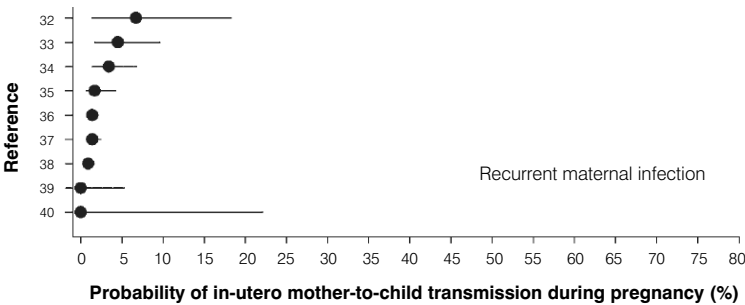


Figure 2 Meta-analysis of studies reporting the prevalence of CMV at birth (congenital infection) among the offspring of CMV seropositive pregnant women, described as the maternal-to-fetal transmission risk following recurrent maternal infection. Lines represent 95%CI. (Adapted from Kenneson et al.¹⁶)

In the same meta-analysis, the combined birth prevalence of congenital CMV reported by 27 worldwide study groups was 0.64% (95%CI 0.60-0.69%), with considerable variability among different populations.¹⁶ Data on the birth prevalence of congenital CMV in the Netherlands are limited (until publication of the work presented in this thesis) to a single study reporting a birth prevalence of 0.09% in a selected sample of newborns with a low proportion of immigrants.¹⁷ In general, the birth prevalence of congenital CMV increases with maternal CMV seroprevalence in the population, with a birth prevalence estimate of about 2% or higher in populations with >95% seroprevalence (Figure 3).¹⁶ Recently, a prospective, serological study has shown that re-infection with new strains occurred in about 8% of seroimmune pregnancies among a population with nearly 100% CMV seroprevalence (Brazil).⁴¹ Risk factors for congenital CMV infection, mainly derived from case-control studies, are summarized in Table 1.

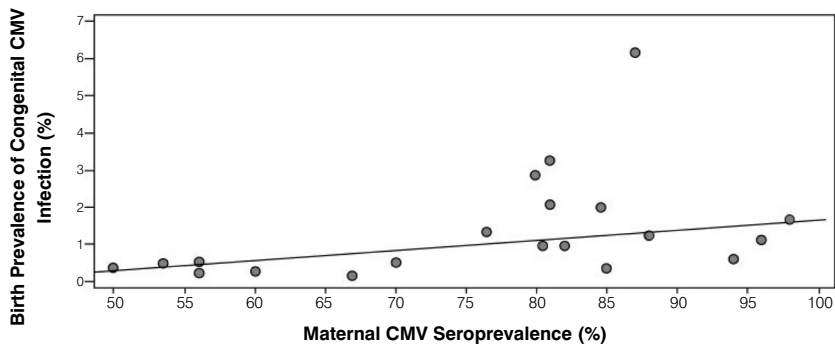


Figure 3 Birth prevalence of congenital CMV is positively correlated with maternal CMV seroprevalence in the population. Each circle represents the birth prevalence estimate from one study group. In linear regression analysis, every 10% increase in seroprevalence corresponded to a 0.26% increase in birth prevalence. The coefficient of determination (R^2) was 0.29^{16,42}, and 0.55 in an earlier report^{16,42}, indicating that maternal seroprevalence accounted for respectively 29%¹⁶ and 55%⁴³ of the variability of birth prevalence of CMV between study populations. (Adapted from Kenneson et al.¹⁶)

Table 1 Factors reported to be significantly associated with congenital CMV infection.

Risk factor	Reference
High maternal seroprevalence in the population	16
Non-white race	16
Low socioeconomic status	16
Caring for preschool children	43
Household size >3 people	43
Maternal age <25 years	43
Onset of sexual activity <2 years before delivery	43
STD during pregnancy	43

STD; sexually transmitted disease

Clinical manifestations of congenital CMV

Approximately 10% of the infants born with congenital CMV infection are symptomatic at birth.^{16,44} Half of these symptomatic infants present with typical and potentially fatal generalized cytomegalic inclusion disease (CID), characterized by hepatosplenomegaly, microcephaly, jaundice, and petechiae, with or without ocular

and auditory damage.^{3,7,45} In total 40-58% of these infants symptomatic at birth will have permanent sequelae.⁴⁴ Because early studies focussed on symptomatic infections, congenital CMV was considered a rare and often fatal disease.⁷ Nowadays, we realize that 10-15% of the infants born with asymptomatic congenital infection develop neurologic complications throughout the first years of life and will have long-term sequelae.⁴⁴ Isolated sensorineural hearing loss (SNHL) is the most common long-term complication of congenital CMV.⁴⁶ Because of the late-onset nature of the hearing loss (Figure 4), up to half of the children with congenital CMV-related hearing loss may not be detected in the newborn hearing screening.⁴⁷ Among children with hearing loss at later ages, the hearing loss is associated with congenital CMV in 15-40% of the cases.^{48,49,50} Other neurologic complications in newborns with congenital CMV are summarized in Table 2, according to symptomatic and asymptomatic status at birth.

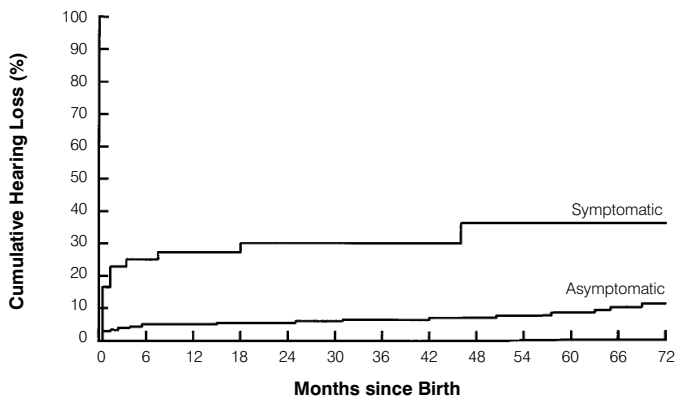


Figure 4 Cumulative hearing loss (>20 dB) in children with congenital CMV infection according to symptomatic and asymptomatic status at birth. (Adapted from Fowler et al.⁴⁷)

Table 2 Frequency of neurologic complications in newborns with congenital CMV infection, according to symptomatic and asymptomatic status at birth. (Adapted from Remington and Klein.⁷⁾)

	Symptomatic at birth	Asymptomatic at birth
Hearing loss	58%	7%
IQ <70	55%	4%
Chorioretinitis	20%	3%
Seizures	23%	1%
Paresis/paralysis	13%	0%
Death	6%	0%

Clinical symptoms are more frequently seen in newborns from preconceptionally seronegative women⁴⁵ (Table 3), indicating that maternal antibodies provide substantial protection against harmful infection in the newborn.

Table 3 Neurologic sequelae in children with congenital CMV infection after primary and non-primary maternal infection. (Adapted from Fowler et al.⁴⁵)

	Primary infection	Non-primary infection
Any sequelae	25%	8%
Sensorineural hearing loss	15%	5%
IQ <70	13%	0%
Chorioretinitis	6%	2%
Microcephaly	5%	2%
Seizures	5%	0%
Paresis or paralysis	1%	0%
Death	2%	0%

Postnatal diagnosis of congenital CMV

The gold standard in the diagnosis of congenital CMV is viral culture of urine, sampled within the first 2-3 weeks of life.^{51,52,53} After this period, congenital CMV infection cannot be differentiated from the generally harmless postnatally acquired CMV infection.⁵³ CMV DNA detection (PCR) in urine, saliva, and blood is mentioned in recent literature and described in guidelines as acceptable alternative for diagnosing congenital CMV.^{54,55,56} Furthermore, CMV DNA detection in dried blood stored on filter paper (dried blood spots, DBS; Guthrie cards) has become of interest and has the advantage that congenital CMV can be diagnosed retrospectively, e.g. when late-onset hearing

loss becomes manifest.^{57,58,59,60,61,62,63} Serological testing for CMV IgM in the newborn lacks adequate sensitivity (range 20%^{16,64,65}-70%⁶⁶) and specificity (about 95%⁶⁶) for the diagnosis of congenital infection. Sensitivity of IgM serology in the congenitally infected newborn is hampered by the time-frame between fetal infection and birth, and the immature immune system at the time of infection. CMV IgM antibodies can be detected in only about 25% of the DBS of newborns with congenital CMV.⁶⁷

Postnatal antiviral therapy

Antiviral treatment of congenitally CMV infected newborns with clinically apparent disease is generally accepted^{54,68,69,70}. Few studies have addressed the efficacy of antiviral treatment on hearing preservation in newborns with symptomatic and asymptomatic congenital CMV infection (Table 4). Results from 1 RCT show that congenitally infected newborns with central nervous system (CNS) disease benefit from ganciclovir with preserved hearing⁷¹ and recent guidelines include the recommendation of antiviral treatment in this specific group of newborns.^{54,56} In addition to preserved hearing, improvement of neuro-developmental status after treatment of newborns with CNS disease has been found.⁷² Treatment of asymptomatic congenitally CMV infected newborns is currently not recommended because of limited data on the efficacy in that specific group of newborns.^{54,56}

Table 4 Studies on the efficacy of antiviral treatment on hearing preservation in newborns with congenital CMV. Case-reports were excluded.

Reference	Newborns	Design	Intervention	Primary outcome	Efficacy	Remarks
Kimberlin et al ⁷¹ (2003)	Symptomatic with CNS disease ^a (≤1 mth)	RCT (N=100)	GCV (6 wks) ^c	Hearing ^d at 1 yr	↓ Worsening (P <.01) Treated: 21% Controls: 68% > Efficacy 69%	-50% Lost to follow-up -No placebo, not blinded -Neutropenia Treated: 63%, Controls: 21%
Lackner et al ⁷³ (2009)	Asymptomatic, normal hearing (≤10 dys)	RCT (N=23)	GCV (3 wks) ^c	Hearing ^d at 4-10 yrs	↓ Hearing loss (NS) Treated: 0% Controls: 11%	-No placebo -Neutropenia: 17%
Amir et al ⁷⁴ (2010)	Symptomatic with CNS disease ^a (≤1 mth)	Retrospective case series, historical controls ⁷¹ (N=23)	ValGCV (1 yr) ^{b,c}	Hearing ^d at 1 yr	↑ Normal hearing (P<.001) 1 yr treated: 76% 6 wks treated: 35%	-Historical controls -Neutropenia: 53%
Baquero-Artigao et al ⁷⁵ (2011)	Symptomatic with CNS disease ^a (age NR)	Retrospective case series (N=55)	ValGCV (median 6 mths, range 1.4-18 mths) ^{b,c}	Hearing ^d at 1 yr	Improved: 36% Worsening: 4% (among impaired)	-No controls -Neutropenia: 53%

^a CNS involvement criteria: (1) microcephaly, (2) intracranial calcifications, (3) abnormal CSF, (4) chorioretinitis, and/or (5) hearing deficits.

^b Preceded by GCV in 67%⁷⁵ to 100%⁷⁴ of the patients.

^c Dosage valGCV: 16 mg/kg twice daily⁷⁵, m³x clearance x 7mg once daily⁷⁴, GCV: 5 mg/kg twice daily⁷⁴, 10 mg/kg daily⁷³, 6 mg/kg twice daily⁷¹

^d BSER^{72,74,75} or OAE⁷⁶

CNS: central nervous system, RCT: randomized controlled trial, GCV: ganciclovir (i.v.), ValGCV: valganciclovir (p.o.), NS: not significant, NR: not reported, CSF: cerebrospinal fluid, BSER: brainstem-evoked response (=auditory brainstem response, ABR), OAE: otoacoustic emissions

Prevention

Preventive programs for CMV infection have been developed by the United States Centers for Disease Control and Prevention (CDC), and the American College of Obstetricians and Gynecologists (Figure 5). Because exposure to saliva and urine of young children is a major cause of CMV infection among pregnant women⁷⁶, it is likely that good personal hygiene can reduce the risk of CMV acquisition. Evidence for the efficacy of hygiene counseling is limited to studies showing a reduced rate of CMV seroconversion of pregnant woman after hygiene counseling.^{76,77,78}



Figure 5 Hygienic measures recommended by the CDC to pregnant women to reduce the risk of CMV infection in pregnancy (www.cdc.gov/features/dscytomegalovirus/).

Newborn screening for congenital CMV

General criteria for screening have been proposed by Wilson and Jungner (Table 5).⁷⁹ In the Netherlands, newborns are routinely screened for 18 metabolic and inherited disorders, including PCR-based screening on cystic fibrosis using DBS (since May 2011). Newborn screening for congenital CMV has only recently been seriously considered, despite earlier appeals for preventive measures for congenital CMV infection.^{80,81} The potential for newborn screening for CMV would lie in the identification of the large proportion of asymptomatic congenitally infected newborns at risk for developing late-onset hearing loss. These newborns at risk could benefit from intervention measures such as extensive audiological follow-up and potentially, antiviral therapy.

Table 5 Criteria for screening as proposed by Wilson and Jungner.⁷⁹

The disease	The condition should be an important health problem The natural history should be well understood There should be a detectable early stage
The screening test	The test should be suitable for the early stage The test should be acceptable to patient and staff Intervals for repeating the test should be determined
The treatment	Early treatment should be of more benefit than at a later stage Adequate health service provision should be made The risks should be less than the benefits The costs should be balanced against the benefits

Outline of the thesis

The aim of this thesis is to study several aspects of congenital CMV infection in general and more specifically in the Netherlands, in order to determine the necessity and feasibility of newborn screening for congenital CMV. The major topics addressed in this thesis are the following.

PART I

The DISEASE BURDEN of congenital CMV infection in the Netherlands.

This topic is addressed in several ways. Firstly, the birth prevalence of congenital CMV in the Netherlands was determined in a cross-sectional study. A large sample of DBS from infants born in the Netherlands was retrospectively tested for CMV DNA (Chapter 2). To address the clinical impact of congenital CMV disease in the Netherlands, the proportion of congenital CMV infections among Dutch children with permanent bilateral hearing loss was determined (Chapter 3). Additionally, to address subpopulations at risk for congenital CMV infection, risk factors for congenital CMV in the Dutch population were analyzed (Chapter 2). Furthermore, maternal immunity to CMV as risk factor for congenital infection was assessed by means of a population-based prediction model (Chapter 4). Finally, awareness of the disease burden of congenital CMV among doctors in the Netherlands was studied using a digital questionnaire sent to doctors involved in mother and childcare. (Chapter 5).

PART II

Postnatal SCREENING TOOLS for congenital CMV were studied by evaluating a large number of DNA extraction methods for dried blood spots (DBS) (Chapter 6 and 7), and by evaluating real-time PCR on urine in the diagnosis of congenital CMV (Chapter 9). Following CMV DNA detection in DBS, the potential to use DBS for genotyping of CMV was assessed (Chapter 8).

PART III

Pros and cons of NEWBORN SCREENING for congenital CMV are summarized and discussed in detail in Chapters 10, 11 and 12. Rationale for potential benefits and disadvantages of newborn screening on congenital CMV are addressed, using the criteria of Wilson and Jungner to summarize the disease burden, the currently available screening tests, and the evidence for intervention options.

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