Chapter 10

Summarizing discussion

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This thesis addresses several aspects of congenital cytomegalovirus (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 studied were

I. the **disease burden** of congenital CMV infection in the Netherlands,

II. postnatal **screening tools** for congenital CMV, and

III. pros and cons of **newborn screening** for congenital CMV.

In this chapter, the implications of our main findings are discussed, overall conclusions are formulated and recommendations for future studies are made.

PART I DISEASE BURDEN OF CONGENITAL CMV INFECTION

The birth prevalence of congenital CMV in the Netherlands was 0.54% (95%CI 0.36–0.72%) (*Chapter 2*)

IMPLICATIONS OF THIS FINDING

In a cross-sectional study, a large sample of dried blood spots (DBS) from infants born in the Netherlands was retrospectively tested for CMV DNA. The birth prevalence of congenital CMV was estimated at 0.54% (95%CI 0.36–0.72%) (*Chapter 2*). This finding, combined with the total number of newborns in the Netherlands (182,765 newborns/ year, 2007)[1], implicated that annually about 1000 children are born with congenital CMV infection in the Netherlands. This annual number of congenitally infected newborns is higher than some other well-known congenital conditions (Figure 1), including Down syndrome and spina bifida, for which prenatal screening is standard care.[2] Moreover, congenital CMV is at least 10 times more frequent than congenital hypothyroidism, and 100 times more frequent than homocystinuria, both disorders for which postnatal screening is standard care nowadays.[3] Based on the current knowledge on the natural history of congenital CMV infection[4], about 125 of these 1000 congenitally infected cases are expected to be symptomatic at birth. Approximately 5 congenitally infected newborns are expected to die each year in the Netherlands because of severe CMV inclusion disease. About 18% (1 out of 5) of the newborns with congenital CMV will develop neurological sequelae.[4] This implies that annually about 180 of these 1000 infected children born in the Netherlands will

eventually suffer from CMV-related sequelae, of whom 87%[4] (157) were asymptomatic at birth. The most frequently encountered sequela related to congenital CMV infection is hearing loss, followed by mental retardation, developmental delay, visual impairment, seizures, and paresis/paralysis. These conditions are known to have profound and life-long impact on the affected children and their families.

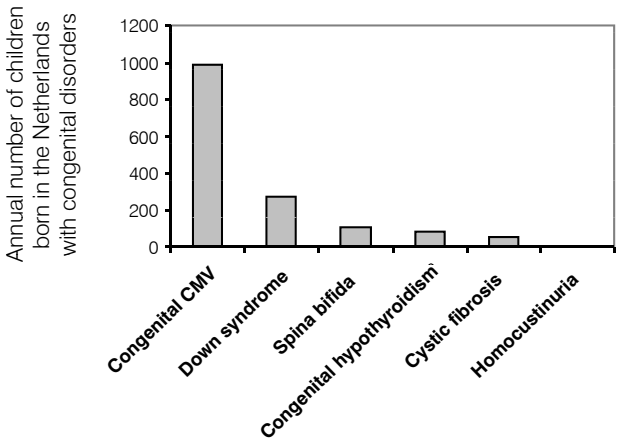

Figure 1 Annual number of newborns with congenital CMV in the Netherlands compared with several other congenital conditions (data from 2007).[2]

Recommendations for future studies

Our calculations of the number of infants with CMV-related sequelae and symptoms at birth were based on previous data on the natural history of congenital CMV infection in the United States.[4] The frequency and severity of clinical symptoms at birth and the long-term sequelae are known to differ among primary and non-primary maternal infections.[5] The proportion of primary and non-primary infections is associated with the seroprevalence in the underlying population (*Chapter 4*), and therefore varies among different countries. Thus, it would be interesting to study the prevalence of CMV-related symptoms and sequelae in the Netherlands, in a prospective study design. Follow-up of neurologic sequelae would be desirable for many years after birth because of the frequent late-onset and progressive nature of the hearing loss associated with congenital CMV.[6,7] Since developmental disorders (e.g. IQ<70) and visual impairment are the second and third most frequently encountered sequelae of congenital CMV infection[5,8], it would be interesting to address the prevalence of

congenital CMV-related mental retardation and more subtle mental, developmental and visual impairment in the Netherlands.
It has been suggested that congenital CMV infection is associated with disorders belonging to the autism spectrum. However, evidence is limited to case reports and a small series of children diagnosed with both autism and congenital CMV.[9] A large study would be necessary to rule out or confirm this speculative association of congenital CMV with autism. One of the major challenges of a retrospective analysis would be the age of diagnosis of autism, in combination with the limited time-frame to retrospectively diagnose congenital CMV using DBS (the storage duration of DBS in the Netherlands is 5 years).

About 1 out of 5 deaf children in the Netherlands was congenitally infected with CMV

2 of the 8 (25%) congenitally infected children with hearing loss at later age had passed the newborn hearing screening (*Chapter 3*)

Implications of these findings

Analyzing a cohort of children in the Netherlands with bilateral hearing loss at a later age (3-5 years), we found that the hearing loss was associated with congenital CMV infection in 1 in 5 deaf children (*Chapter 3*). This would render CMV the leading cause of non-genetic congenital hearing loss. Importantly, 2 of the 8 (25%) infants with both congenital CMV and hearing loss had passed the newborn hearing screening test, probably because of delayed-onset or progressive hearing loss. One should be aware that, in the absence of universal screening for congenital CMV infection, up to half [6] of the children with congenital CMV associated hearing loss at later ages may be missed by newborn hearing screening. Consequently, the Joint Committee on Infant Hearing recommended additional hearing evaluations in children with congenital CMV.[10]

Furthermore, we found that children with both hearing loss and congenital CMV had a greater delay in language comprehension than uninfected infants with comparable degrees of hearing loss. This implies that the delay in language comprehension in the infected infants was the result of a factor additional to the hearing loss, possibly cerebral damage resulting from congenital CMV infection.

Recommendations for future studies

The delayed onset and progressive nature of the hearing loss associated with congenital CMV is remarkable and the pathological mechanisms involved are largely unknown. CMV DNA has been detected in inner ear fluids (perilymph) of congenitally infected children up the age of 7 years, undergoing cochlear implant surgery.[11,12,13,14] The presence of CMV genome in the cochlea up to several years after birth supports the hypothesis of ongoing replication of CMV in the inner ear.[14] Moreover, this would be in line with data on long-term viral shedding in other body fluids of children with congenital CMV infection. The median duration of shedding of CMV in urine has been found to be approximately 4 years in both symptomatic and asymptomatic children.[15] CMV detection in the inner ear is limited to few reports describing a small number of congenitally infected patients. It would be interesting to further unravel the pathological mechanism of hearing loss associated with congenital CMV infection by analyzing the inner ear fluid of a large number of children undergoing cochlear implant surgery. Such a study would also provide insight in the proportion of congenital CMV infections among children with cochlear implants in the Netherlands, and would enable more detailed estimates of the disease burden and costs involved.

Subpopulations in the Netherlands with more young children, and with more non-western immigrants, had a higher risk of congenital CMV infection (*Chapter 2*)

Implications of this finding

Our region based case-control analysis showed that congenital CMV infection was most frequent in subpopulations with a high proportion of young children (a 6 times higher risk), and non-Western immigrants (a 3 times higher risk) (*Chapter 2*). The proportions of young children and immigrants in a population can be seen as demographic markers for environmental factors and behaviors that facilitate CMV transmission. Young children shed CMV in their body fluids, and a CMV shedding child

is a known risk factor for maternal CMV infection.[16] Similarly, CMV seroprevalence is reported to be higher among immigrant mothers than among native Dutch mothers[17], implicating a more frequent exposure to CMV. Factors involved in increased exposure and potentially related to cultural differences include large household size, crowding, certain child care practices, and possibly sexual practices.[8] Assessing subpopulations and indicating (behavioral) risk factors for congenital CMV infection in the Netherlands will provide insight in the transmission of CMV and potential preventive measures. While a vaccine is currently unavailable, prevention of congenital CMV infection is limited to hygiene practices.

Recommendations for future studies

In our study, risk factors were analyzed at a regional level. It would be interesting to assess risk factors for congenital CMV in the Netherlands at the individual level, in a prospective or retrospective design. Identification of risk factors is vital for proposing preventive measures. While there is evidence that hygiene counseling results in a reduced rate of CMV seroconversion among pregnant women[18,19,20], further studies are required to determine whether these measures reduce the rate of congenital CMV infection and disease.

Non-primary maternal CMV infections were estimated to account for the majority of congenital CMV infections (*Chapter 4*)

Implications of this finding

Applying the population-based prediction model we developed, we found that, for populations with CMV seroprevalence of 30% to 95%, non-primary maternal CMV infections accounted for the majority of congenital CMV infections (*Chapter 4*). The proportion of newborns with congenital CMV attributable to non-primary maternal infections was up to 96% in populations with seroprevalence of 95% (95%CI 88-99%). Additionally, the proportion of newborns with sequelae attributable to non-primary infections increased with CMV seroprevalence, and was up to 89% (95%CI 26-97%). These findings stressed the impact of non-primary infections on the disease burden of congenital CMV.

Combining this prediction model (*Chapter 4*) with our findings on the birth prevalence of congenital CMV in the Netherlands (*Chapter 2*), additional estimates could be made on the proportion and number of congenitally infected children born

from seropositive mothers in the Netherlands (Figure 2). Data on maternal CMV seroprevalence in the Netherlands (50%[17,21]) were combined with the annual number of newborns with congenital CMV and CMV-related sequelae in the Netherlands (987 and 177, respectively, *Chapter 2*). This resulted in an estimate of 681 congenitally infected children born from seropositive mothers in the Netherlands annually, of whom about 76 children eventually will be affected by sequelae. The birth prevalence of congenital CMV in the Netherlands as predicted by our model (based on 50% CMV seroprevalence) corresponded with the birth prevalence as detected in our cross-sectional study (0.51% and 0.54%, respectively).

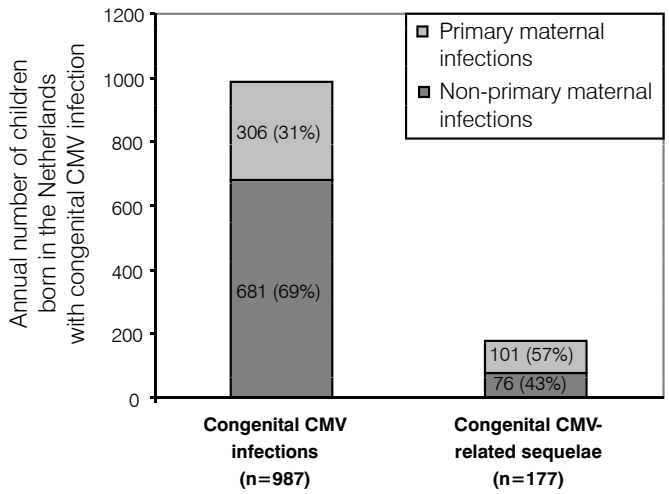

Figure 2 Annual number of children born in the Netherlands with congenital CMV infection and with CMV-related permanent neurological sequelae (at later ages) according to primary and non-primary maternal infection. Estimates were based on the population based predication model (*Chapter 4*) (50% CMV seroprevalence[17,21]) and the annual number of congenitally infected newborns based on the birth prevalence in the Netherlands (*Chapter 2*).

Similarly, additional calculations could be made when combining our model (*Chapter 4*) with seroprevalence data of subpopulations in the Netherlands found to be at higher risk of congenital CMV (*Chapter 3*). CMV seroprevalence data of subpopulations of Dutch and Turkish/Moroccan origin in the Netherlands were used (35% and 96% seroprevalence[17,21], respectively). Among mothers of Turkish/Moroccan origin, non-primary maternal infections were estimated to account for 91% of the congenital infections with long-term sequelae (Figure 3).

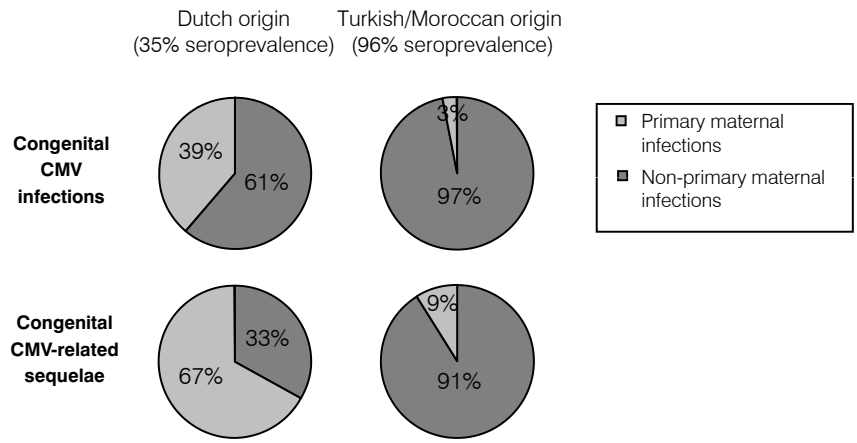

Figure 3 Estimated proportion of children with congenital CMV and CMV-related sequelae in the Netherlands among subpopulations of Dutch and of Turkish/Moroccan origin, according to non-primary and primary maternal infection (seroprevalence of 35% and 96% [17], respectively). Estimates were based on the population based prediction model (*Chapter 4*).

The apparent contradiction of maternal immunity as a risk factor for congenital CMV can be explained by the higher force of (re-)infection in highly seroprevalent (sub)populations.[22] Additionally, maternal re-activations may play a role. Awareness of the risk of seroimmune pregnant women of having a congenitally infected and neurologically affected newborn will have significant consequences for preventive strategies to reduce the disease burden of congenital CMV. Preventive measures such as hygiene counseling should not be limited to seronegative pregnant women. In that case, prenatal maternal serological screening will be futile as long as no adequate intervention option is available. Awareness of the fact that CMV immunity

is only partially protective for congenital infection raises questions on the ratio of re-infections with new strains versus reactivations of latent virus in seroimmune pregnant women. Passive and active immunization efforts should aim at provision of antibodies and vaccines for both seronegative and seropositive women, while, currently, an immunological correlate of full protection against congenital CMV infection and disease seems to be lacking. Recently, a CMV glycoprotein B vaccine has been shown to boost immunity in CMV seropositive women[23], however future studies are needed to determine the capacity of this vaccine to reduce congenital CMV infection and disease.

Recommendations for future studies

Practical data are desired to confirm our theoretical estimates, which were derived from a model, based on data from previous reports. A prospective study with follow-up of a large cohort of pregnant women would deliver data on the (re-)infection rate among pregnant women in the Netherlands. Preliminary data we obtained by means of an additional cross-sectional study in which sera from CMV seropositive pregnant women in the first trimester were assessed for CMV DNA, indicated that a very low proportion of these women was CMV viremic at the time of sampling (1/122, 0.8% of CMV IgG positive sera, data not shown). Furthermore, it would be interesting to distinguish re-infections with new strains from reactivations of latent virus in pregnant women. A recent serological study showed that re-infection with new strains was a major source of congenital infection, occurring in about 8% of seroimmune pregnancies in Brazil.[24] However, the proportion of maternal re-infections versus reactivations resulting in congenital infection is not known and further studies distinguishing CMV strains by means of serology and/or genome analysis (see *Chapter 8*) would be helpful. Immunization studies addressing the capacity of CMV vaccines to reduce maternal-to-fetal transmission rates among both seronegative and seroimmune women are needed. A search for correlates of protection of fetal infection and disease is essential.

Knowlegde of the responding obstetricians and gynecologists (in training) on congenital CMV infection was suboptimal (*Chapter 5*)

Implications of this finding

A digital questionnaire sent to interns, residents, senior doctors, general practitioners and medical researchers involved in mother and child care in the Netherlands, suggested that the responding physicians (in training) had suboptimal knowledge concerning congenital CMV. About half of the responding obstetricians and gynecologists (in training) were not aware of the fact that CMV is not transmitted by air and can be transmitted by kissing young children on the mouth and changing diapers (*Chapter 5*). Furthermore, only the minority of the respondents in pediatrics realized that newborns with congenital CMV may be asymptomatic at birth and that 1 out of 5 congenitally infected newborns will develop long-term sequelae. Our findings imply that congenital CMV infections may not be recognized by these physicians and therefore under-diagnosed with the risk of treatment delay or refrain. Furthermore, these physicians were not likely to be able to optimally advise on the risk of congenital CMV and how this risk may be reduced.

Recommendations for future work

Education of physicians on congenital CMV is expected to result in increased awareness, and awareness of physicians is essential for awareness of pregnant women and policy makers in health care. Increased knowledge and awareness of physicians and pregnant women is expected to improve recognition and care, to stimulate diagnostic investigations and audiological follow-up of infected newborns, and to enhance preventive measures. A two-fold reduction of the risk of seroconversion among pregnant women has been reported[20] after advising mainly three hygiene measures, also promoted by the CDC: 1. hand washing after diaper changes, 2. avoiding kissing young children on the mouth, 3. avoiding sharing utensils. A large study is needed to determine the effect of hygiene measures on the number of (prevented) congenital CMV infections. Overall, it is recommended that educational efforts are increased employing all possible methods to reach all groups involved.

OVERALL CONCLUSIONS OF PART I

Combining insights provided by the findings presented in part I of this thesis as well as from other available data, it can be concluded that the disease burden of congenital CMV in the Netherlands is considerable. Congenital CMV infection is the most frequent congenital disorder and appears to be the leading cause of non-genetic congenital hearing loss. Congenital CMV disease affects all subpopulations in the Netherlands, and seronegative as well as seropositive pregnant women are at risk of having a newborn with congenital CMV-related disabilities. The disease burden is striking when one realizes that a non-negligible part of the congenitally infected newborns with late-onset hearing loss is not detected in the newborn hearing screening, with delayed intervention for hearing loss as a consequence. Uncorrected prelingual hearing loss has profound negative effects on speech and language development, communication and learning, and affects the socio-economic status of the affected children and their families. Though an extensive analysis of the exact costs involved in congenital CMV disease is currently underway, lifetime costs of prelingual bilateral hearing loss, irrespective of etiology, are impressive (>700,000 euros per disabled individual[25,26,27,28]). Moreover, additional to the postnatal disease burden of congenital CMV addressed in this thesis, congenital CMV has been associated with intra-uterine fetal death[29,30,31,32], increasing its impact even further.

Taken together, it can be concluded that **congenital CMV infection can be labeled as an important public health problem**.

PART II POSTNATAL SCREENING TOOLS FOR CONGENITAL CMV

Sensitivity of CMV DNA detection in dried blood spots (DBS) varied widely, depending on the DNA extraction method used (*Chapter 6 and 7*)

IMPLICATIONS OF THIS FINDING

Sensitivity and applicability of several DNA extraction methods for high-throughput usage were assessed by means of *in vitro* experiments using Guthrie cards spotted with CMV positive blood. Significant differences were found between the extraction methods with respect to the sensitivity. Sensitivities ranged up to about 86% for Guthrie cards spotted with CMV DNA loads around the reported[33] median load of 3.4 $\log_{10}$ copies/ml for symptomatic and asymptomatic congenitally infected newborns. When considering the usage of DBS for universal newborn screening for congenital CMV infection, an assay which is sensitive, specific, and applicable for 96-well format testing, while using only a very small amount of dried blood, is required. When evaluating screening assays, the predictive values of screening test results are even more important than sensitivity and specificity. Considering a national congenital CMV birth prevalence of 0.54% (*Chapter 2*), a screening test with a sensitivity of 75% would still result in a negative predictive value as high as 99.8%. Furthermore, the demonstrated association between viral load and outcome[34,35,36,37] suggests that any cases missed would be those with the lowest viral loads and probably the lowest chance of developing severe permanent sequelae. Thus, the clinical sensitivity, based on the detection of children who will eventually develop sequelae, may well be acceptable.[38,39,40]

Recommendations for future studies

When considering universal newborn screening for congenital CMV infection, an assay which is sensitive, specific, and applicable for 96-well format testing is needed. In view of the existing route of the national metabolic screening program, DBS would be the most practical specimen of choice. Experience with DNA detection in newborn screening laboratories is accumulating, in particular in the postnatal screening for cystic fibrosis.[41] Therefore, it is interesting to further optimize DBS DNA extraction protocols, PCR techniques, testing algorithms, and test procedures. Large scale

prospective and retrospective studies have assessed several PCR-based assays for CMV detection in DBS and their results mainly correspond with our findings, reporting sensitivities of 71-100%.[42,43,44,45] In contrast, the widely commented[38,39] study by Boppana et al[46] reported a sensitivity as low as 34% of the specific DBS assay used to screen 20,448 newborns. Exploratory studies in which optimized CMV DBS assays are used for large-scale newborn screening are needed to address remaining analytic and logistic issues.

While further exploring DBS PCR assays, alternative assays with potential for sensitive and high-throughput detection of CMV may be explored. Recently, the use of dried saliva for screening for congenital CMV has been tested and found to be very sensitive. Table 1 summarizes clinical pilot studies on PCR-based newborn screening assays for congenital CMV infection reported to date. Future studies are likely to address the logistic feasibility of materials other than DBS in more detail. Potentially, logistic issues may be more challenging in countries where a large proportion of the children are born and sampled in their home environment.

Additionally, since current metabolic screening is mainly performed using mass spectrometric assays, it would be logistically advantageous to use mass spectrometric detection of CMV in DBS. While it may be difficult to detect relatively low amounts of CMV-specific proteins present in DBS, it would be worthwhile to explore mass spectrometric detection of CMV.

Table 1 Clinical pilot studies on newborn screening for congenital CMV infection reported in literature. Studies using PCR-based screening assays were included and predictive values were calculated.

Reference	Screening test (PCR-based) sample	Comparison (gold standard)	Sensitivity	Specificity	Birth prevalence	PPV	NPV
[43] (2011)	DBS[a]	Urine PCR	100% 96.9%	98.1% 99.0%	23.6% (64/271)[c]	94.1% 96.9%	100% 99.0%
[46] (2010)	DBS[b]	Saliva culture	34.4%	99.9%	0.5% (92/20,448)	91.7%	99.8%
[47] (2011)	Saliva Liquid Dried	Saliva culture	 100% 97.4%	 99.9% 99.9%	0.5% (177/34,989)	 91.4% 90.2%	 100% 99.9%
[48] (2006)	Saliva Urine	Urine and saliva culture[d]	85.7% 92.9%	100% 100%	1.5% (28/1923)	100% 100%	99.8% 99.9%
[49] (2010)	Urine Throat swab DBS	Urine and throat culture	100% 100% 50.0% (1/2)	100% 100% 100%	1.5% (2/137)[c]	100% 100% 100%	100% 100% 99.3%
[50] (2011)	Urine (pooled)	Urine culture	100%	100%	6.3% (10/160)[c]	100%	100%
[51] (2011)	Urine (on filter paper)	[e]	-[f]	-	0.3% (66/21,272)	94.0%	-
[52] (2009)	Urine and/or saliva	[e]	-	-	1.1% (87/8047)	100%	-
[53] (2008)	Urine (on filter paper)	[e]	-	-	0.4% (4/901)	100%	-
[54,55] (2003,2005)	Urine	[e]	-	-	0.7% (14/2000)	90.0%	-
[56] (2009)	Umbilical cord blood	[e]	-	-	0.2% (2/1010)	100%	-
[57] (2006)	Umbilical cord blood	-	-	-	0.5% (2/433)	-	-

[a] QIAamp DNA Blood Mini Kit (Qiagen) extraction using 1 whole DBS,
[b] Qiagen M48 robot (MagAtract) extraction using two 3-mm disks,
[c] Selected population of newborns,
[d] Comparison included a subset (n=100) of screening negative samples,
[e] Only positive screening test results were confirmed with urine and/or saliva culture,
[f] DBS sensitivity 75% (9/12)
PPV; positive predictive value, NPV; negative predictive value, DBS; dried blood spot

The multiplex real-time CMV glycoproteins B and H genotyping assays developed, were efficient, sensitive for detecting mixed infections in plasma, and applicable for usage on DBS (*Chapter 8*)

Implications of this finding

Detection of CMV DNA in DBS has been shown to be a challenge[44,45,42,46] due to the small amount of dried blood available (*Chapter 6*). In spite of this, using our genotype assay, a genotype could be assigned to approximately 75-80% of the CMV DNA positive DBS of congenitally infected newborns. Others have shown that genotyping of CMV has supported the discrimination of reactivation of latent virus from re-infection with new CMV strains in plasma from transplant patients, allowing a better definition of donor-to-recipient transmission patterns.[58] As described in *Chapter 4*, congenital CMV infections mainly result from recurrent maternal infections, comprising re-infections and possibly reactivations. Our genotyping tool might support the discrimination of maternal reactivation from re-infection, reveal mother-to-fetus transmission patterns and the clinical outcome of congenital infection after reactivation versus re-infection. Increased insight into transmission risks of latent and new strains may have significant implications for preventive and therapeutic strategies, including CMV vaccine research.

Recommendations for future studies

Future studies, analyzing a large number of newborns and their mothers should address the frequency of re-infections and reactivations, mother-to-fetus transmission patterns, and the potential role of congenital infections with multiple CMV genotypes. Additionally, it would be of interest to study the presence of genomic variants longitudinally within one human host. Recent genome-wide next-generation sequencing of CMV in urine of congenitally infected newborns suggested that the genomic intra-host variability of CMV (0.2% nucleotide diversity per sample) may be comparable to that of many RNA viruses.[59]

CMV was more frequently detected by real-time PCR than by viral culture of neonatal urine samples (Chapter 9)

Implications of this finding

A retrospective analysis of a large series of neonatal urine samples received for congenital CMV diagnostics, showed that CMV was more frequently detected by real-time PCR than by viral culture. False negative CMV urine culture results have been reported, both in experimental setting[60] and in clinical setting[61,62,63,64], and therefore seem the most likely explanation for our discrepant test results. Loss of viable CMV particles implicated in false negative culture results may be caused by transport at room temperature[65], and/or antiviral therapy. Our results, supported by analytical and clinical data on CMV DNA detection in neonatal urine, suggested enhanced sensitivity of recent PCR techniques when compared to viral culture. These combined findings provide considerable rationale to favor real-time CMV PCR as a gold standard in the diagnosis of congenital CMV infection.

Recommendations for future studies

Data from large-scale studies combining clinical data from newborns with diagnostic inhibition-controlled real-time CMV PCR procedures should address the differentiation between congenitally and postnatally acquired CMV infection, and should determine the exact time-frame for sampling of neonatal urine when using real-time PCR.

OVERALL CONCLUSIONS OF PART II: SCREENING TOOLS

Combining the findings presented in part II of this thesis with data from the literature, the overall conclusion would be that, now that several newborn screening tools for congenital CMV have been studied, PCR-based screening assays using DBS, saliva, and urine appear to be the most attractive tools currently available for newborn screening for congenital CMV. Whereas saliva and urine samples have the advantage of containing high viral loads[53] and potentially high test sensitivity, DBS have the major logistic advantage of being suitable for use in Guthrie card-based metabolic screening. The wide range of sensitivities of DBS PCR assays reported in (clinical pilot) studies including our own, provides the insight that sensitivity data of specific DBS PCR assays cannot be generalized. It appears that a sub-selection of DBS PCR assays with high DNA extraction capacity has the potential to achieve sensitivity and specificity levels approaching those of assays currently used in metabolic screening in the Netherlands (about 100% sensitivity and ≥99.97% specificity[66]). It must be noted that a lower analytical sensitivity may well be acceptable, since the previously demonstrated association between viral load and clinical outcome[34,35,36,37] suggests that any cases missed, would be those with the lowest viral loads and probably the lowest chance of developing severe permanent sequelae.

The use of dried urine samples collected on filter paper placed in diapers has been described as feasible for mass screening and should be explored.[51] When considering the use of (dried) urine samples for newborn screening for congenital CMV, a narrow time-frame for sampling must be taken into account, in order to differentiate congenital from postnatal infection.

Taken together, while currently available screening tools are being optimized and fine-tuned, **the technical stage appears to be set for newborn screening for congenital CMV**.

PART III PROS AND CONS OF NEWBORN SCREENING FOR CONGENITAL CMV

Despite previous appeals for preventive measures for congenital CMV infection[67,68], newborn screening for congenital CMV has only recently begun to be considered seriously. The potential for newborn screening for CMV lies in the identification of the large proportion of asymptomatic congenitally infected newborns at risk for developing late-onset hearing loss or other sequelae. There is growing support for two primary ideas: the benefit of hearing preservation in symptomatic newborns by means of **antiviral treatment**, and the benefit of early identification of late-onset hearing loss by means of extensive **audiological follow-up** in congenitally infected infants. It appears that, after many years of research, congenital CMV infection now satisfies most screening criteria of Wilson and Jungner.[40,69] Pros and cons for newborn screening for congenital CMV are addressed in detail in the following *Chapters 11* and *12*.

From these discussions and combining insights, as provided by the studies presented in this thesis as well as from other available data, the overall conclusion is that a large-scale study on the safety and efficacy of combined newborn screening and antiviral therapy is the necessary next step to take in the long-lasting fight against the damage caused by congenital CMV infections.

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