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

M. J. KORNDEWAL[1,2]*, A. C. T. M. VOSSEN[2], J. CREMER[3],
R. S. VAN BINNENDIJK[3], A. C. M. KROES[2], M. A. B. VAN DER SANDE[1,5],
A. M. OUDESLUYS-MURPHY[4] AND H. E. DE MELKER[1]

[1] *National Institute of Public Health and the Environment (RIVM), Centre 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), Centre 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*

SUMMARY

Congenital cytomegalovirus infection (cCMV) may lead to symptoms at birth and long-term consequences. We present a nationwide, retrospective cohort study on the outcome of cCMV up to age 6 years. For this study we identified cCMV, using polymerase chain reaction, by analysing dried blood spots, which are taken shortly after birth for neonatal screening. The group of children with cCMV were compared to a group of children who were cCMV negative at birth. Data were collected about their health and development up to age 6 years. Parents of 73 693 children were invited to participate, and 32 486 (44·1%) gave informed consent for testing of their child's dried blood spot for CMV. Of the 31 484 dried blood spots tested, 156 (0·5%) were positive for cCMV. Of these, four (2·6%) children had been diagnosed with cCMV prior to this study. This unique retrospective nationwide study permits the estimation of long-term sequelae of cCMV up to the age of 6 years. The birth prevalence of cCMV in this study was 0·5%, which is in line with prior estimates. Most (97·4%) children with cCMV had not been diagnosed earlier, indicating under-diagnosis of cCMV.

Key words: Cytomegalovirus, epidemiology, paediatrics.

INTRODUCTION

Cytomegalovirus (CMV) infection is endemic worldwide and is usually asymptomatic in healthy individuals. However, in immunocompromised patients 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 (cCMV) infection is the most prevalent congenital infection worldwide with a birth prevalence ranging from 0·4% to 2·0% of all newborns [3]. An estimated 10–15% 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

* Author for correspondence: M. J. Korndewal, MD, National Institute of Public Health and the Environment (RIVM), Centre for Infectious Diseases, Epidemiology and Surveillance, Internal Post Box 75, PO Box 1, 3720 BA Bilthoven, The Netherlands. (Email: marjolein.korndewal@rivm.nl)

neurological problems, occur in 40–58% of children who were symptomatic at birth. In addition, about 10–15% of children asymptomatic at birth develop long-term sequelae [4]. These estimates of symptoms and sequelae are based on a meta-analysis by Dollard *et al.* [4], which mostly included studies with a relatively short follow-up of no more than 3 years. Moreover, most of these studies focused only on hearing loss and general developmental delay. Most information on the long-term outcome (>5 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 UK [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 (consequences and risk factors of congenital cytomegalovirus virus) 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 nationwide group of children, retrospectively diagnosed at age 5 years. The design of this study enables 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 1 displays the design of the CROCUS study, comprising the study elements; identification of children with cCMV and collection of study data; and the regular youth healthcare system for all children in The Netherlands provided by Regional Public Health Services.

Identification of children with cCMV 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* = 139 543 births, Statistics Netherlands [6]). All children, living in regions covered by the Regional Public Health Services who were willing to participate (*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, e.g. phenylketonuria, and was stored for 5 years. To test the DBS, a real-time multiplex PCR was chosen, incorporating two independent CMV target genes, i.e. *UL55* and *UL123*, as described by Boeckh *et al.* [7]. This multiplex PCR had a 95% detection limit of 850 copies/ml and was performed 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 larger cCMV-negative control group, matched for gender, 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 complete questionnaires and to give their consent to retrieve data from the youth healthcare records, teachers, general practitioners (GPs) 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 4 years of life the health, growth and development of children are regularly checked at the child health centre. At enrolment, data are collected concerning pregnancy, delivery and the first weeks of
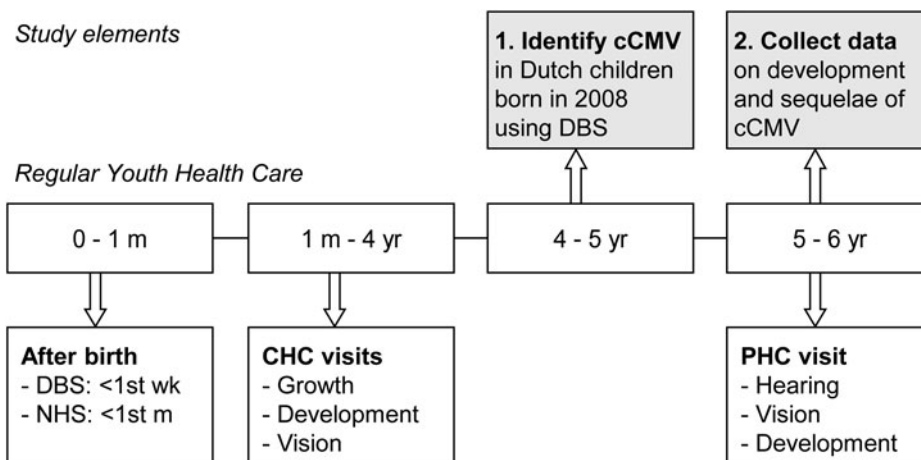
Fig. 1. Design of the CROCUS study. cCMV, Congenital cytomegalovirus; CHC, child health centre; PHC, preventive health check; DBS, dried blood spot; NHS, neonatal hearing screening; wk, week; m, month; yr, year.

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 3 years of life. After the age of 3 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 is performed by a medical specialist or other healthcare provider.

At age 5 or 6 years 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 the data from the child health centre 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 2 years of primary education were collected. School results were divided into quintiles (I, II, III, IV, V), where I represents the 20% highest scores, or into quartiles (A, B, C, D/E) with A indicating the highest 25% scores.

GP and healthcare providers

In The Netherlands the GP provides primary medical care and keeps a record of the entire medical history and medication use of a patient. If the child attended a GP or other healthcare provider, these medical data were retrieved.

Outcome measures

The primary outcome of this study is sensorineural hearing loss up to age 6 years. This is defined as >40 dB 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 6 years of life. Visual impairment is defined as best-corrected visual acuity <0·3 in the better eye. Cognitive impairment, based on the CDI, is defined as a developmental age below –2 standard deviations (s.d.). Values under –2 s.d. 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, 25 000 DBS were required for testing, which meant that 75 000 parents had to be approached for this study.

Ethical and legal issues

This study was approved by the medical ethical committee of the LUMC 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 73 693 children, born in The Netherlands between 1 January and 30 September 2008 (see Fig. 2). Of the parents of 34 105 children who responded (46·3%), the majority (32 486, 95·3%) gave informed consent to have their child's DBS tested for CMV.

DBS of a total of 31 484 children were tested for cCMV. DBS that had been obtained more 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 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 (see Table 1). Of the postal code areas of the non-responders the proportion of migrants, especially non-Western migrants, as well as the proportion with lower incomes, were somewhat higher compared to the postal code areas of responders.

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 (2·6%) of these 156 children were aware that their child had cCMV prior to this phone call, at which time the children were aged 5 years. Informed consent was given for 133 (85%) cCMV-positive children to participate in the second part of the study (Fig. 2).

From the children who were confirmed CMV DNA negative at birth ($n = 31 330$), 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. (Fig. 2).

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 5 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
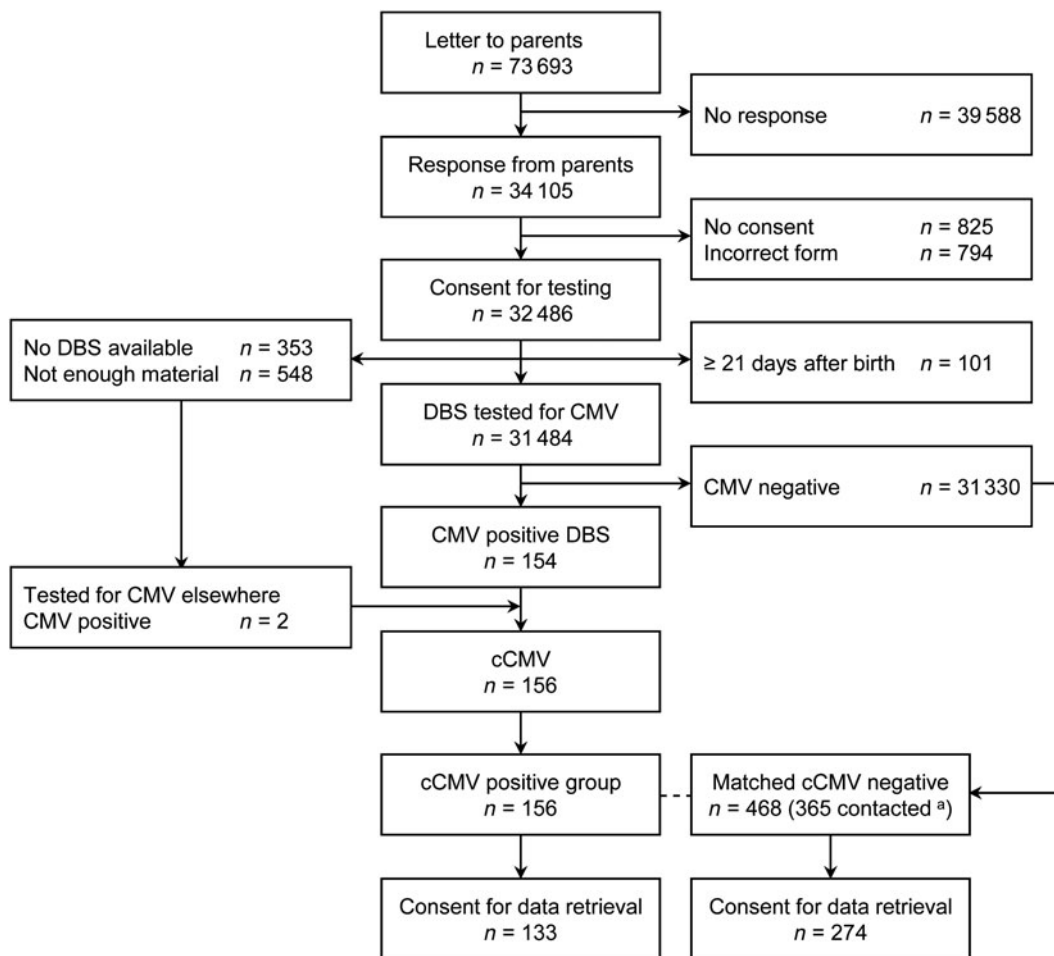
Fig. 2. Flowchart of the CROCUS study. [a] Inclusion of matched controls ceased when informed consent was obtained for two matched controls. DBS, Dried blood spot; CMV, cytomegalovirus; cCMV, congenital CMV infection; n indicates number of children.

[19–21]. It is clear from other studies that some long-term consequences of cCMV become apparent later than 5 years after birth; for example hearing loss may first become obvious up to, or even after, the age of 6 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 in women of childbearing age in The Netherlands of ∼37% [27].

Markedly, 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 contribute 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

Table 1. *Differences in the group of responders and non-responders based on their postal code region*

Characteristic	Responders* (N = 32 486)	Non-responders† (N = 41 207)
Children (aged <5 years)	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.

drawback is the high probability of missing data, for example a complete physical examination 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 could lead to either an over- or under-estimation of the disease burden. There are differences between parents who consented to 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 of 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 post-natal 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/ml, 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 1/1000 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 31 328 children with a cCMV-negative test result. We assumed that most of the children with cCMV, who had not been diagnosed by DBS testing in this study, 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 age 6 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].

DECLARATION OF INTEREST

None.

REFERENCES

1. **Griffiths P, Baraniak I, Reeves M.** The pathogenesis of human cytomegalovirus. *Journal of Pathology* 2015; **235**: 288–297.
2. **Boppana SB, et al.** Intrauterine transmission of cytomegalovirus to infants of women with preconceptional immunity. *New England Journal of Medicine* 2001; **344**: 1366–1371.
3. **Kenneson A, Cannon MJ.** Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. *Reviews in Medical Virology* 2007; **17**: 253–276.
4. **Dollard SC, Grosse SD, Ross DS.** New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. *Reviews in Medical Virology* 2007; **17**: 355–363.
5. **Townsend CL, Peckham CS, Tookey PA.** Surveillance of congenital cytomegalovirus in the UK and Ireland. *Archives of Disease in Childhood (Fetal and Neonatal Edition)* 2011; **96**: F398–403.
6. **CBS Statistics Netherlands.** Statline population and population dynamics; month, quarter and year (http:// statline.cbs.nl/Statweb/publication/?DM=SLEN&PA= 37943eng&D1=0,10-22&D2=221-237&LA=EN&VW=T). Accessed 24 April 2015.
7. **Boeckh M, et al.** Optimization of quantitative detection of cytomegalovirus DNA in plasma by real-time PCR. *Journal of Clinical Microbiology* 2004; **42**: 1142–1148.
8. **Kalpoe JS, et al.** Validation of clinical application of cytomegalovirus plasma DNA load measurement and definition of treatment criteria by analysis of correlation to antigen detection. *Journal of Clinical Microbiology* 2004; **42**: 1498–1504.
9. **Doig KB, et al.** The Child Development Inventory: a developmental outcome measure for follow-up of the high-risk infant. *Journal of Pediatrics* 1999; **135**: 358–362.
10. **Ireton H, Glascoe FP.** Assessing children's development using parents' reports. The Child Development Inventory. *Clinical Pediatrics* 1995; **34**: 248–255.
11. **Korver AM, et al.** DECIBEL study: Congenital cytomegalovirus infection in young children with permanent bilateral hearing impairment in The Netherlands. *Journal of Clinical Virology* 2009; **46** (Suppl. 4): S27–31.
12. **Varni JW, Limbers CA.** The pediatric quality of life inventory: measuring pediatric health-related quality of life from the perspective of children and their parents. *Pediatric Clinics of North America* 2009; **56**: 843–863.
13. **Varni JW, Seid M, Kurtin PS.** PedsQL 4·0: reliability and validity of the Pediatric Quality of Life Inventory version 4.0 generic core scales in healthy and patient populations. *Medical Care* 2001; **39**: 800–812.
14. **Varni JW, Seid M, Rode CA.** The PedsQL: measurement model for the pediatric quality of life inventory. *Medical Care* 1999; **37**: 126–139.
15. **Varni JW, et al.** The PedsQL 4·0 Generic Core Scales: sensitivity, responsiveness, and impact on clinical decision-making. *Journal of Behavioral Medicine* 2002; **25**: 175–193.
16. **Chan KS, et al.** The PedsQL: reliability and validity of the short-form generic core scales and asthma module. *Medical Care* 2005; **43**: 256–265.
17. **Gandek B, et al.** Tests of data quality, scaling assumptions, and reliability of the SF-36 in eleven countries: results from the IQOLA Project. International Quality of Life Assessment. *Journal of Clinical Epidemiology* 1998; **51**: 1149–1158.
18. **Gandek B, et al.** Cross-validation of item selection and scoring for the SF-12 Health Survey in nine countries: results from the IQOLA Project. International Quality of Life Assessment. *Journal of Clinical Epidemiology* 1998; **51**: 1171–1178.
19. **Neto EC, et al.** Newborn screening for congenital infectious diseases. *Emerging Infectious Diseases* 2004; **10**: 1068–1073.
20. **Lebech M, Petersen E.** Neonatal screening for congenital toxoplasmosis in Denmark: presentation of the design of a prospective study. *Scandinavian Journal of Infectious Diseases Supplementum* 1992; **84**: 75–79.
21. **Sander J, Niehaus C.** Screening for rubella IgG and IgM using an ELISA test applied to dried blood on filter paper. *Journal of Pediatrics* 1985; **106**: 457–461.

22. **Fowler KB, et al.** Newborn hearing screening: will children with hearing loss caused by congenital cytomegalovirus infection be missed? *Journal of Pediatrics* 1999; **135**: 60–64.

23. **Foulon I, et al.** A 10-year prospective study of sensorineural hearing loss in children with congenital cytomegalovirus infection. *Journal of Pediatrics* 2008; **153**: 84–88.

24. **de Vries JJ, et al.** Congenital cytomegalovirus infection in The Netherlands: birth prevalence and risk factors. *Journal of Medical Virology* 2011; **83**: 1777–1782.

25. **Ahlfors K, Ivarsson SA, Harris S.** Report on a long-term study of maternal and congenital cytomegalovirus infection in Sweden. Review of prospective studies available in the literature. *Scandinavian Journal of Infectious Diseases* 1999; **31**: 443–457.

26. **de Vries JJ, et al.** The apparent paradox of maternal seropositivity as a risk factor for congenital cytomegalovirus infection: a population-based prediction model. *Reviews in Medical Virology* 2013; **23**: 241–249.

27. **Korndewal MJ, et al.** Cytomegalovirus infection in The Netherlands: seroprevalence, risk factors, and implications. *Journal of Clinical Virology* 2015; **63**: 53–58.

28. **Korver AM, et al.** Awareness of congenital cytomegalovirus among doctors in The Netherlands. *Journal of Clinical Virology* 2009; **46** (Suppl. 4): S11–15.

29. **Pereboom MT, et al.** Maternal cytomegalovirus infection prevention: the role of Dutch primary care midwives. *Midwifery* 2014; **30**: 1196–1201.

30. **de Vries JJ, et al.** Evaluation of DNA extraction methods for dried blood spots in the diagnosis of congenital cytomegalovirus infection. *Journal of Clinical Virology* 2009; **46** (Suppl. 4): S37–42.

31. **Boppana SB, et al.** Dried blood spot real-time polymerase chain reaction assays to screen newborns for congenital cytomegalovirus infection. *Journal of the American Medical Association* 2010; **303**: 1375–1382.

32. **Barbi M, Binda S, Caroppo S.** Diagnosis of congenital CMV infection via dried blood spots. *Reviews in Medical Virology* 2006; **16**: 385–392.

33. **Wang L, et al.** Dried blood spots PCR assays to screen congenital cytomegalovirus infection: a meta-analysis. *Virology Journal* 2015; **12**: 60.

34. **Lanari M, et al.** Neonatal cytomegalovirus blood load and risk of sequelae in symptomatic and asymptomatic congenitally infected newborns. *Pediatrics* 2006; **117**: e76–83.

35. **Boppana SB, et al.** Congenital cytomegalovirus infection: association between virus burden in infancy and hearing loss. *Journal of Pediatrics* 2005; **146**: 817–823.

36. **Rivera LB, et al.** Predictors of hearing loss in children with symptomatic congenital cytomegalovirus infection. *Pediatrics* 2002; **110**: 762–767.

37. **Cannon MJ, et al.** Universal newborn screening for congenital CMV infection: what is the evidence of potential benefit? *Reviews in Medical Virology* 2014; **24**: 291–307.