Consequences of congenital cytomegalovirus infection in early childhood

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Chapter 7

General Discussion

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

Consequences of congenital cytomegalovirus infection

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

Prevalence of cCMV and morbidity (Figure 7.1)
Stored dried blood spots of 31,484 children, born between January and September 2008, were tested for cCMV for the CROCUS-study. This is 17% of the entire birth cohort of 2008 in the Netherlands, which consisted of 184,634 children. The birth prevalence of cCMV in the Netherlands as estimated in this study was 0.5%. When this is extrapolated to the entire birth cohort of 2008 this means that around 915 children would have had cCMV in the Netherlands. [Chapter 3] In the next paragraphs this group of children is described in more detail.
Based on the results of this study, of these 915 cCMV-positive children, almost 20% (179) will be symptomatic at birth and about 80% (736) will have no symptoms at birth. However, it is important to keep in mind that the same or similar symptoms at birth are also commonly found in children without cCMV (12.4%). Based on the risk difference between these groups it is expected that approximately 66 children with cCMV have symptoms at birth that are attributable to cCMV. Nonetheless, it is in general impossible to determine which children with cCMV and symptoms at birth have these symptoms as a result of cCMV. [Chapter 4]
It is expected that about 25% of children with cCMV (227) will develop some moderate-to-severe long-term impairment in the first six years of life. Of these children, 96 (42%) will have been symptomatic at birth and 131 (58%) will have been asymptomatic at birth. However, only a proportion of this long-term impairment is actually attributable to cCMV given that 12% of the cCMV-negative children have similar problems. Approximately 75 (41.8%) of the 179 symptomatic children and 43 (5.8%) of the 736 asymptomatic children will have long-term impairment attributable to cCMV. [Chapter 4]
About one-third of symptomatic children with long-term impairments will have impairments in multiple domains (19.2% of all symptomatic children with cCMV), which indicates more severe disease. In the group of children who were asymptomatic at birth, almost half of the children with long-term impairment will have problems in multiple domains (8.4% of all asymptomatic), while only 1.8% of the children without cCMV will have impairments in multiple domains.

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

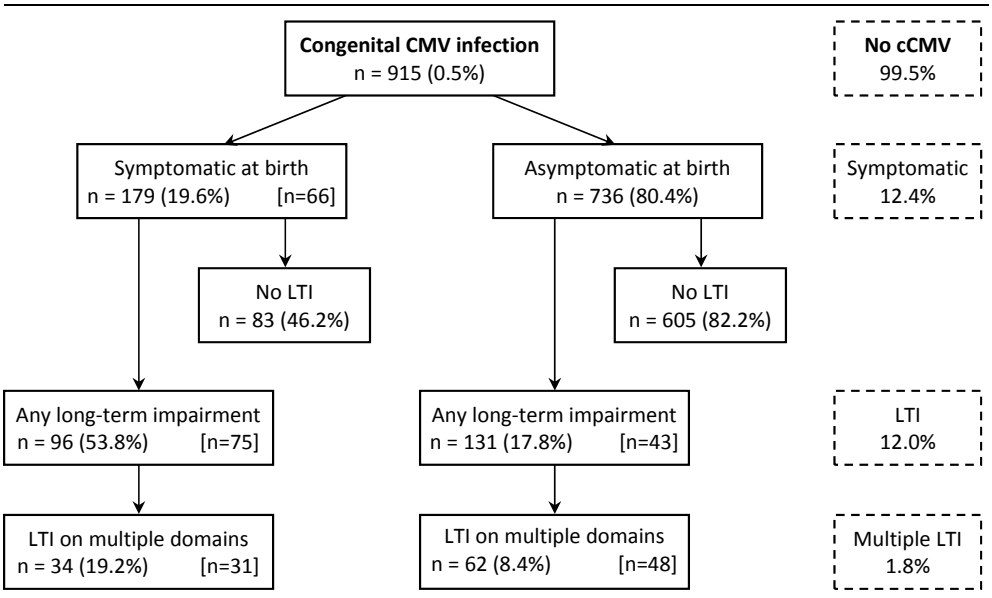

Figure 7.1 - Results of the CROCUS-study extrapolated to the entire birth cohort of 2008 (n=184,634). The figures represent number and percentages (%).The figures between square brackets represent the number of children with symptoms or impairment attributable to cCMV. The numbers in the dashed text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

Clinical, daily life and economic consequences of cCMV (Figure 7.2)
The CROCUS-study demonstrated that cCMV can lead to a broad array of long-term impairments. Besides sensorineural hearing loss, which is the most well-known and a much studied consequence of cCMV, we found other less-studied impairments to be much more common in children with cCMV than in the control group. The largest risk differences between children with and without cCMV were seen for motor, cognitive and speech-language impairment. [Chapter 4] Some of these problems, such as balance problems [1-3], have recently been described in other studies. Others, such as sensory processing disorders and developmental coordination disorders, have not been reported previously and require further investigation.

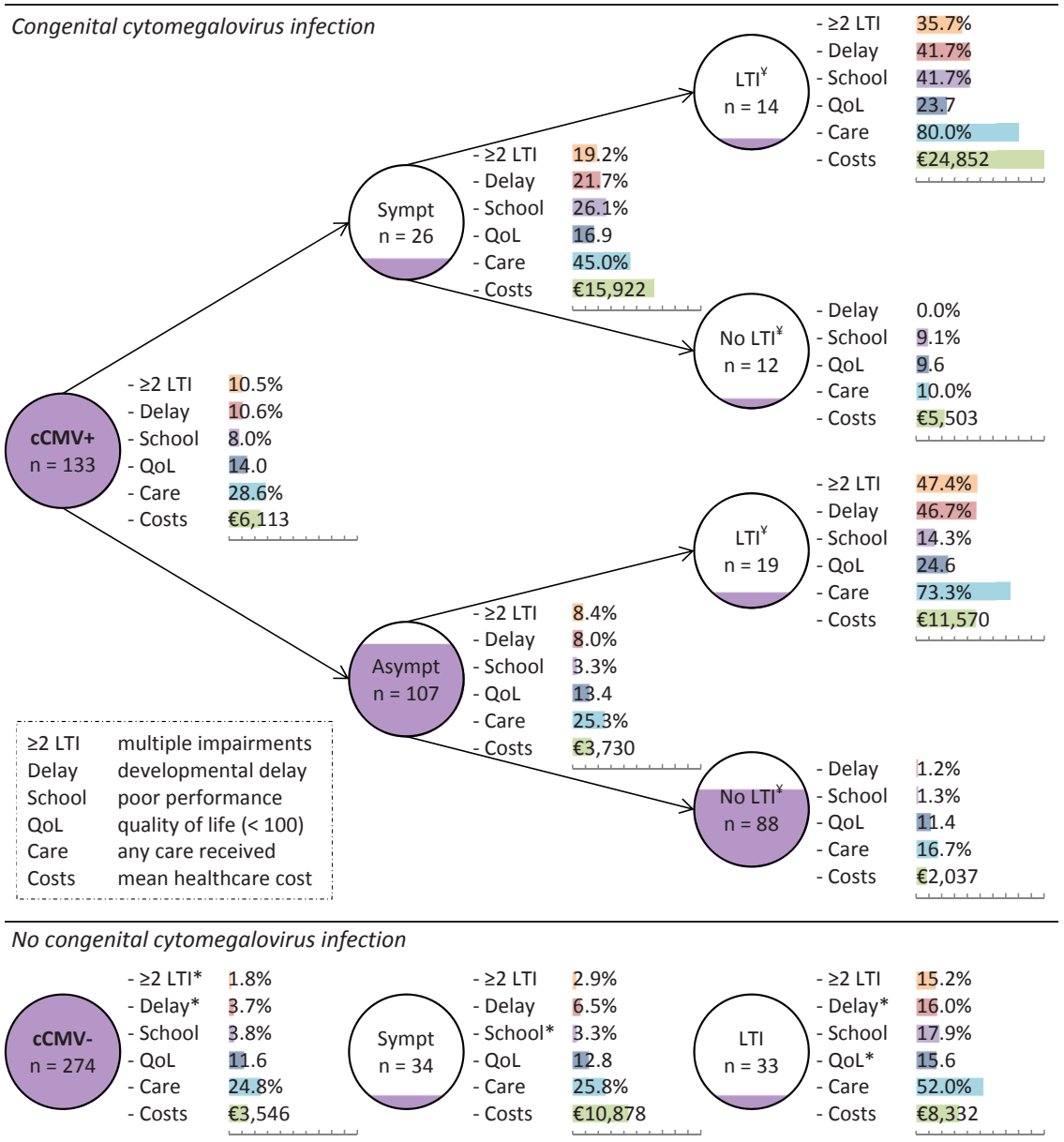

Figure 7.2 - Overview of the consequences on different aspects of daily life and on the average costs of healthcare.

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

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

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

Children with cCMV were expected to incur higher healthcare costs because they had higher healthcare consumption than children without cCMV. Indeed, the mean healthcare costs for children with cCMV were more than € 2,500 higher than for children without cCMV in the first six years of life. Especially the costs for physical and speech therapy and visits to a rehabilitation center were higher. The difference in average healthcare costs between children with and without cCMV is not statistically significant, due to the large range of costs. Moreover, the costs for children with cCMV are probably underestimated, as explained in chapter 6.
When the total costs were extrapolated to the entire birth cohort of 2008, the total healthcare costs for children with cCMV were estimated at 5.6 million euros, of which 2.3 million euros are expected to be attributable to cCMV. Almost 50% of the total costs of children with cCMV could be accounted for by the 20% of children with symptoms at birth.

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

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

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

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

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

Methodological considerations

Why design another study on long-term consequences?

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

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

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

Methodological limitations and challenges

Estimation of the birth prevalence

- Participation rate of Regional Public Health Services

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

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

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

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

- Testing of dried blood spots

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

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

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

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

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

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

This means that the actual birth prevalence of cCMV would be 0.58% instead of 0.5%, thus the birth prevalence is presumably slightly underestimated in our study. However, with a chance of 29/31328 (0.1%), it is very unlikely that cCMV-positive children ended up in the cCMV-negative control group (n = 274). Because the population tested was very large, and the great majority of children had a negative test-result both the positive predictive value and the negative predictive value would be very good, being 98.7 and 99.9% respectively. (Table 7.1)
Furthermore, it is known that the sensitivity of this test depends on the viral load. In the case of a very low viral load the chance of missing a child with cCMV becomes higher. Missing some children with a low viral load could mean that the children identified as having cCMV are those with a somewhat higher viral load. Because a higher viral load is possibly related to a worse outcome [14-16] this could lead to an overestimation of the proportion of children with long-term consequences of cCMV.

Estimation of the burden of disease of cCMV

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

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

Definitions of symptoms at birth and long-term impairment

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

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

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

Missing data

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

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

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

Implications of the limitations
It is difficult to say what the overall effect is of these limitations and biases.
It is likely that the birth prevalence has been underestimated. Taking the sensitivity of the test into account, the estimated birth prevalence of cCMV in the Netherlands is probably 0.58%.
Overall, for the estimation of long-term outcome, it is unclear whether all above-mentioned limitations and biases lead to over- or underestimation of the long-term consequences. Presumably, the overestimation possibly resulting from recall bias and diagnostic suspicion bias would be balanced by the underestimation due to the missing data or onset of disease after the data collection.

What should we do about cCMV?

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

Awareness

As discussed in the introduction current awareness of cCMV, in the Netherlands and many other countries, is generally low, among healthcare professionals as well as among women of reproductive age. Awareness is the first step; people need to know about cCMV before they can act upon it. Pregnant women need to know about the risk of cCMV and the measures they can take to minimize this risk. Doctors and midwives need to be knowledgeable about cCMV in order to give advice to women who want to become pregnant or who are already pregnant. Healthcare providers also need to be aware of the consequences of cCMV in order to recognize this condition.
The low level of awareness of cCMV is even more frustrating when it is realized that cCMV-related morbidity in children is at least as common as several better-known childhood disorders such as Down syndrome, fetal alcohol syndrome and spina bifida. [23, 24] In addition, the prevalence of cCMV is much higher than that of several disorders currently included in newborn screening in Europe. [25] Furthermore, due to the media attention, probably many more women of reproductive age currently know about congenital Zika virus infection than about cCMV.

How can we increase awareness of cCMV?

There are two important groups of people who should know about cCMV, the healthcare providers and women who are pregnant or who want to become pregnant.
Education is the key for the first group. Not only should they be aware of cCMV, they also need information about horizontal and vertical transmission risks and about ways to prevent transmission and they should be able to give adequate information to women of reproductive age. This means that medical students should learn about cCMV and that it should be a standard subject in their curriculum. A start has already been made to increase the knowledge of midwives about cCMV. An e-learning program has been developed by students of the Midwifery Academy Amsterdam Groningen. If this e-learning program would be incorporated in the curriculum and continuing education, it could increase the knowledge of all midwives concerning cCMV.
A first step has also been taken for the second group, i.e. women of reproductive age. In the brochure "Pregnant!", a paragraph has recently been added on cCMV. This brochure is produced by the Dutch Association for Obstetrics and Gynaecology (NVOG), Royal Dutch Organization of Midwives (KNOV), Dutch College of General Practitioners (NHG), Association of Doctors Practising Obstetrics (VVAH), National Information Center for Heredity, Pregnancy, and Medical Biotechnology (Erfocentrum), Royal Dutch Organization for promoting Pharmacy (KNMP), Children in Hospital Foundation (K&Z), and National Institute of Public Health and the Environment (RIVM). It is routinely handed to pregnant women by midwives and gynecologists. However, cCMV is usually not mentioned in other generally available resources (books, internet, etc.) women use to find information about pregnancy.

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

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

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

Primary prevention

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

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

Secondary prevention

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

If screening would be introduced the reason for screening and the results of the test would need to be discussed during the consultation with the midwife or gynecologist. Advice about hygienic measures should then be given at the same time, to both seronegative and seropositive women.
Yet, screening might lead to a false sense of security in women who are seropositive. It is important that gynecologists and midwives are made aware of the fact that antibodies against CMV are not fully protective against cCMV. Moreover, women with a reactivation or reinfection cannot be diagnosed by IgM or IgG testing. This means that about half of the cases of cCMV would still be missed by this screening method.
Furthermore, besides follow-up, little can currently be done to influence the transmission of cCMV from mother to fetus when a primary maternal infection is detected, as hyper-immune globulin was reported to be ineffective [37]. Yet, some studies found encouraging effects of hyper-immune globulin in improving the outcome in cases of fetal CMV infection (tertiary prevention), but this requires further investigation.
Because no proven effective therapeutic option is available and screening would not lead to a different approach towards seropositive or seronegative women, screening cannot be justified, unless as part of a research project. Women who are diagnosed with a primary CMV infection during pregnancy, in such a research project, could participate in trials for evaluation of promising treatment options. In addition, these research projects could provide valuable data about potential predictive factors.

Tertiary prevention

Universal neonatal screening
According to the criteria of Wilson and Jungner for screening, the evaluated condition should be an important health problem, which cCMV most certainly is. However there should also be a generally accepted treatment for the condition. It is debatable whether this is true for cCMV.
There is general consensus on the benefit of treatment of children with cCMV who have neurologic signs and symptoms at birth. These children should be treated with (val)ganciclovir as this has been demonstrated to be effective in preventing deterioration of hearing loss. [38, 39] However, there is no evidence for antiviral treatment in children with only mild, reversible or solitary symptoms or in children without symptomatic disease at birth.
On the other hand, screening children for cCMV can have some benefits. The most important benefit is the early identification of children with cCMV enabling early treatment of potential impairment. A clear example of this is late-onset hearing loss, which commonly occurs in children with cCMV and which is missed by neonatal hearing screening in probably one third to half of the cases.[40] Early detection and initiation of intervention for hearing loss, such as hearing aids or cochlear implants, [41, 42] can lead to improved speech- and language development. [43, 44] Repeated audiological follow-up should be standard care in children with cCMV, because of the unstable nature of the hearing loss. [45-48] Even though this might not prevent disease, the audiological follow-up could be considered as an intervention and it can prevent or limit harm such as impaired language development.
However, universal neonatal CMV screening also has some disadvantages. At present there are no highly predictive prognostic markers available. Even though we know that half of the symptomatic children will develop long-term impairment, we cannot predict who will

develop these problems. The same is true for the even larger group of asymptomatic children. It would be difficult to tell a parent that his or her child has cCMV without knowing the prognosis for that specific child other than a 50% or 20% chance of impairment.
Another potential disadvantage of screening is the detection of children who have no clinically apparent disease at birth, but who could be classified as symptomatic at birth. These include children who have isolated cranial ultrasound abnormalities, transient thrombocytopenia and neutropenia, or isolated sensorineural hearing loss. Even though these children could be classified as symptomatic at birth, it is not yet clear whether they would benefit from treatment. This problem is a point of discussion at international conferences.
The combination of screening with research could provide an answer for this problem. The children who would be identified by a research related screening program would receive the best possible care, including audiological follow-up, and promising interventions could be evaluated in randomized controlled trials. Furthermore this screening based research could provide valuable information about prognostic markers. This approach could also give more insight in the effects of treatment of mildly symptomatic children as has been mentioned above.

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

Recommendations for the care of children with cCMV

Some recommendations based on current evidence are available when a child has been diagnosed with cCMV. Firstly a child diagnosed at birth with cCMV and having neurological signs and symptoms should be treated with (val)ganciclovir, since antiviral therapy can influence the course of hearing loss in some symptomatic cCMV children. [38, 39, 50]
Secondly, audiological follow-up until the age of six years is recommended for children with cCMV, because of potential late-onset hearing loss and possible progression and fluctuation of hearing problems. [45-47]
All available current advice is focused on hearing loss. However, we also recommend care and support for other consequences of cCMV based on the results of our study.
We advise regular evaluation of motor and speech and language development, preferably by a physical therapist and speech therapist. These evaluations could be scheduled around various developmental milestones which the child is expected to reach.
Furthermore, it would be convenient if one central professional would be the first port of call for a child and its family. This would preferably be a pediatrician who could manage and assess the care for this child. This case manager could also keep an eye on the quality of life and potential problems in the daily life of the child and the parents.
Ideally a register of all children with known cCMV should be kept, for instance by a pediatrician. This would facilitate the detection and evaluation of specific problems, for example behavioral problems, that are possibly related to cCMV. Furthermore, such a registry could be used to evaluate the long-term effects of various interventions.

Recommendations for future research

Clear definitions of symptoms and sequelae of cCMV

There is a need for a universal definition of being symptomatic at birth. In order to achieve this, the first step is to decide which signs and symptoms should be included in the definition. Subsequently the definitions of the separate signs and symptoms, such as microcephaly and conjugated hyperbilirubinemia, should be agreed on by researchers in this field. The same is true for the overall long-term impairment due to cCMV which needs to be defined in more detail. In addition, the various impairments need to be more clearly defined and the cut-off values for diverse conditions should be used more consistently.
Universal definitions will improve the comparability of different studies, create the possibility of performing meta-analyses in order to get more reliable estimates, and are essential for international registries on cCMV. It may even be an option to define different categories of symptomatic children, for example using a scoring system for clinically apparent abnormalities, neurological abnormalities, and abnormal laboratory parameters or abnormal imaging findings. Registration of these findings could provide valuable information for making clearer prognoses and choosing an individual treatment program.

Research on recurrent infections

In chapter 2 we identified risk factors for a high seroprevalence of CMV. In women of reproductive age with a high seroprevalence the chance of a primary infection during pregnancy is relatively low, whereas the risk of reactivation or reinfection is fairly high. The risk of a child with cCMV in this group is relatively high as there is a positive correlation between maternal CMV seroprevalence and birth prevalence of cCMV. However, it is not known what proportion of recurrent infections is due to reactivation and what proportion to reinfection. The differentiation between reactivation and reinfection is important for preventive strategies.
Primary infection and presumably reinfection can be partially prevented by hygienic measures. Therefore, if most recurrent infections would be reinfections then preventive hygienic measures should be pursued in seropositive women. However, if most of the recurrent infections would be due to reactivation of the virus, then prevention of cCMV by hygienic measures would be of little help to seropositive women. Also the specifications of a future vaccine, whether a vaccine only needs to prevent CMV infections arising from external sources or if it also needs to prevent CMV reactivation, depends on this outcome. One way to study the relative contribution of reinfections and reactivations is by using the information collected in the PIENTER-study on CMV-seroprevalence in modelling studies.

Evaluation of potential vaccination strategies

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

Research on prognostic factors

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

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

Follow-up of the CROCUS-study

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

Final conclusion

The long-term consequences of cCMV include clinical impairment, impact on the quality of life and increased healthcare costs compared to children without cCMV. Altogether, it has a considerable effect on the life of children and parents.
This justifies taking action with preventive measures that are readily available. The first step is creating awareness along with implementing existing preventive measures. Women who are, or want to become, pregnant should learn about cCMV. Increased knowledge and awareness among healthcare professionals would enable the education of pregnant women or women who want to become pregnant. The education should consist of advice on hygienic measures, in order to prevent primary infections and reinfections. Of course, development of a vaccine against CMV should continue to have a high priority, but in the meanwhile the focus should remain on education of women and healthcare providers. Screening pregnant women for CMV and universal neonatal screening for cCMV could provide benefits, but they also have disadvantages. Further research, on potential treatment options and prognostic factors is essential for the implementation of these programs. In order to optimize and take advantage of the benefits of screening programs, they should be combined with research into treatment options and prognostic factors.
Furthermore, the availability of information about the broad range of impairments justifies an update of guidelines on the care of children with cCMV, with more attention for their motor, cognitive and speech-language development, as well as for the consequences in the daily life of the children and their families.

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