Viral infections in young infants : epidemiologic and diagnostic aspects of ToRCH, enterovirus and human parechovirus

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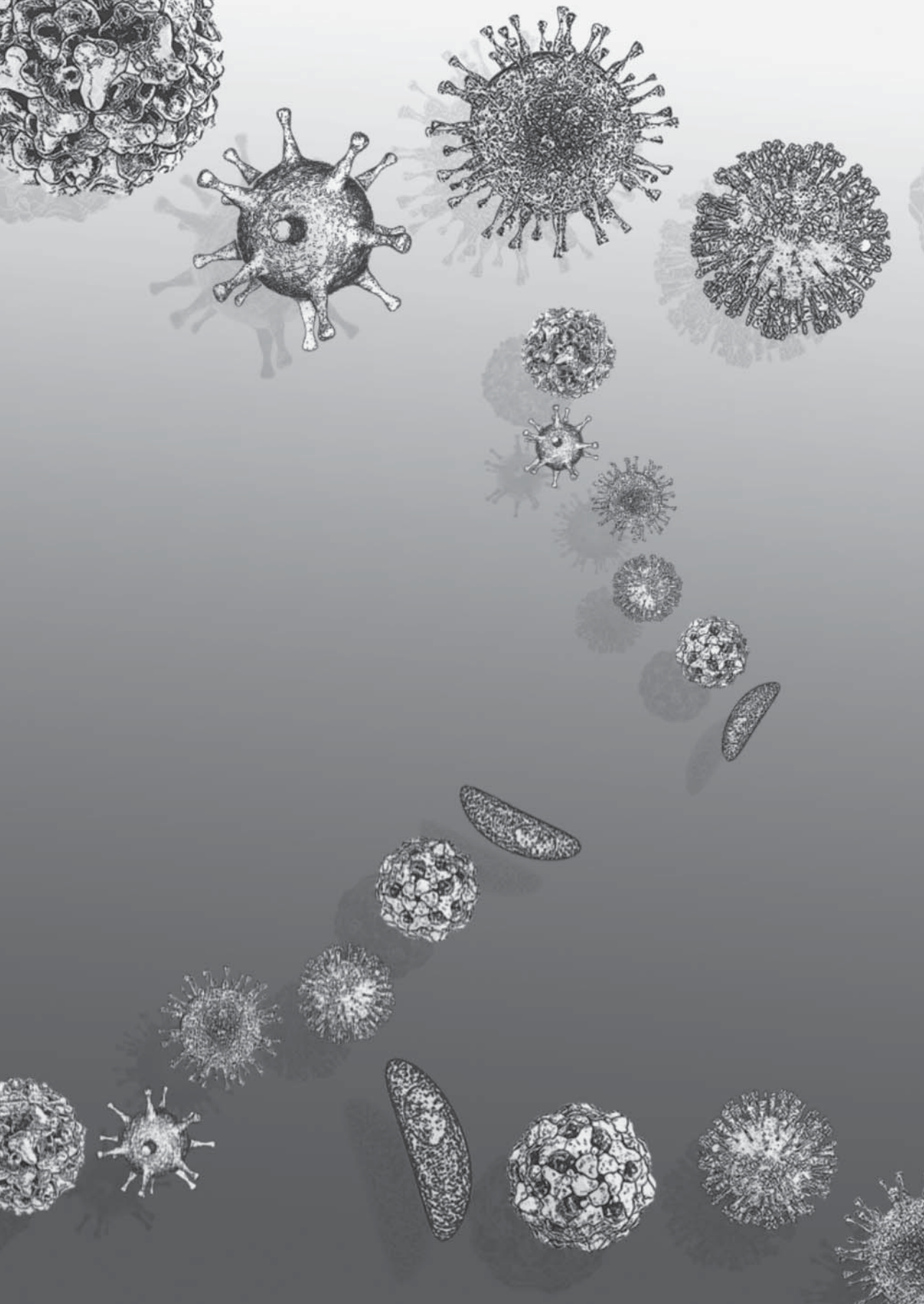
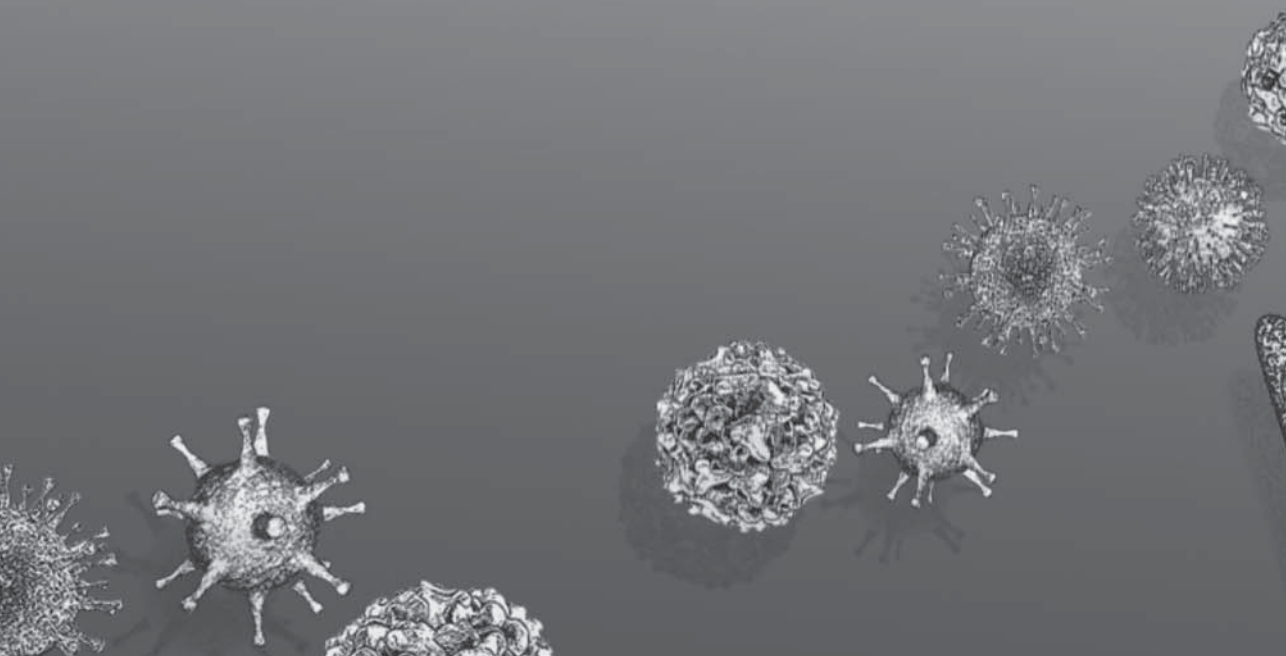


PART C



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GENERAL DISCUSSION

'Viral infections in young infants' is an immense topic that could (and should) never be completely discussed in one thesis. Therefore, choices to limit to the scope of this thesis were made and we decided to discuss two different groups of paediatric viral infections; first 'ToRCH' infections (part A) and secondly Enterovirus (EV) and Human Parechovirus infections (HPeV) (part B).

Congenital (ToRCH) infections have a relatively low incidence in The Netherlands but are frequently tested for a variety of different indications. Two frequent indications for 'ToRCH testing', lenticulostriate vasculopathy (LSV) and small for gestational age (SGA), were evaluated in chapter 2 and 3, respectively. General diagnostic considerations on ToRCH-testing from a pathophysiology and laboratory perspective were reviewed in chapter 4. ToRCH-testing is requested relatively often in daily clinical practice, but EV- and HPeV testing is not always integrated in standard of care, even though they occur frequently in young infants with sepsis-like illness. Therefore, this diagnosis can be easily missed. The added knowledge from our large epidemiology study (chapter 5) and smaller follow-up studies from a non-intensive care point of view (chapters 6 and 7) will improve the general paediatrician's capability to provide adequate follow-up after EV or HPeV infection and better inform parents about what to expect after hospital release.

In this general discussion, the main findings and the methodological challenges of the studies in this thesis are mentioned and put in a broader scientific perspective. This is followed by a review of the clinical consequences of the conclusions of this thesis. Finally, suggestions for further research are provided.

PART A: TORCH INFECTIONS

Epidemiology

The ToRCH acronym was first proposed in 1971 in order to include congenital infections in the differential diagnostic considerations of several signs and symptoms that the pathogens in the acronym have in common, such as small for gestational age (SGA) or several brain anomalies [1]. An infection with one of these pathogens generally causes no or only mild maternal symptoms, but vertical transmission causing foetal infection can lead to severe birth defects. These similarities make the ToRCH acronym a very practical one, but the pathogens included, i.e. *Toxoplasma Gondii*, rubella, CMV and herpes simplex virus, also have some quite different characteristics. For example, the vertical transmission rates and associated long term morbidity vary considerably. Table 8.1 provides a summary.

Table 8.1: Epidemiology of the ToRCH group pathogens

	Prerequisite for foetal infection	Vertical transmission (% of congenitally infected new-borns after proven maternal infection)	Neonatal symptomatic disease (% of infected new-borns)	Long-term morbidity (% of infected new-borns)	Dutch birth prevalence (number/1,000 new-borns)	Estimated number of infected infants in the 2017 Dutch birth cohort
Toxoplasma gondii	Maternal primary infection	< 20% below 24w GA; rising to ~70% at 36w GA [2 3] *Increasing vertical transmission rate with increasing GA*	Hydrocephalus: 9% Chorioretinitis: 14% *Decreasing severity of symptoms with increasing GA*	Retinal scarring, chorioretinitis: 15% [2 4] Any symptom: 20%	1.8/1,000 [5]	n = 306
Rubella virus	Maternal primary infection	~80% during first trimester [6], *Decreasing vertical transmission rate with increasing GA*	CRS: 85% (if infected in first trimester [7] *Decreasing severity of symptoms with increasing GA*	N/A	~0/1,000^	n = 0^
Cytomegalovirus	Maternal primary infection, viral re-activation or re-infection	Maternal primary infection: 32% Recurrent infection: 1.4%	Any symptom: 10-15% [8], ~50% develop permanent impairment [9] *Decreasing severity of symptoms with increasing GA*	SNHL: 13% [8] Moderate-to-severe developmental impairment: 18% [9]	5.0/1,000 [10]	n = 849
Herpes Simplex Virus	Maternal primary infection, re-infection or re-activation	Maternal primary infection: 50% New, non-primary infection: 30% Viral reactivation: 2%	Disseminated: 50% Encephalitis: 30% SEM: 20% [11]	Disseminated: 29% mortality, 9% severe impairment. Encephalitis: 4% mortality, 39% severe impairment. SEM: 78% unknown, 22% complete recovery [11]	0.3/1,000 [12] *Of whom: 5% congenital infection, 85% perinatal, 10% postnatal*	n = 46 *(90% of total birth incidence)*

GA: gestational age; CRS: congenital rubella syndrome, SEM: Skin-Eye-Mouth disease; N/A: not applicable; SNHL: sensorineural hearing loss. ^Burden of disease for rubella is not estimated, the reported numbers from the national registry 2017 are shown [13].

Due to the differences in epidemiology and morbidity, comparing the burden of disease of the pathogens of the ToRCH group is difficult. The last column of Table 8.1 uses the Dutch 2017 birth cohort (n = 169,836 newborns) as a calculation example. By multiplying this number with the birth prevalence of each of the pathogens of the ToRCH group, an estimation of the number of infants affected by a pathogen of the ToRCH group can be made. These calculations show that an estimated 1200 infants have been symptomatically affected by one of the ToRCH pathogens in 2017. Due to the success of rubella vaccination, no infants were affected by Rubella virus. This is in stark contrast to the relatively high birth prevalence of congenital CMV (n = ~ 849) and toxoplasmosis (n = ~ 306), which is higher (6.8/1,000) than the birth prevalence of non-chromosomal congenital heart disease (5.4/1,000) [14] and trisomy 21 (1.4/1,000) [15], respectively. The birth prevalence of herpes simplex virus infection is much lower (0.3/1,000), comparable to infants born with cleft palate (0.4/1,000) [16]. In the last decades, diagnostic accuracy has been improved for toxoplasmosis and evidence of treatment improving outcome has been provided [17-19]. Thus, more awareness of congenital cytomegalovirus and toxoplasmosis should be created by promoting prevention and screening methods.

Prevention

As is shown in Table 8.1, rubella vaccination has led to a virtual extinction of rubella virus infections in our and other countries that implemented rubella vaccination. There is some reason for concern though, vaccination rates are declining and since 2013 the Dutch vaccination coverage is below the 95% target. In 2017 overall rubella vaccination coverage was 92.2%, with 2.2% of the Dutch population living in an area with < 90% coverage. This led to the World Health Organisation (WHO) assigning The Netherlands as at intermediate risk for a rubella outbreak [13]. This risk has been observed during the 2004–2005 rubella epidemic in our country, when the reported incidence of congenital rubella infection was 2.6/1,000 newborns and cases of congenital rubella syndrome were reported. These cases occurred only in children of unvaccinated women [20 21]. This means that the declining vaccination coverage could lead to an increase of congenital rubella syndrome. Clinicians' suspicion for congenital rubella infection should rise, especially in sub-populations that have a low vaccination coverage.

For toxoplasmosis, CMV and herpes simplex virus no registered vaccine exists to date [17 22], although several study groups are working on its development. Hygienic measures are another type of primary prevention. Awareness of congenital infections can be effectively increased by active counselling of pregnant women. Preventive measures are shown in Table 8.2. These measures have been shown to be successful in decreasing the risk for maternal

Table 8.2: Preventative measures

	Primary prevention
Toxoplasma gondii	- Avoid ingestion of raw meat products - Avoid contact with cat faeces (litter box and gardening) - Development of cat vaccination
Rubella	- Vaccination of general population - Advise vaccination for unvaccinated women of childbearing age (one month prior to conception)
CMV	- Hand washing (15–20 seconds) after contact with saliva or urine of young infants - Avoid sharing food, drinks, pacifiers, toothbrush etc. with young infants
Herpes simplex	- Caesarean section in case of maternal vaginal herpes lesions (prevents peripartum infection) - Hand washing after contact with a person with labial herpes and frequent hand washing when having labial herpes - Avoid kissing and sharing food, drinks, pacifiers, toothbrush etc. with people with labial herpes

infection and active counselling can reduce the seroconversion rate of CMV in pregnant women by 6.4% [23]. Advice on how to prevent congenital toxoplasmosis has been reported to be extremely successful; repeated information given to pregnant women decreased the seroconversion rate of pregnant women by more than 90% [24]. But the effect can be easily over-estimated by selection bias. Nevertheless, the possible positive effect can be considerable. Another strategy that should be studied is implementation of cat vaccination, as they spread the oocysts that lead to human infection [25].

Although not implemented in The Netherlands, other countries have implemented maternal CMV and/or toxoplasmosis screening as a secondary prevention measure. Advantages are that awareness of both patients and physicians is increased, which has been shown in France where screening pregnant women for CMV is common [26,27]. However, screening for CMV can lead to false reassurance because of viral reactivation or reinfection that cannot be detected by determining maternal IgG and IgM antibodies. In The Netherlands this would lead to missing about half of all congenital CMV cases by maternal screening [28]. Furthermore, if maternal CMV infection is detected, no means of preventing foetal infection is available. For toxoplasmosis, the accuracy, speed and cost of screening methods are improving, thus cost-effectiveness of early diagnosis of congenital infection is improving for toxoplasmosis [19,29]. In 2009 a study reported that the burden of congenital toxoplasmosis in The Netherlands has been underestimated previously, adding to the importance of increasing awareness [5]. Also, intrauterine and early postnatal treatment regimens have been shown to improve

outcome [30]. Thus, maternal screening for toxoplasmosis need be considered and studies to determine its cost-effectiveness should be performed.

Clinical manifestations of ToRCH infections

In the general introduction and in chapter 4 of this thesis, the clinical signs and symptoms for each of the ToRCH pathogens have been extensively described, thus, in this general discussion those will not be repeated. For a quick reference Table 1.1 of the general introduction can be used.

It is important to remain aware that some signs and symptoms are more specific for one of the pathogens of the acronym than others, such as chorioretinitis for toxoplasmosis or signs of extramedullary haematopoiesis for CMV. Other symptoms are nonspecific, such as (minor) cerebral abnormalities or being born small for gestational age (SGA). Targeted diagnostic testing for one or more of the ToRCH pathogens should of course be planned based on these clinical signs and symptoms, as is described in chapter 4 of this thesis. But especially for these more nonspecific clinical signs and symptoms, the yield and cost of extensive microbiologic testing should be clear to the clinician.

Small for gestational age and ToRCH screening

Infectious diseases account for 5–10% of SGA cases [31 32], but SGA is rarely an isolated manifestation of congenital infection with ToRCH agents [32]. In chapter 3 we found that the co-occurrence of congenital ToRCH infections in asymptomatic SGA neonates is extremely low [33]. Only 2/112 (2%) of infants with SGA were diagnosed with congenital CMV infection. No co-occurrence of SGA and any of the other pathogens of the ToRCH group has been detected. In the epidemiological context of congenital infections in The Netherlands, in cases of isolated and unexplained SGA, a complete ToRCH screening is not necessary and can be limited to screening for CMV, using urine.

Since the publication of our study, four more studies have added evidence that screening for congenital ToRCH infections in infants with isolated SGA is not cost-effective. In 2014 Wei et al. [34] investigated 232 SGA neonates of whom about 50% were screened for either ToRCH serology or CMV in urine: 2 neonates were CMV positive and treated with ganciclovir. Both also had other findings on physical examination suggesting a congenital CMV infection, such as hepatosplenomegaly. In a large study from Australia 415/3,437 SGA (birth weight < 10th percentile) neonates had urine CMV PCR tests performed and only 1 neonate (0.4%) was CMV positive. This infant also had other symptoms suggestive of neonatal infection [35]. Chung et al. [36] screened 119 SGA neonates for ToRCH infections, none tested positive. And

recently Espirutu et al. [37] investigated 386 neonates with a birth weight < 10th percentile, 325 were tested for congenital CMV (either urine PCR and/or IgM in serum) and none tested positive.

At the time of its publication we drew cautious conclusions due to the limitations of our study, such as the retrospective design, possibility of selection bias in our tertiary population and the missing data. But when combining our results with that of the newer studies on the co-occurrence of SGA and congenital infections, the evidence is now much stronger. This means that in countries with a low incidence of congenital infections, a complete routine ToRCH screening in neonates with isolated, unexplained SGA is unnecessary and should be limited to testing for CMV in urine by PCR. In 2017 the European Expert consensus statement on congenital CMV also advised to implement this policy [18], and it has since been integrated in the Dutch CMV guideline [38] and Dutch obstetric guideline for foetal growth restriction [39]. The focus should be on implementing this policy in local protocols and daily routine of neonatal units throughout The Netherlands. New studies could evaluate the cost-effectiveness of this policy.

Neurologic abnormalities associated with ToRCH infections

The pathogens of the ToRCH group have all been associated with various neurologic abnormalities, an overview has been given in Table 1.1 of the general introduction. To decide if cerebral imaging is necessary, a division can be made between those that cause none or mild impairments, such as germinolytic cysts, micro-calcifications and lenticulostriate vasculopathy (LSV), and those that cause severe neurodevelopmental impairments, such as cerebral atrophy, microcephaly, migration abnormalities, large cystic abnormalities and white matter injury. For CMV a European expert consensus statement and advise on cerebral imaging has been formulated, suggesting that in an infant with congenital CMV and neurologic signs or symptoms, a cerebral MRI should be performed [18], and it should be strongly considered in any infant with congenital CMV [18 40]. Depending on the chosen imaging modality, some abnormalities cannot be detected though. For example, polymicrogyria is best detected on cerebral MRI, but LSV only on cerebral ultrasound [41 42]. A combination of both imaging modalities should be considered and consultation of a paediatric (neuro-) radiologist can help to determine their optimal timing and required imaging parameters.

For the other pathogens of the ToRCH acronym, no such advice exists to date and there is not much evidence available. Deciding on cerebral imaging is performed on a case-to-case basis. Table 8.3 shows the type of cerebral lesions that have been described at different gestational ages in foetal infection with one of the ToRCH pathogens. The severity of the possible lesions and their therapeutic or prognostic consequences should be leading

Table 8.3: Brain abnormalities of congenital ToRCH infections by gestational age

	Gestational age (GA) at congenital infection	Described brain abnormalities
Toxoplasmosis [40 43]	< 20 weeks	Ventriculomegaly (hydrocephalus) Severe parenchymal destruction Large diffuse calcifications
	20–30 weeks	Ventriculomegaly (hydrocephalus) Parenchymal destruction/volume loss Mild, less diffuse, calcifications
	> 30 weeks	Small calcifications (periventricular / parenchymal) Lenticulostriate vasculopathy Subependymal (pseudo-)cysts Encephalitis
Rubella virus [40 44]	< 10 weeks	Near-to-total brain destruction Microcephaly
	Later in pregnancy	Ventricular enlargement Myelination abnormalities Periventricular signal abnormalities Basal calcifications Subependymal (pseudo-)cysts
Cytomegalovirus [40 45 46]	< 20 weeks	Migration disorders: agyria/pachygyria Ventriculomegaly Cerebellar hypoplasia Calcifications Microcephaly
	20–27 weeks	Migration disorders: polymicrogyria/schizencephaly Less severe ventriculomegaly Calcifications White matter disease Pseudocysts LSV Microcephaly
	>27 weeks	Myelin delay or myelin destruction White matter disease (predominantly posterior distribution) Pseudocysts LSV
Herpes simplex virus [47]	No gestational age specific data	*Early signs:* Loss of gray-white differentiation Cortical gray matter blurring Cerebral oedema Gyral or leptomeningeal enhancement (after contrast) Haemorrhaging
		Late signs: Leukomalacia Severe diffuse multicystic encephalomalacia Parenchymal, punctate or gyral calcifications Ventricular enlargement Microcephaly Subependymal (pseudo-)cysts (predominantly anterior-temporal)

factors in deciding when to perform cerebral imaging. Consultation of a paediatric (neuro-) radiologist can help to determine the optimal timing and imaging modality. A similar approach as advised for congenital CMV should be taken. In case of neurologic signs or symptoms and confirmed congenital infection, neuroimaging should be performed. In case of no neurologic symptoms and confirmed congenital infection, it should only be performed if finding any abnormalities has therapeutic or prognostic consequences. If an infant has neurologic signs or symptoms and no diagnosis has been made yet, neuroimaging can be part of the diagnostic work-up.

Lenticulostriate vasculopathy and ToRCH

One of the minor cerebral abnormalities that can be found in congenital infections, LSV has been the topic of our study in chapter 2 of this thesis. We found no association between congenital ToRCH infections and the appearance of isolated LSV on cerebral ultrasound examination in our study population. But, when combining our patient data with the data from all previously published studies on the subject, the overall incidence of congenital infections was 7% (32/442), of whom the majority had a congenital CMV infection (25/32, 78%) [48-57]. We therefore concluded that in cases with isolated LSV, routine screening for all infections included in the ToRCH group yields a poor result and does not justify the incurred costs. Diagnostics should be limited to urinary screening for CMV only. But we were careful in our conclusions due to the retrospective nature and absence of a control group in our study.

Since the publication of our patient series, Maayan-Metzger et al. [58] have reported the results of urinary screening for congenital CMV in 84 premature neonates with isolated LSV on cerebral ultrasound. All infants were tested for congenital CMV, but none tested positive, confirming that the yield of screening neonates with isolated LSV for congenital CMV is poor.

Whether isolated LSV in CMV positive neonates is associated with an increased risk of developing sensorineural hearing loss, has been a topic of recent research. Amir et al. [59] and Bilavsky et al. [60] described an increased incidence of hearing loss in infants with congenital CMV and isolated LSV compared to those without LSV on cerebral ultrasound. Giannattasio et al. [61] investigated this recently in a large prospective case-control study and found isolated LSV on cerebral ultrasound exams in 14% of healthy neonates and in 67% of infants with confirmed congenital CMV. Although this difference in the incidence of LSV between the two groups is statistically significant, this study found no association of LSV with neurodevelopmental delay or sensorineural hearing loss. Thus, study results are contradicting, and no consensus has been reached on whether or not isolated LSV is a predictor for congenital CMV and, more importantly, if it is a predictor for symptomatic disease and thus has treatment consequences.

Urinary PCR to test for congenital CMV remains warranted in cases of isolated LSV, but a complete diagnostic work-up for the other pathogens in the ToRCH group is redundant. Urinary PCR for CMV is a non-invasive test and a positive result has clinical consequences. If a child has congenital CMV infection, follow-up of his or her ability to hear is necessary as congenital CMV is the leading cause of sensorineural hearing loss [62]. And if congenital CMV is or becomes symptomatic, early treatment with ganciclovir prevents hearing deterioration [63 64].

When and how to use diagnostic testing for ToRCH infections

Many neonates are evaluated for being SGA, or because of abnormalities found on cerebral ultrasound exams. Infection with one of the ToRCH micro-organisms is a common diagnostic consideration in these cases. It is important for the clinician to be aware that ToRCH 'screening' is not one single serologic test and that multiple serologic and PCR tests are required. And sometimes repeated testing is necessary as is explained in chapter 4 of this thesis. Maternal serologic status can also be informative about the possibility of congenital infection. Knowing maternal serologic status can sometimes prevent neonatal blood drawing and thus reduce the risk for phlebotomy-induced anaemia in the newborn, for which the premature infant is most at risk [65]. Therefore, paediatricians should inquire with their obstetric colleagues if maternal testing has been or can be performed. First trimester serologic status is most informative. In case of a first trimester seronegative or unknown serologic status, maternal (re-)testing can be performed to investigate maternal seroconversion, and if the mother remains seronegative, congenital infection can be excluded. If maternal seroconversion is found, maternal infection has occurred and neonatal testing should be performed to determine if vertical transmission has taken place. In chapter 2 and 3 of this thesis we described that 44% and 50% (respectively) of mothers had been tested for one or more of the pathogens of the ToRCH group, thus inquiring with the obstetrician will prevent many neonatal blood drawing. Figure 8.1 provides a flow-chart of implementing maternal serologic status into detecting congenital infection.

International consensus to determine which clinical condition in a newborn is an indication for ToRCH testing is not available, because it is dependent on various factors, such as local epidemiology, first-trimester maternal antibody status (local obstetric policy) and clinical signs and symptoms of the mother and neonate. These must be taken into account before deciding which laboratory test is useful for diagnostic purposes.

It is important to note though, that the ToRCH acronym was not meant to be exclusive for toxoplasmosis, rubella, CMV and herpes virus. Since its first description in 1971 by Nahmias

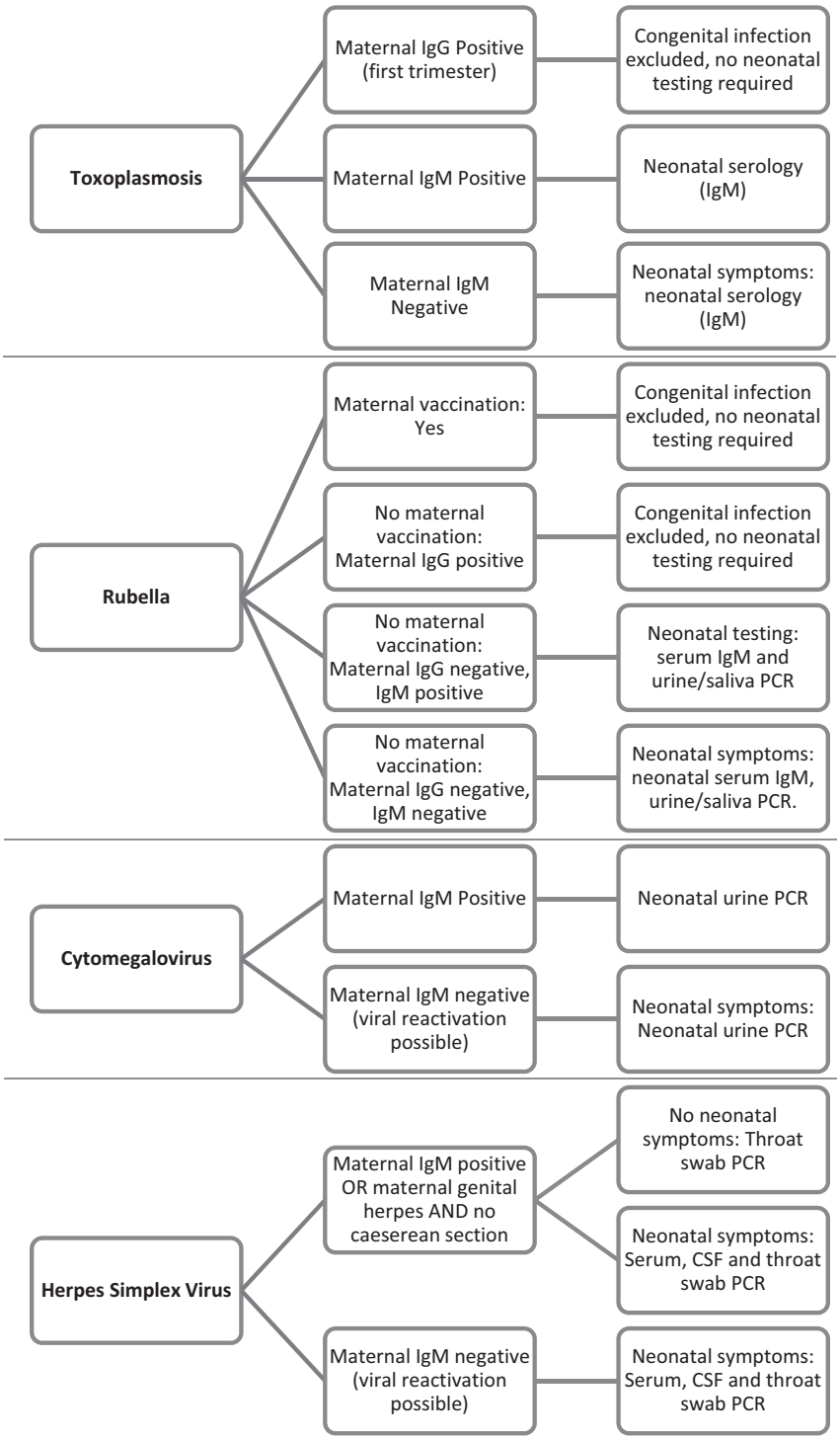

Figure 8.1: Maternal serologic status and detecting congenital infection.

et al. [1], proposals have been made to use the 'o' of the acronym for 'other pathogens'. Other pathogens have been added at different times to the acronym by different authors. Varicella zoster virus, treponema pallidum, enterovirus, parvovirus B19, lymphocytic choriomeningitis virus (LCM), parechovirus and many more have been suggested [66 67]. And more pathogens still can be proposed to be added, for example Zika virus [68]. Its emergence has caused an epidemic, especially in Latin-America, of neonates with microcephaly, ocular abnormalities and sensory neural hearing loss (SNHL) [40 69]. This stipulates the importance of not only relying on the traditional pathogens of the ToRCH group. If a clinician should use the acronym in composing a differential diagnosis, it is important that she or he embraces the 'o' of the acronym into their diagnostic considerations.

Future research perspectives on ToRCH infections

The imaging modalities (MRI and cUS) and their diagnostic usefulness in congenital ToRCH infections should be investigated. Determination of the appropriate timing of imaging and repeated examinations are necessary. To do this, (inter)national collaboration to obtain large enough study populations is very important.

Whether or not LSV is a marker for cerebral damage or CNS involvement in congenital CMV should be investigated in a large prospective cohort study, with an appropriate control group. Infants with congenital CMV with and without isolated LSV and a control group of healthy infants with and without isolated LSV should be compared for clinical outcomes.

Also, other frequent indications for ToRCH testing should be evaluated on their cost-effectiveness in similar ways as we evaluated those with LSV and SGA. The indications that could be considered are thrombocytopenia, hepatosplenomegaly, and/or elevation of liver enzymes.

PART B: ENTEROVIRUS AND HUMAN PARECHOVIRUS

Epidemiology

The first description of enterovirus sepsis-like illness in young infants dates back to 1956, when Crawford and colleagues published a case series of 10 young infants. All had irritability, a rash and fever, and an enteric virus was found in viral cultures [70]. The reported incidence of EV and HPeV infections in young infants varies depending on the studied population. A study from Germany included children under three months of age with aseptic meningitis during a ten year study period [71]. The incidence of EV-meningitis was 7.7% and the same

incidence was found for HPeV-meningitis. But when all infants with sepsis-like illness (including aseptic meningitis) are included, the incidence for both viruses is much higher. In 1999 the overall yearly incidence of EV induced sepsis-like illness in infants from the USA aged less than 90 days was reported to be 25%, and increased up to 50% during an epidemic season (Summertime) [72].

In Chapter 5 of this thesis we confirm this high incidence for the Dutch population. We included all infants with sepsis-like illness with and without meningitis. These inclusion criteria were chosen because these are the most frequent clinical presentations in this age group. We did use a strict definition of sepsis-like illness (see Table 5.1 for the details). EV was detected in 132/353 (37%) and HPeV in 52/353 (15%) infants as the causative pathogen. Figure 8.2 shows the relative distribution of pathogens in young infants with sepsis-like illness in The Netherlands (data based on Chapter 5 of this thesis).

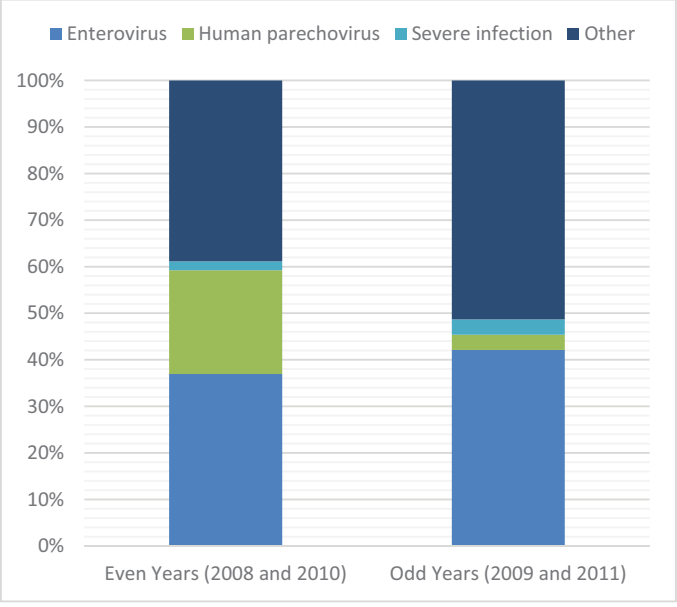

Figure 8.2: Pathogens causing sepsis-like illness in infants aged 0–90 days in even and odd years.
Data based on the study reported in Chapter 5 of this thesis [73].

Our data shows that EV and HPeV infection are the cause of sepsis-like illness in approximately half of the infants aged 0–90 days studied. Combined, they cause 59% of cases of sepsis-like illness in our study population in even years and 45% in odd years. This high incidence stipulates the necessity of adding EV and HPeV PCR in both serum and cerebrospinal fluid (CSF) to the standard work-up of young infants with sepsis-like illness.

EV and HPeV also have distinct seasonal epidemiology. EV has a yearly peak incidence during summer and HPeV has a biannual summertime peak in Europe in even years [74-76]. Our study population also demonstrated this specific biannual pattern [73]. A possible hypothesis is that herd immunity for a specific circulating genotype is gained in epidemic years and protects newborns the year thereafter. This biannual epidemiologic phenomenon is yet to be clarified.

It is important to note though, that during non-epidemic months or years both viruses are never completely absent and thus performing EV and HPeV PCR in a young infant with sepsis-like illness is never redundant.

One of the limitations of our study was that some sample sizes were too small for adequate viral typing. Complete typing of all samples could have given more specific clinical information about the circulating viral types in our study period and their clinical presentation. Among the samples that were typed, type B enterovirus and HPeV type-3 were most frequently identified.

The circulating EV and HPeV genotypes have been shown to vary from year to year and by geographic location [77]. Different genotypes can also express different clinical signs and symptoms in specific age groups. For example, in children up to 3 years of age the percentage of children that develop symptomatic infection due to HPeV is much lower and the severity of the disease is milder than in infants up to 90 days of age. The predominant genotype affecting these children is also different, explaining these differences. In older children (up to 3 years of age), HPeV type-1 is most frequently identified [78], whereas in the younger, more severely ill, infants that were studied in this thesis, HPeV type-3 was most frequently the causative agent.

Different EV types can also cause differences in clinical manifestation. For example, EV genotype D68 can cause severe respiratory symptoms, but is mostly detected in older children [79 80]. Enterovirus A71 has been associated with flaccid paralysis and polio-like syndrome, with more severe symptoms reported from Asian subtypes than in those found in Europe [81-87]. Both EV D68 and A71 illustrate that not only poliovirus (EV type C1, 2 and 3) but also a variety of other types of picornaviruses, can cause severe disease, stipulating the need for picornavirus surveillance and detection of emerging genotypes and their clinical manifestations [88].

Prevention

If infection with EV and HPeV in young infants could be prevented, many cases of hospital admission could be avoided, which would have many positive consequences. However, since EV and HPeV are mostly spread through viral droplets or the faecal-oral route, this

has many practical obstacles. Advocating basic hygienic measures, such as frequent hand washing and avoiding contact with toys or saliva of children with an infection, could possibly prevent some infections in these young infants. But no studies evaluating the effects of such hygienic measures on transmission of EV or HPeV exist.

Vaccination could be another option for prevention of infection and is available for two specific types of enteroviruses, poliovirus and EV-71. Poliovirus vaccine has been available for several decades. Since worldwide poliovirus vaccination started in 1988 [89], its burden of disease has been tremendously reduced [90]. In recent years, three enterovirus-71 vaccines have been licenced in China [91-93], and cost-effectiveness of implementing childhood EV-71 vaccination for the Chinese population is promising [94]. But the Chinese situation, where the EV-71 disease burden is high, is not representative for Europe with a much lower burden of EV-71 related disease. The development of EV-71 specific vaccines does show that developing vaccines against specific types of picornaviruses is possible. However, the EV and HPeV types associated with sepsis-like illness are too numerous for vaccine development. Furthermore, the much lower burden of disease for the EV and HPeV genotypes that cause sepsis-like illness and their generally favourable long term outcome makes development of such vaccines not a priority. Early diagnosis of EV or HPeV can lead to tertiary prevention by recognition of those infants with severe disease. Early interventions to prevent long term disability, where possible, should therefore be the primary focus of clinicians.

Clinical manifestations of enterovirus and human parechovirus

Chapter 5 describes the clinical manifestations of EV and HPeV sepsis-like illness in infants under 90 days of age in detail. Combined with other reports, we now understand the manifestations of EV and HPeV induced sepsis-like illness very well [75 95-101]. The signs and symptoms suggestive of EV or HPeV in these young infants consist, besides the classic signs of infection in infancy, of a rash in about 16% of cases, abnormal behaviour (lethargy or agitation) in 77%, a short duration of illness before presentation (< 1 day), and mildly increased infectious parameters in blood and/or CSF. Our study also confirms that it is important to remain aware that there are no clinically significant differences, between infants with EV or HPeV and those with a serious bacterial infection, neither in medical history, physical examination or infectious parameters. Considering the high incidence of EV and HPeV as causative pathogens, it is important to add EV and HPeV PCR in serum and CSF to the standard of care during a sepsis work-up in young infants.

One of the limitations of our study is that we did not perform other additional viral testing to discover the occurrence of dual viral infections in our study population. Although this

was not our objective, it could have provided additional information on the epidemiology of viral infections in young infants. Hypothetically, those infants with multiple pathogens could have a longer or more severe course of illness. Other limitations in our study were missing data and insufficient sample sizes to perform EV and HPeV PCR on the material in some infants. Although this number of infants was small and represents the obstacles of daily clinical practice, it could have led to over- or underestimation of the incidence of EV and HPeV infections.

Cardiac involvement in EV or HPeV induced sepsis-like illness

In chapter 7 of this thesis, myocardial involvement in young infants with sepsis-like illness was studied. Timely diagnosis of acute myocarditis is difficult as clinical signs and symptoms are often non-specific. Approximately 60% of patients has prodromal symptoms [102] and 77% has had an antecedent illness diagnosed [103], most commonly an upper respiratory infection. Table 8.4 provides an overview of clinical signs of acute myocarditis and demonstrates the relative non-specificity of these symptoms. Chest pain is only reported in literature in children over 10 years of age [103]. Of course young infants cannot express themselves and complain of this phenomenon, but it can be hypothesized that insoluble crying or agitated behaviour in young infants are signs of chest pain.

None of the infants with sepsis-like illness in our study developed clinical signs of acute myocarditis. There were no clinically relevant differences between cardiac markers, ECG and echocardiographic findings in both EV/HPeV positive and EV/HPeV negative infants. Moreover, in both groups some infants had elevated cardiac enzymes or high voltage QRS complex on the first ECG, suggesting that other viral infections in the young infant are also capable of causing these abnormalities. Although none of the studied infants had abnormalities at echocardiography, we did detect a subclinical improvement in left ventricular performance in the EV/HPeV positive group. Although statistically significant, this minor improvement cannot be regarded clinically relevant. Our findings therefore did not support cardiac screening in all infants with EV or HPeV sepsis-like illness. Clinical awareness should remain the clinicians approach.

The occurrence of myocardial involvement in acute viral myocarditis is thought to occur through two pathophysiological processes: (1) direct myocyte damage from viral activity and (2) host inflammatory response [103]. Although little is known about the early stages of acute viral myocarditis [105], we do hypothesise that the minor improvement in %SF, EF and GLS 4Ch in our EV/HPeV positive patients, 4 weeks after infection, can be a sign of minor cardiac involvement during the acute stage of illness. Due to viral entry into the myocardium and activation of the immune response, ventricular function may show a (minor) decrease,

Table 8.4: Possible signs and symptoms of children with confirmed acute myocarditis [102-104]

	Sign or symptom at diagnosis	Percentage of children reported
Patient history	Antecedent illness	73–77%
	Breathing difficulties	43–69%
	Feeding difficulties	13–39%
	Vomiting	9–48%
	Lethargy	18–39%
Physical examination	Tachypnea	60–68%
	Hepatomegaly	36–50%
	Fever	30–45%
	Respiratory distress	47–68%
	Cardiogenic shock	20–29%
Electrocardiogram (%)	Sinustachycardia	13–73%
	ST-wave abnormalities	32–60%
	T-wave abnormalities	31–60%
	Low QRS voltage	13%
	Axis deviation	53%
	Ventricular hypertrophy	20–41%
	Bundle branch block	10–33%
	Arrhythmia	6–7%
	AV-block	5–6%
	Prolonged QT-Interval	5%
Laboratory results	Elevated CRP	27%
	Elevated troponin	38–54%
	Elevated CK-MB	73–77%
	Elevated NT-pro-BNP	96%
Echocardiography	Valvular regurgitation	93%
	Left ventricular dilatation	53–76%
	Pericardial effusion	15–40%
	Wall motion abnormality	7%
	Pulmonary hypertension	24%
	Decreased ventricular function	73–87%

which recovers after clearance of the virus [105]. In our study population this was of transient nature and did not lead to overt myocarditis with left ventricular failure. Possibly, viral-host interactions and specific host genotypes lead to some infants developing overt myocarditis. Further studies are needed to investigate both of these topics.

Limitations of our study are its small sample size and missing data, and, although this represents daily practice, strong conclusions about cardiac involvement in EV and HPeV, and other viral, infections are therefore impossible.

Neurologic sequelae after EV or HPeV induced sepsis-like illness

Chapter 6 reports on cerebral imaging and neurodevelopmental outcome of young infants after admission to a medium care unit with EV or HPeV induced sepsis-like illness. We investigated the presence of neurologic signs and symptoms up to one year of age. Neurodevelopment was not different from age equivalent, healthy Dutch children [106].

Previous studies did report neurodevelopmental delay, but these studies were dated or performed in a population that was admitted to a neonatal or paediatric intensive care unit [107-109]. Presumably, these infants were severely ill at the time of the infection, and therefore incomparable with our study population. Recently, a large retrospective cohort study form Australia described neurodevelopment of infants that had been admitted with HPeV induced sepsis/meningitis [110]. In this large study of 145 infants, 23% had to be admitted to an ICU. Outpatient follow-up was planned for 77/145 (53%) of the study population, in all other cases this was not deemed necessary. Of the 77 children assessed, 11 had reported sequelae of whom 8 needed ICU treatment at the time of infection. The 3 infants that did not need ICU admission, had gross and/or fine motor delay at 1–2 years of age. This study emphasizes the need for good quality follow-up studies of a large cohort. A large percentage of the study population was not followed-up at all and this can lead to a selection bias and over-estimation of the possible sequelae. Follow-up visits were not standardized, which can have led to under-diagnosis of possible sequelae, and firm conclusions were therefore not possible.

Our study also has its limitations, especially its small cohort size and missing data. Although previous studies included cohorts of similar size, the number of patients is still too small to allow firm conclusions, especially in terms of HPeV infection. Also, the duration of our follow-up was only one year and therefore it is possible that some neurodevelopmental problems have been missed or will (dis)appear over time. Another important issue is that MRI scanning was performed 4–6 weeks after hospital admission and used 5 mm thickness scans, so it is possible that we missed small, subtle abnormalities and those that disappear after the acute phase of illness.

When and how to use diagnostic testing for EV and HPeV

The high incidence of EV and HPeV detection in young infants with sepsis-like illness stipulates the necessity of adding EV and HPeV PCR in both serum and cerebrospinal fluid (CSF) to the standard work-up of young infants with sepsis-like illness. As an alternative, a stool sample could be used to detect the virus [111]. It is important to be aware that viral shedding in the gastro-intestinal tract can be detected several weeks after infection, thus

if an infant experienced EV or HPeV infection in the weeks before presenting with sepsis-like illness to the hospital, PCR in faeces could lead to false positive results. However, most young infants did not experience prior EV or HPeV infections in their early life. Another positive benefit of collecting stool samples is that sample size is usually large enough for viral typing, which is a problem in serum and CSF. EV and HPeV PCR in serum, CSF and stool should be integrated into the standard sepsis work-up.

A British study showed recently that rapid PCR results for EV and HPeV by performing them daily influences clinical decisions. They describe that early diagnosis can reduce both the use of broad antibiotic and duration of hospital stay by 2 days [112]. Others have shown that rapid EV and HPeV PCR results decrease the use of long-term broad-spectrum antibiotics and also reduces the number of invasive and costly investigations [113 114], which is both more patient friendly and reduces overall healthcare costs. Some caution should be exercised about the discontinuation of antibiotic therapy. This should only be done when the clinical condition of the infant is stable, his/her infectious parameters do not show a significant increase over time and there is a positive PCR result for EV or HPeV.

Future research perspective on EV and HPeV

The European non-polio enterovirus network (ENPEN) [115] was recently established in Europe to perform viral tracking and surveillance of non-polio enteroviruses. This is important to help predict a next epidemic and should include both case-based and environmental testing. Newly identified viral types and subtypes, and human parechovirus, should be investigated in both a laboratory and clinical setting to better understand their pathophysiology and clinical manifestations. The EPNEN initiative is a laboratory-based collaboration. Clinical researchers should join this initiative to provide clinical input as this could help in early identification of (emerging) viral genotypes that may cause severe disease. Because HPeV can cause severe disease in young infants, HPeV surveillance should be incorporated in the EPNEN initiative.

The safety and cost-effectiveness of discontinuation of antibiotic therapy in infants with positive PCR for EV or HPeV without signs or symptoms of a serious bacterial infection should be established in the Dutch population. Genotyping of the viruses, especially in infants admitted to an intensive care unit, should be performed as this could help in discovering which viral genotypes are a risk factor for causing severe disease.

More evidence to support the cautious conclusions of our studies is needed. A larger cohort and a control group of both healthy infants and infants with a non-EV/HPeV induced sepsis-like illness are pivotal to draw firm conclusions. Genotyping of the viruses found in

infants with subclinical myocardial involvement and those with overt myocarditis should be added to the study protocol so that specific risk of various viral genotypes can be identified.

Future research regarding neurodevelopmental follow-up is essential and should focus on optimal methodology, a standardized follow-up regime, and a longer duration of follow-up. This will make stronger conclusions possible about neurodevelopment after EV or HPeV induced sepsis-like illness in young infants that did not need intensive care treatment in the acute stage of disease.

MAIN CONCLUSIONS OF THIS THESIS

EV and HPeV

- EV and HPeV are the causative pathogens of sepsis-like illness in young infants in about 50% of cases; testing for EV and HPeV should be standard of care.
- Viral genotyping should be performed in all EV and HPeV research projects.
- No clinically significant differences between infants with EV or HPeV and those with a serious bacterial infection exist; not in medical history, physical examination or infectious parameters.
- In our small follow-up cohort, no abnormalities on cerebral imaging were found and neurodevelopment was not different from age equivalent, healthy Dutch children.
- Our findings do not support cardiac screening in all infants with EV or HPeV sepsis-like illness.

ToRCH infections

- In cases of isolated LSV, urinary PCR testing for congenital CMV is warranted. A complete diagnostic work-up for the other pathogens in the ToRCH group is redundant.
- A complete routine ToRCH screening in neonates with isolated, unexplained SGA is unnecessary in countries with a low incidence of congenital infections. It should be limited to CMV testing in urine by PCR.
- Maternal serologic status must be taken into account before deciding which laboratory tests are useful to diagnose congenital infection with one of the ToRCH pathogens.
- Clinicians using the ToRCH acronym should use the ‘o’ of the acronym representing ‘other pathogens’ in their diagnostic considerations.

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