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

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GENERAL INTRODUCTION

INFECTIOUS DISEASES IN PAEDIATRICS

Diagnosis and treatment of infectious diseases are a large part of everyday work of paediatricians. About 35% of all paediatric emergency room consultations concern febrile children [1-3], and in children under one year of age, infectious diseases account for approximately 10% of hospital admissions in the Netherlands [4]. When visiting the emergency room due to febrile illness, the incidence of hospital admission increases with decreasing age of the child. In addition, younger age is a risk factor for more severe illness [5]. Besides infection of the young infant, vertical transmission of micro-organisms from mother-to-foetus during pregnancy or childbirth can lead to morbidity in the neonate and cause late onset symptoms. A more thorough understanding of the epidemiology, diagnostic options and clinical spectrum of infectious diseases, both prenatal, perinatal and postnatal, will contribute towards improvements in treatment and follow-up.

The first part of this thesis investigates toxoplasmosis, rubella virus, cytomegalovirus (CMV) and herpes simplex virus (HSV), also know under the acronym 'ToRCH', a group of micro-organisms that can cause severe symptoms when vertically transmitted from mother to foetus. The second part of this thesis describes the epidemiology, and clinical signs and symptoms of Enterovirus and Human Parechovirus induced sepsis like illness and their cardiac and neurologic sequelae.

TORCH

Pathophysiology and epidemiology

The 'ToRCH' acronym was first proposed in 1971 by Nahmias et al., and groups the micro-organisms *Toxoplasma gondii*, rubella, CMV and HSV because vertical transmission and concurrent neonatal symptoms have been described in all pathogens included in the acronym [6].

Toxoplasma gondii

Although *Toxoplasma gondii* is a protozoan parasite and not a virus, it has been included in this acronym since its first appearance in the literature and although we are aware of this 'non-viral pathogen' in this 'viral' thesis, we decided not to exclude *Toxoplasma gondii* from it.

Vertical transmission of *Toxoplasma gondii* can occur if the mother has a 'primo' infection during her pregnancy. The highest risk of giving birth to a child with symptomatic congenital

toxoplasmosis (about 10%) is when seroconversion occurs at 24–30 weeks' gestation [7 8]. The classic triad of symptoms consists of chorioretinitis, hydrocephalus and intracranial calcifications. Since description of this triad, several other signs and symptoms have been associated with *Toxoplasma gondii* infection. Clinical signs and symptoms of congenital toxoplasmosis, if present, are not always recognised at birth and ocular sequelae sometimes go unrecognised until school age [9]. Congenital toxoplasmosis results in chorioretinitis and retinal scarring in approximately 12% of children and neurological abnormalities, such as cerebral calcifications and hydrocephalus, are seen in about 12%–16% of cases [10-12].

Rubella virus

When primary maternal rubella infection occurs during the first trimester of pregnancy, the virus will cross the placenta and cause foetal infection in about 80% of cases. The risk for foetal infection declines thereafter, as does the risk for congenital defects [13]. The features of congenital rubella syndrome were originally described as a triad of cataracts, heart defects and sensorineural hearing loss (SNHL) [14]. Since then, almost every foetal organ has been described to be infected by rubella and the clinical spectrum ranges from miscarriage or stillbirth, to severe multiple birth defects, to no apparent defect at birth [15].

Cytomegalovirus

The risk of in utero transmission of CMV is highest (approximately 32%) following primary maternal infection. But, in contrast to congenital rubella and toxoplasmosis, the relative immunocompromised state of pregnancy can result in maternal re-infection (with a different strain) or viral reactivation which can also lead to congenital infection [16 17]. About 10%–15% of congenitally infected newborns have symptoms of disease at birth, including low birth weight, central nervous system (CNS) damage, liver involvement and ocular or auditory damage (sensorineural hearing loss, SNHL) [18-20]. Blueberry muffin spots are another symptom of congenital CMV, indicative of extramedullary haematopoiesis. Approximately half of infected children who are symptomatic at birth eventually show CNS involvement [18]. However, almost 90% of the congenitally infected children are asymptomatic at birth and an estimated 13.5% of them will develop long term neurological sequelae, predominantly SNHL [21].

Herpes simplex virus

True primary HSV infection (a first infection with HSV in the individual) has the highest risk for vertical transmission, about 50% [22]. Neonatal infection with HSV is symptomatic in almost all cases and is categorized into localised, CNS disease, and disseminated disease. Localised congenital HSV infection is limited to the skin, eye or mouth, whereas CNS disease results in encephalitis and disseminated disease leads to multiple organ involvement [9].

Lenticulostriate vasculopathy and ToRCH infections

Lenticulostriate vasculopathy (LSV) is one of the cerebral signs that has been associated with ToRCH infections. LSV is an echodensity of the lenticulostriate branches of the middle cerebral arteries in the region of the basal ganglia and/or thalamus. It is thought to be a non-specific marker of a previous insult to the developing brain [24]. LSV occurs in approximately 5% of neonates [24-26]. The clinical significance on long-term neurodevelopmental outcome is not clear. LSV has been associated with a wide variety of diseases including chromosomal disorders, perinatal asphyxia, non-immune foetal hydrops, twin-twin transfusion syndrome, congenital heart disease and metabolic disorders [27-29]. Some authors also suggest a clinical co-occurrence of congenital infections and LSV and advise routine ToRCH screening in all infants with LSV [30-32]. However, this co-occurrence of ToRCH infections and LSV is based on few casuistic reports or small case series, most of which lack a control group [25-27 29-35]. To date, there is no consensus regarding the relevance of efforts to diagnose congenital infections in newborns with LSV detected on cerebral ultrasound scans. In chapter 2 the prevalence of congenital infections was investigated in a large series of neonates with LSV, detected on routine cerebral ultrasound examinations, to determine the role of ToRCH testing in neonates with LSV.

Small for gestational age and ToRCH screening

The exact definition of small for gestational age (SGA) is an ongoing discussion in scientific literature [36-39]. For this thesis we decided to use a neonatal birth weight below 2 standard deviations (SD) for gestational age. Congenital ToRCH infections are one of many signs, symptoms and diseases that have been associated with SGA [40]. But since congenital infections are a diagnostic consideration in SGA neonates, some authors have suggested that ToRCH screening should be part of the routine diagnostic work-up in SGA neonates, but this suggestion is based on limited data [41-44]. The objective of the study in chapter 3 was therefore to evaluate the co-occurrence of congenital ToRCH infections in a larger series of neonates with SGA and describe the yield of ToRCH screening in SGA neonates.

ToRCH screening

As is summarized in Table 1.1, not all pathogens of the ToRCH acronym cause the same symptoms, but 'ToRCH screening' has been increasingly used during the last decades and questions have been raised concerning the indications and cost-effectiveness of ToRCH testing [40 45 46]. We aimed to answer whether ToRCH screening as a 'package' screening tool for a wide scale of symptoms is really necessary and what is the yield of this screening?

Table 1.1: Signs and symptoms of pathogens of the ToRCH acronym

	Neurologic	Cardiac	Abdominal	Sensory organs	Other organs
Toxoplasmosis *Classic triad: chorioretinitis, hydrocephalus and intracranial calcifications.*	Hydrocephalus (ventriculomegaly), intracranial calcifications, LSV*, subependymal (pseudo-)cysts, encephalitis		Hepatosplenomegaly	Chorioretinitis, retinal scarring	Erythroblastosis, hydrops foetalis, lymphadenopathy
Rubella virus *Classic triad: cataract, heart defects and sensorineural hearing loss.*	Subependymal (pseudo-)cysts, microcephaly	Congenital heart defects#		Congenital cataract, sensorineural hearing loss	Small for gestational age
Cytomegalovirus	Hydrocephalus (ventriculomegaly) calcifications, LSV*, microcephaly		Extramedullary haematopoiesis (blueberry muffin spots), liver involvement	Sensorineural hearing loss, ocular damage	Small for gestational age
Herpes simplex virus	Subependymal (pseudo-)cysts, meningoencephalitis	Sepsis		Localised infection; skin, eye and mouth infection.	Disseminated HSV infection; multiple organ failure.

* LSV; lenticulostriate vasculopathy.
# Congenital heart defects most commonly described are pulmonary artery stenosis and patent ductus arteriosus [23].

In chapter 4 the indications and interpretations of ToRCH testing are reviewed in further detail in light of the signs and symptoms of each.

ENTEROVIRUS AND HUMAN PARECHOVIRUS

Viral classification and epidemiology

Enterovirus (EV) and human parechovirus (HPeV) are both genera of the viral family Picornaviridae. This is a viral family that contains small, 'pico', RNA-viruses [47]. More than 80 different EV serotypes can cause infections in humans, among them are coxsackievirus A (classified amongst EV type A), coxsackievirus B (classified amongst EV type B), rhinoviruses (classified as type A, B and C) and polioviruses (classified amongst EV type C) [48]. Echoviruses 22 and 23 initially belonged to the Enterovirus genus [49], but were later reclassified into

the parechoviruses (PeV) and re-named HPeV-1 and HPeV-2 respectively [50 51]. HPeV's are currently classified as parechovirus A species. Currently, 19 different types (HPeV-1 – HpeV-19) of HPeV are known [52].

Studies regarding epidemiology of EV and HPeV have shown that both viruses are more prevalent during summer and early fall (May to September). EV shows little annual variation except from a small 4–5 yearly peak in incidence [48]. However, the prevalence of HPeV infection shows a biannual cycle [53 54]. Remarkably, in Europe HPeV is absent during odds years, whereas in the United States, HPeV is absent during even years [54 55].

Clinical manifestations of febrile children with EV and HPeV infections

In the Netherlands, the majority of febrile children has a viral infection. Bacteria are the causing agents of febrile illness in children under one year of age only in about 8% of cases [56]. But in febrile children with fever of unknown origin or sepsis-like symptoms, the distinction between a bacterial or viral pathogen remains difficult as the range of signs and symptoms is similar in both [5]. Therefore, a sepsis work-up is often performed, including determining blood parameters, blood culture, urine sediment analysis, and, if required by the attending paediatrician, a lumbar puncture. Furthermore, the majority of these children is admitted to the hospital for treatment with broad-spectrum antibiotics, although many of them have a viral infection. Viral infections often have mild symptoms, but some can cause serious disease. Approximately half of all infants younger than 90 days of age that are hospitalized with sepsis-like illness have an infection with EV and HPeV [57-59].

EV and HPeV show overlap in symptoms and are often discussed together [53 54]. EV infection may cause minor disease, such as rhinitis and gastro-intestinal symptoms, but numerous EV-types, especially of the enterovirus B species, have been associated with febrile-illness and aseptic meningitis in infants [60 61]. Moreover, EV's are the most common cause of viral myocarditis in young infants, which presents itself with hemodynamic instability and respiratory failure that can warrant intensive care admission. In 30% of cases this is lethal, and in 60% of survivors a cardiomyopathy develops [62 63].

HPeV infection can also cause serious infection. It has been associated with neonatal sepsis and CNS (central nervous system) involvement like paralysis, encephalitis, and meningitis [64-66]. HPeV-1, at the time of this publication [67] known as echovirus 22, has been reported as a fatal cause of myocarditis. HPeV-3 is related to sepsis, meningitis, and encephalitis in young infants [68-71]. HPeV-4 is associated with fever [72]. HPeV-6 has been isolated in a child suffering from Reye's syndrome [73]. HPeV-8 was isolated from faecal samples in Brazil during an outbreak of enteritis [74 75].

Previous studies that describe epidemiology and clinical signs and symptoms of EV and HPeV have been conducted from a laboratory-based perspective or are limited by small sample size [57-59]. We hypothesized that a larger proportion of infants with sepsis-like illness is caused by EV or HPeV infections than has been previously described. To investigate this hypothesis and to describe epidemiologic patterns and clinical characteristics of EV and HPeV infections, we conducted a prospective study that is described in chapter 5 of this thesis.

Neurologic sequelae after intensive care treatment for EV or HPeV infection

Both EV and HPeV have been identified as neurotropic viruses. Neurologic complications and long term neurodevelopmental effects after EV-71 infection have been reported, but in Europe and the United States of America EV infections are mostly caused by other genotypes than EV-71, that cause different symptoms [76-79]. Several studies, performed in paediatric or neonatal intensive care units (PICU/NICU), describe cerebral sequelae, i.e. white matter abnormalities in infants with severe EV and HPeV infection associated with long-term impairment, such as neurodevelopmental delay, cerebral palsy and epilepsy [65 80].

However, most young infants diagnosed with EV or HPeV induced sepsis-like illness do not need intensive care treatment. In this less severely affected population no recent studies about neurodevelopmental follow-up and occurrence of neurologic sequelae exist. In chapter 6 we investigate whether infants who do not need intensive care treatment show neurologic sequelae and describe cerebral imaging and neurodevelopment at three time points in infants who had EV or HPeV induced sepsis-like illness during their first 90 days after birth.

Cardiac sequelae after intensive care treatment

Myocarditis is a rare and severe condition that, in young infants, is often caused by a viral infection. Various types of EV have been associated with myocarditis, amongst them coxsackievirus type B has been described most frequently [62 63]. In a series of 35 cases, 31% of the infants died and 66% of the survivors developed severe dilated cardiomyopathy [62]. HPeV has also been associated with myocarditis, be it less frequently than EV [67]. However, myocardial involvement in young infants with a viral infection will only be diagnosed in case of severe symptoms of heart failure and need of intensive care treatment [81]. It is unknown whether signs of myocardial involvement occur in infants who are less ill because early diagnosis of acute myocarditis is challenging in infants without overt clinical symptoms of heart failure [82].

It can be hypothesized that if EV and HPeV, being such a common cause of illness in young infants, cause myocardial involvement, early detection by screening for EV or HPeV sepsis-like illness can detect myocardial involvement at an early stage in order to enable early treatment and potentially improve prognosis.

In chapter 7 of this thesis, we measured cardiac markers and performed repeated echocardiography and electrocardiogram exams to investigate whether EV or HPeV infection causes cardiac involvement in young infants with sepsis-like illness who do not need intensive care.

OUTLINE OF THIS THESIS

Part A: ToRCH

- Chapter 2; evaluation of the indication for ToRCH screening in infants with LSV on cerebral ultrasound.
- Chapter 3; evaluation of the indication for ToRCH screening in small for gestational age infants.
- Chapter 4; an overview of the pathogenesis, epidemiology and clinical consequences of congenital ToRCH infections and discusses the evidence for the indications and interpretation of ToRCH screening.

Part B: Enterovirus en human parechovirus

- Chapter 5; a large prospective cohort study that reports on the epidemiology and clinical aspects of EV and HPeV induced sepsis-like illness in young infants.
- Chapter 6; will investigate on neurologic sequelae after EV or HPeV induced sepsis-like illness in a non-intensive care population.
- Chapter 7; signs and symptoms of myocardial involvement after EV/HPeV sepsis are described.

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