Human enteroviruses and parechoviruses: disease spectrum and need for treatment in young children
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Chapter 12

Summary
Enteroviruses (EVs) and human parechoviruses (HPeVs) are both members of the *Picornaviridae* family, a large family of small RNA viruses. The *Enterovirus* genus contains both species that do not infect humans, but animals like pigs, cattle and birds, and species that are capable of causing disease in humans. The human EVs are subdivided in the species human rhinoviruses (HRVs) A-C and the EVs A-D and further in over 100 serotypes, causing a wide array of symptoms from mild respiratory and/or gastrointestinal disease to life-threatening infections like meningoencephalitis and myocarditis. The HPeV species is the only species of the genus *Parechovirus* known to infect humans and currently consists of 16 serotypes. The disease spectrum is similar to that of EVs but significant disease is almost exclusively seen in young children. Currently there is no effective treatment against HPeVs and EVs (including HRVs) available.

Since the introduction of new molecular techniques like PCR, numerous new HPeV and EV serotypes and the HRV-C species were discovered. These technology-driven viral discoveries precede our knowledge of interpretation of test results and of associations between clinical disease and laboratory results. In clinical settings the interpretation of positive results is often part of discussion, since the clinical relevance of a positive PCR result is not always clear.

The aim of this thesis is to describe the prevalence and disease spectrum of EVs and HPeVs and to evaluate the need for treatment together with possible options for the development of effective treatment strategies. In the first part of this thesis some important gaps in our current knowledge of relations between positive human picornavirus laboratory test results and clinical outcomes were investigated. The second part of this thesis focused on treatment against EVs and HPeVs: Is there indeed need for such treatment, since most infections seem to be mild or even asymptomatic? If so, what treatment would be best suitable?

The prevalence of HRV in the first years of life in an unselected birth cohort from the Netherlands and relation with severity of respiratory symptoms is described in Chapter 2. HRV was the most prevalent virus in respiratory symptomatic (41%) as well as asymptomatic (26%) children, but was found significantly more frequent in symptomatic children. The rate of (viral) co-infection in symptomatic HRV infected children was high (62%). Overall, respiratory viruses were detected in 86% of symptomatic children with 44% having 2 or more viruses. Children with HRV mono-infection had more severe symptoms, but HRV infections were not associated with occurrence of wheezing. HRV-A and -C were the most commonly detected species in symptomatic children while HRV-B was more often detected in asymptomatic children. There was no association between the different HRV species and occurrence of wheezing or severity of disease. Symptomatic HRV-PCR positive children, in particular wheezing children, had a significantly higher viral load than asymptomatic children, however, a cut-off value for symptomatic disease could not be defined.
In Chapter 3 the clinical relevance of HPeV1 and -3 detection by PCR in stool samples is described. With the introduction of molecular diagnostic methods in clinical virology laboratories, detection of HPeV is reported more frequently. However, the clinical relevance of these positive findings, especially in stool samples, is under debate. In a retrospective study we showed that HPeV3 was seen in younger children and elicited more severe disease, while HPeV1 was associated with mild symptoms in older children, mainly with underlying disease or in combination with other infectious pathogens. The clinical relevance of detection of HPeV1 in stool samples was therefore not always clear, while a positive HPeV3 PCR in stool samples was associated with clinically relevant disease, regardless of the viral load.

The duration of HPeV shedding in stool samples of symptomatic HPeV infected infants was studied in Chapter 4. The duration of shedding varied between 4 and 24 weeks (median 8 weeks) and did not differ between HPeV types. Most children were asymptomatic during the period of shedding. In general, the HPeV viral load decreased gradually over time. At the moment of initial diagnosis, HPeV3 infected children had lower viral loads in stool samples than HPeV1 infected children, while they had more severe disease. This shows that even low viral loads of HPeV3 in stool samples can be associated with severe symptomatic disease and are thus clinically relevant. The HPeV viral load in stool samples could therefore not be used as marker for severity of disease nor for symptomatic disease.

In Chapter 5 the role of water exposure in the incidence of HPeV and EV infections in infants was studied. By means of a questionnaire the exposure to water of infants in the months prior to a symptomatic HPeV or EV infection was assessed and compared to infants without HPeV or EV infection. We could not identify factors associated with the use of environmental water that increased the risk of acquisition of an HPeV or EV infection. However, siblings in the family were potentially associated with a higher risk of acquiring an HPeV or EV infection.

In Chapter 6 the need for treatment and possible treatment options against HPeV infections are discussed and compared with EV infections. Although most HPeV (and EV) infections only cause mild symptoms, HPeV is capable of causing severe disease with long-term sequelae in similarity with EV. Up to now there is no effective anti-viral agent against HPeV or EV available. The development of antiviral agents is focused on EVs, especially EV71. Because of crucial structural differences between HPeV and EV, a potential effective drug against EVs, will not automatically be effective against HPeVs. Another possible treatment intervention could be antibody-based therapy since the humoral immune response is considered important in EV infections. Intravenous immunoglobulin (IVIG) has been given haphazardly in life-threatening EV and HPeV infections with various clinical outcomes. This variance in outcome is probably resulting from differences in neutralizing antibody (nAb) titers of specific EV and HPeV serotypes in IVIG products. The development of specific monoclonal antibodies could be a feasible option for short-term treatment of life-threatening HPeV infections.
The anti-enteroviral compound that has been evaluated most extensively in clinical trials is pleconaril. This capsid inhibitor has a broad activity against EVs and HRVs. Pleconaril was never approved by the FDA because of possible side effects and concerns about the development of resistance. However, pleconaril has been used on a compassionate use basis in immunodeficient patients with severe EV infection, but its effectiveness has not yet been established. In Chapter 7 we describe the use of pleconaril in two patients with X-linked agammaglobulinemia and a chronic enteroviral meningoencephalitis (CEMA). The first patient, infected with echovirus 11 (E11), did not recover during the period described and CSF remained positive for EV PCR, while the other patient, infected with echovirus 13 (E13), recovered completely. The difference in clinical outcome of the two patients could be subscribed to the difference in in vitro pleconaril susceptibility between the two causative echoviruses. In vitro studies can thus be helpful in predicting effectiveness of pleconaril against different EV serotypes.

Remarkably, the patient infected with the pleconaril resistant E11 had never been treated with pleconaril before, indicating a naturally resistant strain. In chapter 8 the possible mechanisms for the occurrence of this natural resistance were tested and discussed. Analysis of the 3D structure of the capsid revealed that pleconaril anchoring was prevented in the hydrophobic pocket. Comparison with other (pleconaril susceptible) E11 strains revealed 64 amino acid substitutions unique for the resistant E11 strain. Using 3D modelling, three of these 64 amino acid substitutions were identified to be involved in the formation of the hydrophobic pocket. These three mutations were considered possible causes for the resistance against pleconaril. In addition pleconaril resistance was induced in a sensitive E11 strain, resulting in 2 other amino acid substitutions. When introduced into a pleconaril sensitive E11 clone, only one mutation (V119M) led to viable and pleconaril resistant clones. These results underline that natural resistance can differ from in vitro induced resistance. This is important to consider when evaluating resistance profiles of new anti-enterovirus medication in vitro.

Chapter 9 describes the successful treatment with IVIG of an infant with a dilated cardiomyopathy. HPeV1 was the only identified cause of disease. Treatment with IVIG was given and the infant recovered completely. The IVIG batch that was used to treat the patient contained high titers of specific nAbs against HPeV1. In addition, a significant increase in aHPeV1 nAb titers was detected in the cured IVIG treated patient. This case suggests that IVIG could be effective in severe HPeV disease (like myocarditis), if IVIG contains high nAb titers against the infecting serotype.

In Chapter 10 specific cell tropism of HPeV1 and HPeV3 was studied in vitro to explore the differences in clinical symptoms elicited by HPeV1 and HPeV3 infections, with the latter often causing central nervous system (CNS) infection. HPeV3 strains indeed showed faster replication in neural cell lines as compared to HPeV1 strains and there was a relation
between increased *in vitro* replication kinetics and CNS symptoms in the patients from whom the HPeV3 strains were isolated. For HPeV1 no relation was found between clinical symptoms and *in vitro* replication kinetics. Subsequently, various IVIG batches were tested for type specific nAbs, showing that HPeV1 nAbs titers were high in all batches while only very low nAb titers against HPeV3 were found. In addition, 2 HPeV3 infected donors showed low nAb titers both at the moment of infection and a year after infection. This indicates that antibody protection and thus use of IVIG as treatment in HPeV3 infections might fail.

To assess the role of (maternal) nAbs in protection against HPeV infection in infants a prospective mother-child case-control study has been set up ([Chapter 11](#)). As cases, infants with a proven HPeV infection and their mothers were included and compared to controls (infants of similar age with a suspected viral infection, but negative for HPeV) and their mothers. We found no difference in seroprevalence of HPeV1 (99%), HPeV4 (76%) and HPeV3 (2%) between the mothers of cases and mothers of controls. Because maternal nAbs are most important in children <3 months we compared type specific nAbs against the infecting HPeV types of the case mothers with the control mothers. Although nAb titers did not differ significantly between case and control mothers, we found only low or absent nAb titers against the infecting HPeV type in the sera of the mothers of children <3 months with HPeV infection (mainly HPeV3). In available sera of HPeV1 and -3 infected children no specific nAbs against the infecting HPeV type were found. HPeV3 infected children were significantly younger and had significantly more severe disease compared to HPeV1 and -4 infected children. The younger age of infection of HPeV3 infected children compared to HPeV1 and -4 can be explained by the observed difference in maternal nAbs against HPeV3 versus HPeV1 and -4. Thus, maternal nAbs protect against disease in young children with an HPeV1 and -4 infection but maternal humoral protection against HPeV3 is missing. Since HPeV3 infection does not seem to elicit a (sustained) nAb reaction, questions remain about the predilection of symptomatic disease for young children.

In conclusion, HPeV and EV are amongst the most prevalent disease-causing viruses in children and elicit a wide range of disease from mild illness to life-threatening infections. NAb play an important role in the host defense against HPeVs and EVs, but cannot explain fully the differences in pathogenesis of disease of various HPeV and EV types. Differences in receptor usage, viral virulence factors and host related responses of the cellular and innate immune system are largely unknown, but probably play an important role in host defense as well. Knowledge of both the pathogenesis and host response against EVs and HPeVs is essential to develop effective treatment strategies.