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ORIGINAL ARTICLE

Human parechovirus meningitis and gross-motor neurodevelopment in young children

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Abstract

This multicenter prospective cohort study describes the impact of human parechovirus meningitis on gross-motor neurodevelopment of young children. Gross-motor function was measured using Alberta Infant Motor Scale. Of a total of 38 eligible children < 10 months of age at onset, nine cases had clinical evidence of meningitis and polymerase chain reaction positive for human parechovirus in cerebrospinal fluid; 11 had no meningitis and polymerase chain reaction positive for human parechovirus in nasopharyngeal aspirate, blood, urine, or feces; and in 18, no pathogen was identified (reference group).

The children with human parechovirus meningitis showed more frequent albeit not statistically significant suspect gross-motor function delay (mean *Z*-score (standard deviation) − 1.69 (1.05)) than children with human parechovirus infection-elsewhere (− 1.38 (1.51)). The reference group did not fall in the range of suspect gross-motor function delay (− 0.96 (1.07)). Adjustment for age at onset and maternal education did not alter the results.

Conclusion: Six months after infection, children with human parechovirus meningitis showed more frequent albeit not statistically significant suspect gross-motor function delay compared to the population norm and other two groups. Longitudinal studies in larger samples and longer follow-up periods are needed to confirm the impact and persistence of human parechovirus meningitis on neurodevelopment in young children.

What is Known:
- *Human parechovirus is progressively becoming a major viral cause of meningitis in children.*
- *There is keen interest in the development of affected infants with human parechovirus meningitis.*

What is New:
- *This study describes prospectively gross-motor functional delay in children with both clinical evidence of meningitis and polymerase chain reaction positive for human parechovirus in cerebrospinal fluid.*
- *It shows the importance of screening young children for developmental delay in order to refer those with delay for early intervention to maximize their developmental potential.*

Keywords Human parechovirus · Meningitis · Children · Neurodevelopment

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Abbreviations

AIMS	Alberta Infant Motor Scale
CI	Confidence interval
CNS	Central nervous system
CP	Cerebral palsy
CSF	Cerebrospinal fluid
EV	Enterovirus
GMF	Gross-motor function
HPeV	Human parechovirus
n	Number
NPA	Nasopharyngeal aspirate
RT-qPCR	Reverse transcriptase real-time quantitative polymerase chain reaction
SD	Standard deviation of the mean
T0	Presenting at emergency or outpatient departments of the participating hospitals
T6	Follow-up 6 months after visiting the emergency or outpatient departments of the participating hospitals

Introduction

Human parechovirus (HPeV) is progressively becoming a major viral cause of meningitis in children [3, 7, 8, 17]. Especially HPeV-genotype 3 (HPeV-3) is an important cause of central nervous system (CNS) infection in young children [3, 7, 13], HPeV-3 strains showed faster replication in neural cells and HPeV-3 receptor seems to facilitate entry of virus into neonatal CNS cells [30] and may cause both temporary and persistent white matter damage in the CNS [2, 4, 14, 21, 28], leading to functional disorders as cerebral palsy (CP), developmental delay, tonus-regulation disorder, gross-motor function (GMF) delay, and retardation [5, 6, 19, 24, 28, 29].

There is a paucity of cohort studies that have prospectively evaluated short- and long-term motor and neurocognitive development of children with clinical evidence of meningitis and reverse transcriptase quantitative real-time polymerase chain reaction (RT-qPCR) positive for HPeV in cerebrospinal fluid (CSF) [6, 19, 20, 24, 28, 29]. So far, the follow-up studies that reported at least three children with HPeV-meningitis had focused primarily on neurological symptoms [5, 6, 8, 28, 29], neuropsychological deficits using standardized neurodevelopmental scales [28], or standardized parental self-report-questionnaires [5, 6]. To our knowledge, none of these studies has yet performed a systematic GMF-assessment [5, 28, 29]. This is important since GMF-delay can be a good predictor of generalized developmental delay in young children. It is crucial to detect early signs of GMF-delay in order to refer children with delay for early intervention to maximize their development [12, 20].

The objective of this study was to prospectively investigate the GMF in a cohort of young Dutch children, 6 months after presenting with clinical evidence of meningitis and RT-qPCR positive for HPeV in CSF (HPeV-meningitis) and to compare this with children from the same cohort with no clinical evidence of meningitis and RT-qPCR positive for HPeV in nasopharyngeal aspirate (NPA), blood, urine, or feces (HPeV-infection-elsewhere) and those in whom no pathogens were identified (reference group).

Methods

Summary of initial study

This study is part of a multicenter prospective cohort study conducted between March 2008 and September 2011 to investigate the incidence, clinical features, diagnostic methods, and prognosis of HPeV- and enterovirus (EV)-infections in Dutch children. The study methods have been extensively described previously [9, 10]. The study inclusion is shown in the flowchart of Fig. 1. Children 0–16 years with clinical suspicion of viral infection were eligible. Parents and/or legal guardians of eligible children received verbal and written information about the study and were invited to participate. Only those whose parents/legal guardians gave written informed consent were included. After inclusion, the experienced consultant pediatrician on call at the hospital of presentation helped the patient and the parents complete a questionnaire on the birth and medical history, did physical examination and collected NPA, blood, urine, and feces specimens for HPeV and EV RT-qPCR, feces, and NPA specimens for viral culture [9]. If the experienced pediatrician clinically suspected the child to have meningitis or meningoencephalitis, a lumbar puncture was performed according to routine clinical practice and CSF specimen collected for chemistry, RT-qPCR for HPeV, EV, and other common neurotropic viruses, including herpes simplex viruses 1 and 2, varicella zoster virus and adenovirus, viral culture, and bacterial and fungal culture. Children with any viral pathogen than HPeV or any other bacterial or fungal pathogen and those with non-infectious explanation for their clinical picture, age at presentation > 16 years, or non-consenting parents were excluded. Preterm-born children were also excluded because it is an important risk factor for GMF-delay, even in those without peri- and postnatal infection. The inclusion and exclusion criteria of the initial study are summarized in Table 1.

Assignment of study-children into subgroups

Based on medical history, presenting symptoms and finding on physical examination by the experienced pediatrician that saw the children at inclusion and the microbiology test results, the participating children were assigned into three study-subgroups. The assignment took place after inclusion, 6 months prior to the present study to test the GMF of the younger

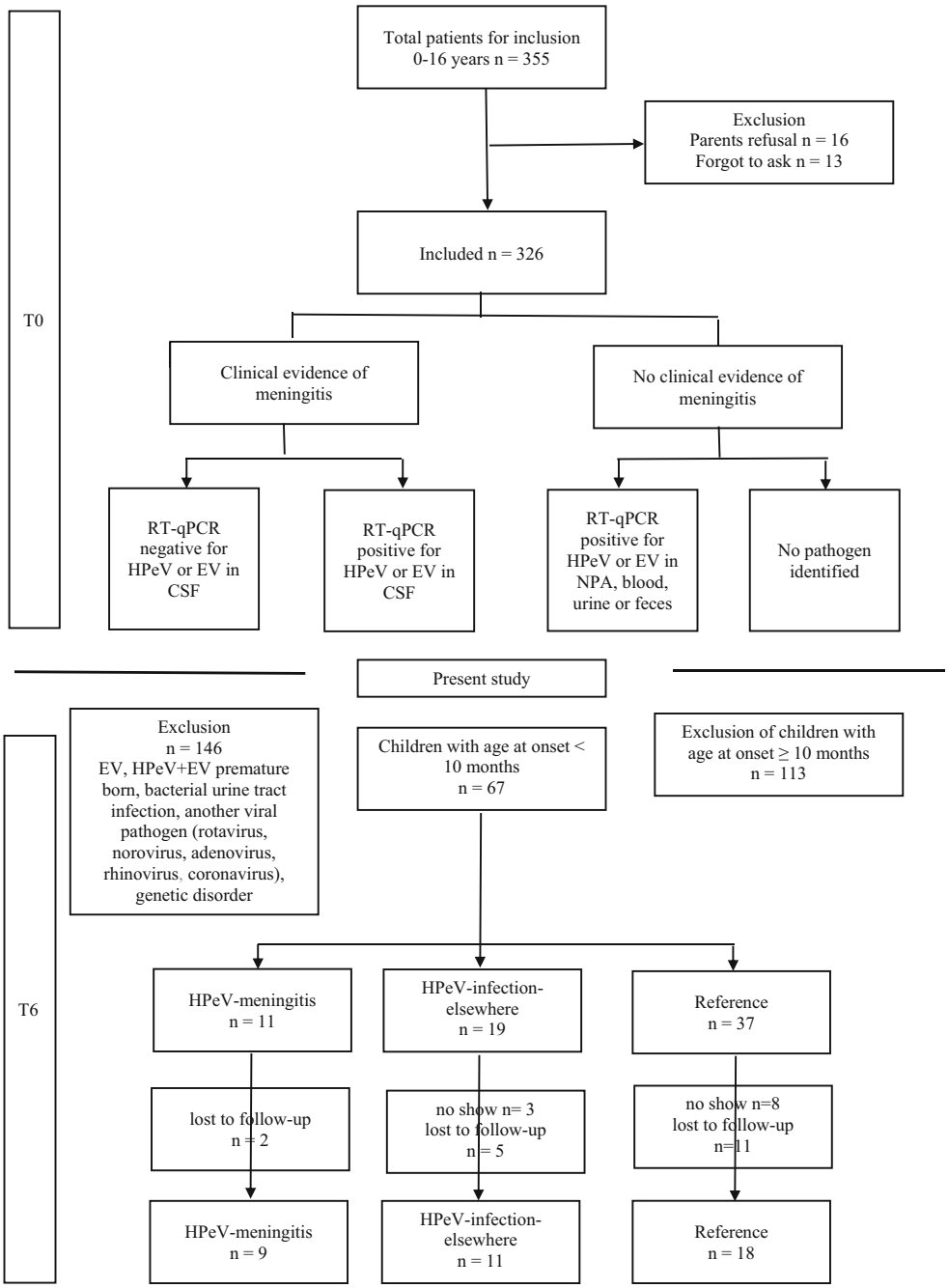

Fig. 1 Flowchart inclusion of the children and follow-up 6 months after visiting the emergency or outpatient departments of the participating hospitals

children aged < 10 months at the initial study inclusion. The method of assignment during the initial study has been published elsewhere [9]. Children in whom their pediatrician found clinical evidence of meningitis [26] at presentation or thereafter during clinical observation and RT-qPCR positive for HPeV in CSF were assigned to the "HPeV-meningitis group"; those whose pediatrician found other clinical symptoms but no clinical evidence of meningitis at presentation or thereafter and RT-qPCR negative for HPeV in CSF, but RT-qPCR positive for HPeV in any of the other tested specimens (NPA, blood, urine, or feces) were included in the "HPeV-infection-elsewhere group" and those in whom their pediatrician found other clinical symptoms but no clinical evidence of meningitis at presentation or thereafter and RT-qPCR negative for HPeV, EV, and any of the other neurotropic viruses tested in CSF, feces, NPA, blood or urine specimens, and negative viral, bacterial, and fungal cultures were included in the "reference group."

The medical ethics committee of each participating center approved both study phases (NL-21361.008.07).

In the present follow-up substudy, we only invited eligible children aged < 10 months at the initial inclusion to participate. We sent an invitation letter to participate in this follow-up. In case of no response, we

Table 1 Inclusion and exclusion criteria of the total study

Inclusion criteria
Children 0–16 year of age with at least one of the following clinical signs and symptoms:
1.Fever (temperature ≥ 38.0 °C or ≥ 100.4 °F)
or
2.Clinical evidence of meningitis including headache, photophobia, nuchal rigidity, irritability, lethargy, nausea, vomiting, drowsiness, positive sign of kernig or brudzinski[a]
or
3.Other clinical signs and symptoms of infection: hypothermia, vomiting, diarrhea, anorexia, cough, myalgia, rash, hypovolemia or shock[b]
and
4.Signed informed consent by the parent(s)/legal guardian(s)
Exclusion criteria
1.Other proven infectious cause of the clinical symptoms
2.Other non-infectious cause of clinical symptoms: e.g., neoplasm, auto-immune diseases, rheumatic diseases, endocrinological diseases, gastro oesophageal reflux
3.Known severe psychomotor retardation, metabolic diseases with neuromuscular and/or cognitive abnormalities
4.Intra-uterine and perinatal problems or traumatic head injury
5.Preterm-born (gestational age < 37 weeks)
6.Children > 16 years of age

[a] At least 2 of these signs or symptoms must be present

[b] At least 3 of these signs or symptoms must be present

followed this up with telephone invitations on at least two occasions. If after the afore-mentioned attempts a parent could still not be reached, we attempted to find out their forwarding addresses or telephone numbers via the practice of their GP in our hospital database. Prior to the visit, parents/legal guardians were instructed not to reveal the study-subgroup to which their child was assigned during the initial study to the blinded independent pediatric physical therapist that tested their GMF.

During the follow-up, the GMF was tested in the pediatric outpatient departments of the participating hospitals by the same pediatric physical therapist. Before starting GMF-assessment, each child was scored accordingly as alert, cooperative, actively moving, showing little activity, struggling, and/or crying. By lack of cooperation from the child, the GMF-assessment was rescheduled on another day. Each child's body mass, length, and head circumference were recorded. Parents/legal guardians were asked to complete a questionnaire on their child's general health, use of medication, presence of recent traumatic head injury, and early intervention (particularly pre-speech- and GMF-training). According to Statistics Netherlands-criteria, maternal education was classified as low (primary school or lower vocational education), middle (middle vocational education), and high (higher vocational education or university degree).

GMF

We assessed the GMF with a norm-referenced observational and performance-based instrument: the Alberta Infant Motor Scale (AIMS) [22]. The AIMS [22], originally developed to assess GMF in Canadian children, from birth to independent walking, has been shown to be discriminative and valid for cross-cultural use [11]. It is suitable for detecting minor GMF-delay in children aged 4–15 months [11].

A higher raw score indicates a more mature GMF. The GMF raw scores were converted to age-adjusted standard deviation (SD) scores (*Z*-score) for each age-month [22]. A *Z*-score of ≤ − 1.30 (equivalent to the 10th percentile of the population norm (AIMS)) is considered suspect GMF-delay [11], while one *Z*-score difference is used to indicate a clinically relevant difference in GMF between groups.

Statistical analysis

The statistical analyses were performed with R-statistics (R-version 3.4.0, R Foundation for Statistical computing, Vienna, Austria). The analysis of categorical variables was performed with the Fisher exact test and continuous variables, including the *Z*-scores of the GMF-tests, with the one-way analysis of variance with post hoc Bonferroni correction and the Kruskal Wallis test with post hoc Mann-Whitney *U* test in case of non-normally distributed data. Linear regression was used to adjust for age at onset, maternal education and early intervention. A p value below 0.05 was considered to indicate statistical significance for all comparisons.

Results

Of the 213 children < 10 months at initial inclusion, 67 were eligible for participation in the follow-up study 6 months later and only 38 were included (Fig. 1). Eighteen were lost to follow-up because they had moved elsewhere without any contact address or telephone number. Eleven failed to appear after repeated invitations; 146 were excluded for different reasons shown in Fig. 1.

Study-population characteristics

Table 2 shows the baseline characteristics. Nine children (24%) had been assigned into the HPeV-meningitis group, 11 (29%) into the HPeV-infection-elsewhere group, and 18 (47%) into the reference group. There were no differences in baseline characteristics of the groups, except the age at onset. Children in the "HPeV-meningitis group" were younger than those in the other groups ($p = 0.01$) (Table 2). Of the 9 children in the HPeV-meningitis group, 8 (89%) were infected by HPeV-3. In 1 (11%), the HPeV-genotype was non-typeable. In the HPeV-

Table 2 Baseline characteristics of the included children attending follow-up visit

	HPeV-meningitis $n = 9$	HPeV-infection-elsewhere $n = 11$	Reference $n = 18$
HPeV-1	0 (0)	3 (27)	
HPeV-3	8 (89)	6 (55)	
HPeV non-typeable	1 (11)	2 (18)	
Male	8 (89)	6 (55)	9 (50)
Age at onset (days)			
Mean (SD)*	31.0 (17.2)	123.0 (73.4)	97.2 (90.5)
Min/max	13/68	16/275	14/251
Hospital stay (days)			
Mean (SD)	3.6 (0.7)	2.9 (1.6)	2.9 (2.2)
Min/max	3.0/5.0	0.0/4.0	0.0/8.0
Maternal education			
Low[1]	1 (11)	1 (9)	3 (17)
Middle[2]	5 (56)	5 (46)	3 (17)
High[3]	3 (33)	5 (45)	12 (66)
Clinical features			
Fever	9 (100)	10 (90.9)	16 (88.9)
Irritability*	7 (77.8)	6 (54.5)	5 (27.8)
Lethargy	2 (22.2)	4 (36.4)	4 (22.2)
Seizures	0 (0.0)	0 (0.0)	0 (0.0)
Dyspnea	1 (11.1)	2 (18.2)	1 (5.6)
Rash	3 (33.3)	6 (54.5)	5 (27.8)
Vomiting	1 (11.1)	4 (36.4)	6 (33.3)
Diarrhea	1 (11.1)	5 (45.5)	9 (50.0)
Poor feeding	5 (55.6)	6 (54.5)	8 (44.4)
CSF findings			
WBC (mm^{-3}) mean (SD)	5.7 (10.8)		
Min/max	1/30		
Protein (g/L) mean (SD)	0.45 (0.11)		
Min/max	0.29/0.61		
Glucose (mmol/L) mean (SD)	2.98 (0.42)		
Min/max	2.60/3.60		
Pleocytosis	0 (100.0)		

*$p < 0.05$

Numbers indicate absolute frequencies and relative frequencies between brackets unless otherwise indicated

Abbreviations: *SD*, standard deviation; *CSF*, cerebrospinal fluid; *HPeV*, human parechovirus; *min/max*, minimum to maximum; *n*, number of children attending follow-up visit

Education level of the parents: [1] primary school and lower vocational education; [2] middle vocational education; [3] higher vocational education or university degree, according to the classification of the Dutch Central Bureau of Statistics

Definitions:

Pleocytosis: the presence of elevated leukocyte count for age in the CSF. The age-specific reference values used were CSF white blood cell (WBC) count > 22/μL for infants ≤ 4 weeks of age, > 15/μL for infants 4–6 weeks of age, and > 7/μL for children > 6 weeks of age

HPeV-meningitis: children with clinical evidence of meningitis and RT-qPCR positive for HPeV in CSF

HPeV-infection-elsewhere: children with no clinical evidence of meningitis and RT-qPCR HPeV-positive in NPA, blood, urine, or feces

Reference: children with no clinical evidence of meningitis and in whom no pathogen was identified

infection-elsewhere group, 6 children (55%) were infected by HPeV-3, 3 (27%) by HPeV-1 and in the remaining 2 (18%), the HPeV-genotype was non-typeable. Of the most frequent presenting clinical symptoms, only irritability was significantly higher in the HPeV-meningitis group. (Table 2).

Results of GMF-assessment, 6 months after presentation

At the follow-up 6 months after presenting at emergency or outpatient departments, parents complete a questionnaire. The

groups did not differ in anthropometrical parameters, general health condition, medication use, and presence of a recent traumatic head injury at the time of GMF-assessment. Seven children had positional nonsynostotical plagiocephaly, 1 in the HPeV-meningitis- and 2 in the HPeV-infection-elsewhere- and 4 in the reference group. They all had a history of preferential supine position with asymmetric position of the head in bed.

Norm-referenced GMF of the study-children

Table 3 summarizes the norm-referenced GMF scores of the included children. Six of the 9 children (67%) in the HPeV-meningitis group had suspect GMF-delay (*Z*-score ≤ − 1.30), compared with 45% in the HPeV-infection-elsewhere and 44% in the reference groups.

Both HPeV-infection groups had a mean GMF *Z*-score in the range of a suspect GMF-delay. Children with HPeV-meningitis had notably, albeit not statistically significant lower mean GMF *Z*-scores (− 1.69 versus − 1.38 and 0.96 for the HPeV-infection-elsewhere and reference groups, respectively).

Association between HPeV-infection and GMF

Table 4 shows that, generally, children in the HPeV-meningitis group had lower GMF scores compared to the children in the HPeV-infection-elsewhere group and in the reference group) (regression coefficient beta (β) (95% confidence interval (CI) − 0.74 (− 1.74 to 0.26). Adjustment for age at onset and maternal education did not alter the results for the HPeV-meningitis group (β (95% CI) respectively: − 0.72 (− 1.81 to 0.36); − 0.84 (− 1.89 to 0.21)). Early intervention had no influence on the association HPeV-meningitis and GMF (β (95% CI) − 0.74 (− 1.71 to 0.23)).

Discussion

In this study, we investigated the GMF outcome of young Dutch children in the HPeV-meningitis group and compared this with those of children in the HPeV-infection-elsewhere group and those in the reference group, 6 months after presentation. A higher percentage of the children in the HPeV-meningitis group showed more frequent albeit not statistically significant suspect GMF-delay (*Z*-score of ≤ − 1.30, equivalent to the 10th percentile of the population norm (AIMS-test)) than the children in the other two groups. Adjustment for age at onset, maternal education, and early intervention did not alter the results. As with previous studies, all the children in the HPeV-meningitis group were ≤ 3 months of age at inclusion and HPeV-3 was the most often isolated genotype [3, 7, 8].

Our study results are noteworthy in the context of and given support by a number of broader emerging literature in this area that reported variable neurodevelopmental outcomes, ranging from normal to CP in young children with HPeV-3-meningitis [5, 6, 19, 24, 28, 29]. One of the hypotheses is that abnormalities in white matter seen during HPeV-infection may lead to minor and major motor functional delay [5, 28, 29]. In this study, children in the HPeV-meningitis group with suspect GMF-delay showed problems mainly in the domain of postural control, a possible early sign for CP or developmental coordination disorder (DCD) [25, 27]. These are not enough follow-up data available to predict if some of these children may end up with CP or DCD at a later age. Barnett et al. have suggested the need for a long follow-up period of at least 5 years in children, in order to detect any late neurodevelopmental abnormalities [1]. The development of the human brain is a dynamic process and the nervous system changes continuously even after the first years of life [16, 18, 23]. The GMF might be variable over the course of the first years of life [16, 20]. This

Table 3 Norm-referenced gross-motor functioning of the study-children 6 months after visiting the emergency or pediatric outpatient departments

	HPeV-meningitis *n* = 9	HPeV-infection-elsewhere *n* = 11	Reference *n* = 18
Children with GMF-delay	6/9 (67)	5/11 (45)	8/18 (44)
Mean GMF *Z*-score (SD)[a]	− 1.69 (1.05)	− 1.38 (1.51)	− 0.96 (1.07)
Min/max	− 3.14/− 0.24	− 4.56/0.25	− 3.50/0.46

Total sample: children with age at onset 13 to 275 days

Number of children with GMF-delay/total number of children tested

Numbers indicate absolute frequencies, relative frequencies between brackets unless otherwise indicated

[a] Norm-referenced GMF *Z*-scores. *Z*-scores of ≤ − 1.30 (equivalent to the 10th percentile of the population norm, measured with the Alberta Infant Motor Scale) are generally accepted to indicate a suspect GMF-delay

Abbreviations: *SD*, standard deviation; *GMF*, gross-motor function; *HPeV*, human parechovirus; *min/max*, minimum to maximum; *n*, number of children attending follow-up visit

Definitions:

HPeV-meningitis: children with clinical evidence of meningitis and RT-qPCR positive for HPeV in CSF

HPeV-infection-elsewhere: children with no clinical evidence of meningitis and RT-qPCR HPeV-positive in NPA, blood, urine, or feces

Reference: children with no clinical evidence of meningitis and in whom no pathogen was identified

Table 4 Association between human parechovirus meningitis and gross-motor function

Determinant	Unstandardized β[a] (95% CI)	p value model[b]
Unadjusted model		0.31
HPeV-meningitis	− 0.74 (− 1.74 to 0.26)	
HPeV-infection-elsewhere	− 0.43 (− 1.37 to 0.51)	
Model adjusted for age at onset		0.51
HPeV-meningitis	− 0.72 (− 1.81 to 0.36)	
HPeV-infection-elsewhere	− 0.43 (− 1.39 to 0.53)	
Age at onset	− 0.01 (− 0.01 to 0.01)	
Model adjusted for maternal education		0.42
HPeV-meningitis	− 0.84 (− 1.89 to 0.21)	
HPeV-infection-elsewhere	− 0.49 (− 1.45 to 0.47)	
Maternal education high	− 0.30 (− 1.14 to 0.54)	
Model adjusted for early intervention		0.15
HPeV-meningitis	− 0.74 (− 1.71 to 0.23)	
HPeV-infection-elsewhere	− 0.32 (− 1.24 to 0.60)	
Early intervention	− 0.77 (− 1.67 to 0.11)	

[a] Unstandardized regression coefficient beta (β) indicates the differences in mean Z-score for HPeV-meningitis-group and HPeV-infection-elsewhere group compared to children in the reference group (reference category)

One Z-score difference is used to indicate a clinically relevant difference in GMF between groups

[b] Model F-statistic p value

Abbreviations: *HPeV*, human parechovirus; *GMF*, gross-motor function; *SD*, standard deviation; *min/max*, minimum to maximum

Definitions:

HPeV-meningitis: children with clinical evidence of meningitis and RT-qPCR positive for HPeV in CSF

HPeV-infection-elsewhere: children with no clinical evidence of meningitis and RT-qPCR HPeV-positive in NPA, blood, urine, or feces

Reference: children with no clinical evidence of meningitis and in whom no pathogen was identified

means that GMF-delay, detected shortly after HPeV-meningitis, may become reversible with time. Seven children, distributed between the three study groups, presented with positional nonsynostotical plagiocephaly during the follow-up study. These were not caused by HPeV-infection but resulted from preferential supine positioning with asymmetrical position of the head in bed. The target of the early intervention was instruction on daily head positioning.

It is difficult to compare our results with the results of some previous findings with regard to severity [5, 28, 29]. This is mainly due to differences in gestational ages of participating children in the different studies. Follow-up studies that reported at least three children with HPeV-meningitis, such as those by Britton et al. ($n = 13$), Verboon et al. ($n = 10$) and Vergnano et al. ($n = 19$) all included a mixture of pre- and full-term-born children (born between 25 and 40 weeks) [5, 28, 29]. It is known that preterm-born children are more likely to develop GMF-delay during the first 18 months of life, even in the absence of a HPeV-meningitis [15]. Therefore, we excluded preterm-born children to limit its potential bias in our results. We recruited study-children from three general hospitals. They were generally mildly to moderately ill. None of them was admitted in an intensive care unit during the study. This contrasts with the afore-mentioned studies in which a majority of patients were admitted in a neonatal- or pediatric intensive care unit [5, 28, 29]. Unlike those studies, the children in our study are more representative of the general population of young Dutch children with HPeV-meningitis, presenting at emergency or outpatient departments.

Maternal education and other environmental factors may influence the GMF of young children [20]. There was no baseline difference in maternal education level between the three groups. This variable did not alter the effects of HPeV-meningitis on GMF in the multivariate regression analysis.

One of the strengths of this study is the use of two control groups, pooled from the same source-population of febrile children aged < 10 months, included in the initial study. Experienced pediatricians assigned the subjects to their study groups based on their history, clinical symptoms, and physical examination and laboratory results in the way it is done in the daily clinical practice. Neither the blinded assessor of the GMFs nor the statistician that performed the statistical analyses of this study was involved in the initial group assignments. This is the first multicenter prospective study comparing GMF of children with proven HPeV-meningitis with two control groups of children with HPeV-infection-elsewhere and a reference group with no pathogen isolated, respectively, from the same cohort. Despite the lack of difference in baseline characteristics, except for age at onset, the children in the HPeV-infection-elsewhere group also had a mean GMF Z-score in the range of a suspect GMF-delay. We cannot completely exclude the possibility of a child in the HPeV-meningitis group presenting without clinical evidence of meningitis at inclusion and being therefore misclassified and assigned into the HPeV-infection-elsewhere group. However, the fact that

most children were hospitalized and clinically observed for an average of 3 days would make this unlikely. We did not consider it ethically acceptable to perform lumbar puncture in all children, irrespective of signs of clinical evidence of meningitis. We think it is unlikely that children in the reference group with meningitis were missed at inclusion, with no signs of clinical evidence of meningitis and negative RT-qPCR and culture for HPeV in all their body specimens. The decision not to perform neuroimaging was based on pediatric literature that has shown discrepancies between brain MRI and neurodevelopmental outcome variables [5, 6, 19, 24, 28, 29]. Most of the reported brain abnormalities seen in the acute phase of infection either disappeared spontaneously or did not correlate to the GMF.

Among the strengths of this study is the use of a norm-referenced GMF measurement (the AIMS). It is widely accepted for detecting (suspect) GMF-delays in young children. Information bias is unlikely because the pediatric physical therapist that conducted the GMF-test was blinded to the assigned study groups of the children during the initial inclusion. The inclusion and exclusion criteria were strictly predefined and study-children were recruited consecutively, thereby reducing selection bias. A high proportion of children of the reference group had GMF-delay. These children had severe symptoms that required a hospital visit. This may have influenced their GMF-development as well, and therefore induced the lack of difference in GMF we found between the HPeV-meningitis and reference groups. We carried out the GMF-testing after 6 months of infection to limit any effect of fever and hospitalization at the initial presentation on the GMF of the study groups.

This study also has several limitations. Firstly, the number of children with HPeV-meningitis was low. This limitation is not specific for our study. Other prospective studies conducted in Western countries have consistently found low incidences of HPeV-meningitis in children [3–5, 7, 19, 24, 28]. Secondly, as with many studies in neonates and young children, it is impossible to adjust for all existing genetic, immunologic, and environmental variables that could play a role in pregnancy, birth, and perinatal development. We excluded those variables known to affect neurodevelopment, including preterm-birth, congenital and birth defects, and infections. In the multivariate analysis model, we adjusted for the influence of age at onset of infection, maternal education level, and early intervention. Children in the HPeV-meningitis group were younger than those in the other two groups. Adjustment for the age at onset did not alter the association between HPeV-meningitis and GMF (Table 4). Lastly, we limited our neurodevelopmental testing to the GMF because it is one of the earliest signs of generalized neurodevelopmental delay in young children. We did not measure language, neuropsychological, cognitive, and behavioral developments. With the plasticity of the young brain, it is possible that GMF can still vary within the first years of life leading to late impairment [18, 23]. We therefore recommend a longer period of longitudinal follow-up in similar cohorts of children until school-going age, with the additional testing of language, neuropsychological, cognitive, and behavioral skills. This will ascertain if a suspect GMF-delay is of a temporary or permanent nature and involvement of other parts of the brain.

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Definitions HPeV-meningitis: children with clinical evidence of meningitis and RT-qPCR positive for HPeV in CSF

HPeV-infection-elsewhere: children with no clinical evidence of meningitis and RT-qPCR HPeV-positive in nasopharyngeal aspirate swab, blood, urine, or feces

Reference: children with no clinical evidence of meningitis and in whom no pathogen was identified

No show: did not show after 3× reminded

Lost to follow-up: moved to another address, changed telephone number, parents too busy to come

Authors' contributions Mrs. van Hinsbergh, MSc., conceptualized and designed the follow-up study. She was responsible for the data collection, carried out the initial statistical analyses, drafted the initial manuscript, revised the manuscript, and approved the final manuscript.

Dr. R. Lindeboom interpreted the statistical data, reviewed the manuscript, and approved the final manuscript.

Prof. Dr. van Furth, Dr. de Crom, and Dr. Obihara conceptualized and designed the study, interpreted the data, reviewed the manuscript, and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved of by the medical ethics committee of each participating center (NL-21361.008.07). This article does not contain any studies with animals performed by any of the authors.

References

1. Barnett A, Mercuri E, Rutherford M, Haataj L, Frisone MF, Henderson S, Cowan F, Dubowitz L (2002) Neurological and perceptual-motor outcome at 5 - 6 years of age in children with neonatal encephalopathy: relationship with neonatal brain MRI. Neuropediatrics 33:242–248. https://doi.org/10.1055/s-2002-36737
2. Belcastro V, Bini P, Barachetti R, Barbarini M (2014) Teaching neuroimages: neonatal parechovirus encephalitis: typical MRI findings. Neurology 82:e23. https://doi.org/10.1212/WNL.0000000000000040

3. Benschop KS, Schinkel J, Minnaar RP, Pajkrt D, Spanjerberg L, Kraakman HC, Berkhout B, Zaaijer HL, Beld MG, Wolthers KC (2006) Human parechovirus infections in Dutch children and the association between serotype and disease severity. Clin Infect Dis 42:204–210. https://doi.org/10.1086/498905
4. Berk MC, Bruning AHL, van Wassenaer-Leemhuis AG, Wolthers KC, Pajkrt D (2018) Human parechovirus meningitis with adverse neurodevelopmental outcome: a case report. Pediatr Infect Dis J 37: e256–e257. https://doi.org/10.1097/INF.0000000000001984
5. Britton PN, Dale RC, Nissen MD, Crawford N, Elliott E, Macartney K, Khandaker G, Booy R, Jones CA, PAEDS-ACE Investigators (2016) Parechovirus encephalitis and neurodevelopmental outcomes. Pediatrics 137:e20152848. https://doi.org/10.1542/peds.2015-2848
6. Britton PN, Khandaker G, Khatami A, Teutsch S, Francis S, McMullan BJ, Jones CA (2017) High prevalence of developmental concern amongst infants at 12 months following hospitalised parechovirus infection. J Paediatr Child Health 54:289–295. https://doi.org/10.1111/jpc.13728
7. Cabrerizo M, Díaz-Cerio M, Muñoz-Almagro C, Rabella N, Tarragó D, Romero MP, Pena MJ, Calvo C, Sonia R, Antonio M, Rienda IM, Otero A, Trallero G (2017) Molecular epidemiology of enterovirus and parechovirus infections according to patient age over a 4-year period in Spain. J Med Virol 89:435–442. https://doi.org/10.1002/jmv.24658
8. Chakrabarti P, Warren C, Vincent L, Kumar Y (2018) Outcome of routine cerebrospinal fluid screening for enterovirus and human parechovirus infection among infants with sepsis-like illness or meningitis in Cornwall, UK. Eur J Pediatr 177:1523–1529. https://doi.org/10.1007/s00431-018-3209-8
9. de Crom SC, Obihara CC, de Moor RA, Veldkamp EJ, van Furth AM, Rossen JW (2013) Prospective comparison of the detection rates of human enterovirus and parechovirus RT-qPCR and viral culture in different pediatric specimens. J Clin Virol 58:449–454. https://doi.org/10.1016/j.jcv.2013.07.017
10. de Crom SC, Rossen JW, de Moor RA, Veldkamp EJ, van Furth AM, Obihara CC (2016) Prospective assessment of clinical symptoms associated with enterovirus and parechovirus genotypes in a multicenter study in Dutch children. J Clin Virol 77:15–20. https://doi.org/10.1016/j.jcv.2016.01.014
11. Darrah J, Bartlett D, Maguire TOR, Avison WR, Lacaze-Masmonteil T (2014) Have infant gross motor abilities changed in 20 years? A re-evaluation of the Alberta Infant Motor Scale normative values. Dev Med Child Neurol 56:877–881. https://doi.org/10.1111/dmcn.12452
12. Doyle LW, Anderson PJ, Battin M, Bowen JR, Brown N, Callanan C, Campbell C, Chandler S, Cheong J, Darlow B, Davis PG, DePaoli T, French N, McPhee A, Morris S, O'Callaghan M, Rieger I, RobertsG SAJ, Wolke D, Woodward LJ (2014) Long term follow up of high risk children: who, why and how? BMC Pediatr 14:279. https://doi.org/10.1186/1471-2431-14-279
13. Fischer TK, Midgley S, Dalgaard C, Nielsen AY (2014) Human parechovirus infection, Denmark. Emerg Infect Dis 20:83–87. https://doi.org/10.3201/eid2001.130569
14. Gupta S, Fernandez D, Siddiqui A, Tong WC, Pohl K, Jungbluth H (2010) Extensive white matter abnormalities associated with neonatal parechovirus (HPeV) infection. Eur J Paediatr Neurol 14:531–534. https://doi.org/10.1016/j.ejpn.2009
15. van Haastert IC, de Vries LS, Helders PJ, Jongmans MJ (2006) Early gross motor development of preterm infants according to the Alberta Infant Motor Scale. J Pediatr 149:617–622. https://doi.org/10.1016/j.jpeds.2006.07.025
16. Hadders- Algra M (2000) The neuronal group selection theory: a framework to explain variation in normal motor development. Dev Med Child Neurol 42:566–572
17. Harvala H, Calvert J, Van Nguyen D, Clasper L, Gadsby N, Molyneaux P, Templeton K, McWilliams Leitch C, Simmonds P (2014) Comparison of diagnostic clinical samples and environmental sampling for enterovirus and parechovirus surveillance in Scotland, 2010 to 2012. Euro Surveill 19:1–9. https://doi.org/10.2807/1560-7917.ES2014.19.15.20772
18. Johnston MV, Ishida A, Ishida WN, Matsushita HB, Nishimura A, Tsuji M (2009) Plasticity and injury in the developing brain. Brain Dev 31:1–10. https://doi.org/10.1016/j.braindev.2008.03.014
19. de Jong EP, Holscher HC, Steggerda SJ, Van Klink JMM, van Elzakker EPM, Lopriore E, Walther FJ, Brus F (2017) Cerebral imaging and neurodevelopmental outcome after entero- and human parechovirus sepsis in young infants. Eur J Pediatr 176:1595–1602. https://doi.org/10.1007/s00431-017-2981-1
20. Morgan C, Darrah J, Gordon AM, Harbourne R, Spittle A, Johnson R, Fetters L (2016) Effectiveness of motor interventions in infants with CP: a systematic review. Dev Med Child Neurol 58:900–909. https://doi.org/10.1111/dmcn.13105
21. Pariani E, Pellegrinelli L, Pugni L, Bini P, Perniciaro S, Bubba L, Primache V, Amendola A, Barbarini M, Mosca F, Binda S (2014) Two cases of neonatal human parechovirus 3 encephalitis. Pediatr Infect Dis J 33:1191–1193. https://doi.org/10.1097/INF.0000000000000412
22. Piper MC, Darrah J (1994) Motor assessment of the developing infant, 1rd edn. WB Saunders, Philadelphia
23. Rice D, Barone S (2000) Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ Health Perspect 108(Suppl 3):511–533. https://doi.org/10.1289/ehp.00108s3511
24. Skram MK, Skanke LH, Krokstad S, Nordbø SA, Nietsch L, Døllner H (2014) Severe parechovirus infection in Norwegian infants. Pediatr Infect Dis J 33:1222–1225. https://doi.org/10.1097/INF.0000000000000456
25. Speedtsberg MB, Christensen SB, Andersen KK, Bencke J, Jensen BR, Curtis DJ (2017) Impaired postural control in children with developmental coordination disorder is related to less efficient central as well as peripheral control. Gait Posture 51:1–6. https://doi.org/10.1016/j.gaitpost.2016.09.019
26. Tapiainen T, Prevots R, Izurieta HS, Abramson J, Bilynsky R, Bonhoeffer J, Bonnet MC, Center K, Galama J, Gillard P, Griot M, Hartmann K, Heininger U, Hudson H, Koller A, Khetsuriani N, Khuri-Bulos N, Marcy SM, Matulionyte R, Schöndorf I, Sejvar J, Steele R, The Brighton Collaboration Aseptic Meningitis Working Group (2007) Aseptic meningitis: case definition and guidelines for collection, analysis and presentation of immunization safety data. Vaccine 25:5793–5802. https://doi.org/10.1016/j.vaccine.2007.04.058
27. Van Balen LC, Dijkstra LJ, Bos AF, Van Den Heuvel ER, Hadders-Algra M (2015) Development of postural adjustments during reaching in infants at risk for CP from 4 to 18 months. Dev Med Child Neurol 57:668–676. https://doi.org/10.1111/dmcn.12699
28. Verboon-Maciolek MA, Groenendaal F, Hahn CD, Hellmann J, van Loon AM, Boivin G, de Vries LS (2008) Human parechovirus causes encephalitis with white matter injury in neonates. Ann Neurol 64:266–273. https://doi.org/10.1002/ana.21445
29. Vergnano S, Kadambari S, Whalley K, Menson EN, Martinez-Alier N, Cooper M, Sanchez E, Heath PT, Lyall H (2015) Characteristics and outcomes of human parechovirus infection in infants (2008-2012). Eur J Pediatr 174:919–924. https://doi.org/10.1007/s00431-014-2483-3
30. Westerhuis BM, Koen G, Wildenbeest JG, Pajkrt D, de Jong MD, Benschop KS, Wolthers KC (2012) Specific cell tropism and neutralization of human parechovirus types 1 and 3: implications for pathogenesis and therapy development. J Gen Virol 93:2363–2370. https://doi.org/10.1099/vir.0.043323-0