Enterovirus, parechovirus, adenovirus and herpes virus type 6 viraemia in fever without source

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► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/archdischild-2019-317382).

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These data have been presented as an oral presentation at the 2018 Infectious Diseases Society of America (IDSA) annual meeting (IDweek) in San Francisco, USA.

Received 8 April 2019
Revised 29 July 2019
Accepted 7 August 2019

To cite: L'Huillier AG, Mardegan C, Cordey S, *et al*. *Arch Dis Child* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/archdischild-2019-317382

ABSTRACT

Objectives To evaluate the potential associations between fever without a source (FWS) in children and detection of human enterovirus (HEV), human parechovirus (HPeV), adenovirus (AdV) and human herpesvirus type 6 (HHV-6) in the plasma; and to assess whether the detection of viruses in the plasma is associated with a reduced risk of serious bacterial infection (SBI) and antibiotic use.

Design and setting Between November 2015 and December 2017, this prospective, single-centre, diagnostic study tested the plasma of children <3 years old with FWS. Real-time (reverse-transcription) PCR for HEV, HPeV, AdV and HHV-6 was used in addition to the standardised institutional work-up. A control cohort was also tested for the presence of viruses in their blood.

Results HEV, HPeV, AdV and HHV-6 were tested for in the plasma of 135 patients of median age 2.4 months old. At least one virus was detected in 47 of 135 (34.8%): HEV in 14.1%, HHV-6 in 11.1%, HPeV in 5.9% and AdV in 5.2%. There was no difference in antibiotic use between patients with or without virus detected, despite a relative risk of 0.2 for an SBI among patients with viraemia. Controls were less frequently viraemic than children with FWS (6.0% vs 34.8%; p<0.001).

Conclusions HEV, HPeV, AdV and HHV-6 are frequently detected in the plasma of children with FWS. Antibiotic use was similar between viraemic and non-viraemic patients despite a lower risk of SBI among patients with viraemia. Point-of-care viral PCR testing of plasma might reduce antibiotic use and possibly investigations and admission rates in patients with FWS.

Trial registration number NCT03224026.

What is already known on this topic?

- Although only 10%–25% of cases are due to serious bacterial infections, the rest are likely due to common viruses.
- Fever without source is a frequent cause of paediatric consultations, requiring invasive investigations, hospital admission and administration of empirical antibiotics.

What this study adds?

- Viraemia is frequent during fever without source and associated with similar antibiotic use despite a fivefold lower risk of serious bacterial infection.
- Point-of-care PCR testing for viruses in the blood could potentially reduce admission rates and antibiotic use.

INTRODUCTION

Fever without a source (FWS) is defined as a fever for which neither an extensive medical history nor a clinical examination can identify a cause.[1] Although most children <3 years old presenting with FWS have a self-limiting viral infection, up to 10%–25% have a serious bacterial infection (SBI).[1–3] Therefore, many children require diagnostic laboratory tests to identify the few patients with an SBI.[1 4 5] Besides blood tests, more invasive laboratory procedures, such as lumbar puncture and urinary catheterisation, are often required, followed by the empirical administration of broad-spectrum antibiotics, especially to younger patients who have an increased risk of an SBI and often a non-specific clinical presentation.[1 4 5] Consequently, identifying systemic viral infections in this population could potentially reduce unnecessary invasive investigations, hospital admissions and antibiotic administration.

Four ubiquitous viruses have been shown to play a predominant role in FWS: human enterovirus (HEV), human parechovirus (HPeV), adenovirus (AdV) and human herpesvirus type 6 (HHV-6).[6] HEV and HPeV are RNA viruses which have a seasonal summer pattern in the northern hemisphere,[7 8] whereas AdV and HHV-6 are DNA viruses with yearly circulation.[9 10] All these viral infections can present as sepsis or FWS in children of all ages,[8–12] although HPeV is diagnosed mainly in infants <3 months old.[8] Because of the frequent asymptomatic respiratory or enteral carriage of these viruses,[13–15] using nasopharyngeal or stool specimens is suboptimal for determining their role in FWS. Consequently, our study aimed to assess how often HEV, HPeV, AdV and HHV-6 viraemia were detected in children <3 years old presenting with FWS and whether patients with viraemia differed from non-viraemic patients in terms of clinical presentation, rates of SBI and management.

PATIENTS AND METHODS

Study design

Participants for this prospective, single-centre, epidemiological diagnostic study were enrolled in the emergency room (ER) division of the Geneva University Hospitals. Inclusion criteria were (1)

clinical diagnosis of FWS defined as a temperature of ≥38°C measured at home or in the ER in acutely ill children <3 years old with no identified focus of infection after a thorough history and clinical exam; and (2) <7 days of fever. Exclusion criteria were (1) unavailable blood; and (2) comorbidities predisposing to infections using chart review (cancer, primary or secondary immunodeficiency, and iatrogenic immunosuppression).

Study specimen collection

Besides usual blood investigations for the normal clinical care of children <3 years old presenting with FWS, plasma was tested by real-time reverse-transcription (RT)-PCR for HEV and HPeV, as well as real-time PCR for AdV and HHV-6 (online supplementary methods).

Definitions

For the study's purposes, the virus-positive group included patients with either HEV, HPeV, AdV or HHV-6 detected in their plasma, whereas the virus-negative group included patients with negative RT-PCR results. SBIs included documented bacteraemia requiring antibiotic treatment (blood cultures interpreted as contaminants were not considered as SBI), bacterial meningitis, osteomyelitis, pneumonia or urinary tract infection (UTI) (online supplementary methods).

Control group

To confirm that viraemia was not an incidental finding, we performed real-time RT-PCR for HEV, HPeV, AdV and HHV-6 on the serum of 50 control children <3 years old consulting at dental or fracture clinics, and enrolled through the Canadian Laboratory Initiative on Paediatric Reference (CALIPER) study in Toronto, Canada.[16]

Ethics

This study was approved by Geneva's (15-082) and Toronto's (1000010867) ethics committees. No investigations were performed before the signature of the informed consent by the parent or legal guardian (online supplementary methods).

Statistics

Continuous variables were compared using Student's t-test or Mann-Whitney U test, depending on variable distribution. Dichotomous variables were compared using χ^2 tests. Statistics were calculated using SPSS V.23.0 software.

RESULTS

Demographics

Over 2 years (from 1 November 2015 to 31 December 2017), we enrolled 135 patients and tested their plasma for HEV, HPeV, AdV and HHV-6 using real-time RT-PCR. The demographics, symptoms and clinical examinations undergone by the study patients are described in table 1.

Viraemia is frequent among patients with FWS

Of 135 patients, 47 (34.8%) had at least one virus detected in their plasma. More specifically, HEV was detected in 19 patients (14.1%), HHV-6 in 15 (11.1%), HPeV in 8 (5.9%) and AdV in 7 (5.2%). Coinfection with more than one virus was detected in two patients (AdV/HEV and AdV/HPeV). There was a trend for an increase in the percentage of positivity with age (p=0.518) (figure 1). The percentage positivity by season is described in online supplementary figure 1.

Clinical presentation, physical examination or laboratory investigations

There were no significant differences in demographics between virus-positive and virus-negative patients (table 1). Moreover, there were no differences in clinical presentations or findings between the groups, except for a faster respiratory rate in virus-negative patients (p=0.038). There were also no significant differences in the diagnostic investigations performed on the two groups (table 1). The breakdown of clinical presentation by virus is detailed in online supplementary table 1.

Initial laboratory results and management

Virus-positive patients had lower leucocyte (p<0.001), neutrophil (p=0.002), lymphocyte (p<0.001) and C reactive protein values (p=0.013) than virus-negative patients (table 2).

After medical history, clinical examination and initial laboratory results, the anticipated risk of SBI was higher among virus-negative patients (p=0.029). Moreover, virus-positive patients were less likely to be admitted (p=0.006) and those admitted had shorter lengths of stay (p=0.048). However, there was no difference between the two groups in the likelihood of receiving antibiotics, taking into account that physicians were blinded to viral RT-PCR results. Among patients who did receive antibiotics, virus-positive patients were more likely to be treated with oral antibiotics (p=0.001) and had shorter durations of therapy (p=0.003) (table 2).

SBIs were rare among patients with viraemia

Twenty study patients (14.8%) were diagnosed with an SBI. One patient had *Haemophilus influenzae* (type f) bacteraemia with concomitant meningitis. Two other patients with positive blood cultures labelled as contaminants were not considered as having an SBI (*Staphylococcus epidermidis* (n=1), *Streptococcus parasanguinis* and *Streptococcus salivarius* (n=1)). Another patient had *Pseudomonas aeruginosa meningitis* (no known comorbidities) (table 2). All these patients belonged to the virus-negative group. Eighteen patients had a confirmed UTI (16 from the virus-negative group), whereas 10 had a possible UTI (9 from the virus-negative group) (table 2). All patients with urine colony counts matching our confirmed or possible UTI criteria showed positive urinalysis. After excluding blood and urine culture contamination, as well as non-confirmed UTIs, SBIs were significantly more frequent in virus-negative (20.5%, 18/88) patients than in virus-positive patients (4.3%, 2/47; p=0.011) (table 2). The relative risk of an SBI was 0.21 among virus-positive compared with virus-negative patients (95% CI 0.05 to 0.86).

Associations with other viral infections

Among patients with any virus detected, regardless of the anatomical compartment (cerebrospinal fluid (CSF), nasopharyngeal swab (NPS), stool or plasma), the relative risk of an SBI was 0.13 among virus-positive patients (3.2%, 2/63) when compared with virus-negative patients (25.0%, 18/72; p<0.001; 95% CI 0.03 to 0.53). Among the 19 patients with HEV detected in the plasma, HEV was also detected in the CSF (n=7), NPS (n=2) and stools (n=1); among 8 patients with HPeV detected in the plasma, HPeV was also detected in the CSF in 1 patient.

Prevalence of viraemia in control children <3 years old

Among control children tested for the presence of HEV, HPeV, AdV and HHV-6, only 6% (3/50) had at least one virus detected in their blood, compared with 34.8% of children with FWS

Table 1 Description of study patients presenting with fever without source and comparison of virus-negative and virus-positive groups

	All study patients (N=135)	Virus-negative (n=88)	Virus-positive (n=47)	P value
Demographics				
Median age, months (IQR)	2.4 (1.3–9.8)	2.4 (1.2–7.2)	2.9 (1.4–16.5)	0.169
Male sex, n (%)	80 (59.3)	54 (61.4)	26 (55.3)	0.496
Symptoms				
Interval between fever onset and ER consultation (hours), n (%)				
<6	29 (21.5)	20 (22.7)	9 (19.1)	0.776
6–12	21 (15.6)	12 (13.6)	9 (19.1)	
12–24	42 (31.1)	30 (34.1)	12 (25.5)	
24–48	15 (11.1)	10 (11.4)	5 (10.6)	
48–72	14 (10.4)	8 (9.1)	6 (12.8)	
72–96	5 (3.7)	2 (2.3)	3 (6.4)	
>96	7 (5.2)	4 (4.5)	3 (6.4)	
Unknown	1 (0.7)	1 (1.1)	0	
Peak temperature, median °C (IQR)	39 (38.7–39.8)	39.0 (38.6–39.8)	39.1 (38.8–40.0)	0.174
Lethargy, n (%)	30 (22.2)	20 (22.7)	10 (21.3)	0.847
Irritability, n (%)	51 (37.8)	32 (36.4)	19 (40.4)	0.643
Decreased intake, n (%)	63 (46.7)	38 (43.2)	25 (53.2)	0.292
Clinical examination				
Toxic appearance, n (%)	22 (16.3)	18 (20.5)	4 (8.5)	0.088
General condition, n (%)				
Good	11 (8.1)	8 (9.1)	3 (6.4)	0.310
Conserved	50 (37.0)	32 (36.4)	18 (38.3)	
Bad	74 (54.8)	48 (54.5)	26 (55.3)	
Median temperature, °C (IQR)	38.4 (38.0–39.0)	38.3 (38.0–38.9)	38.5 (38.0–39.1)	0.356
Median respiratory rate per minute (IQR)	40 (34–52)	42 (36–55)	37 (29–50)	0.038
Median heart rate, beats per minute (IQR)	160 (149–177)	160 (149–172)	160 (148–182)	0.635
Median blood pressure, mm Hg (IQR)	95/57 (86/49–102/66)	93/57 (85/49–100/66)	99/61 (89/54–105/67)	0.402
Median saturation on room air (IQR)	100 (99–100)	100 (99–100)	99 (98–100)	0.051
Investigations performed, n (%)				
CBC	135 (100)	88 (100)	47 (100)	1.000
CRP	135 (100)	88 (100)	47 (100)	1.000
PCT	98 (72.6)	63 (71.6)	35 (74.5)	0.891
Blood culture	130 (96.3)	86 (97.7)	44 (93.6)	0.342
Urinalysis	129 (95.6)	83 (94.3)	46 (97.9)	0.664
Urine culture	127 (94.1)	83 (94.3)	45 (95.7)	1.000
Lumbar puncture	35 (25.9)	23 (26.1)	12 (25.5)	0.939
Chest X-ray	12 (8.9)	10 (11.4)	2 (4.3)	0.215

CBC, complete blood count; CRP, C reactive protein;ER, emergency room; PCT, procalcitonin.

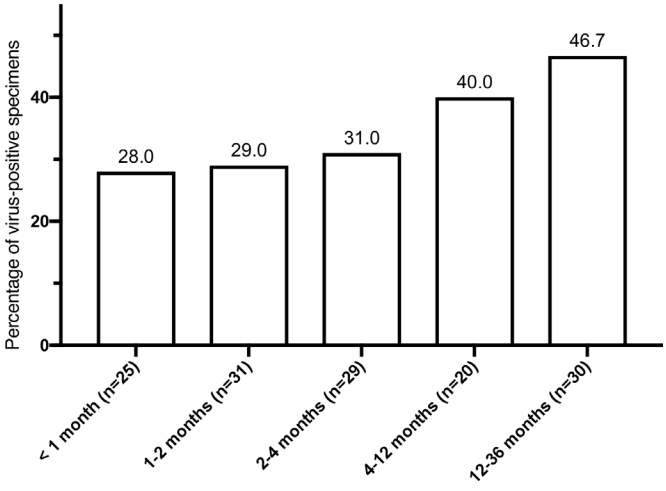

Figure 1 Percentage of virus-positive specimens in children with fever without source by age group.

(p<0.001) (table 3). Precisely, HPeV and HHV-6 were detected in 2 (4%) and 1 (2%) case, respectively, and viral loads were lower than in study patients (table 3). As most controls were enrolled in the autumn, the analysis was repeated after excluding patients enrolled in other seasons, and this confirmed that patients with FWS were still more likely to be viraemic (36.1%, 13/36) than controls (5.0%, 2/40; p=0.001).

Subgroup analysis for patients ≤3 months old

These patients usually benefit from the most conservative approach because of their higher likelihood of SBI and non-specific presentation. Among the 79 patients ≤3 months old, 24 (30.4%) were virus-positive and 55 (69.6%) virus-negative. There was no significant difference between virus-positive and virus-negative patients in the likelihood of receiving antibiotics (62.5% (15/24) vs 72.7% (40/55); p=0.363) and the likelihood of being admitted (54.2% (13/24) vs 70.9% (39/55); p=0.149). The likelihood of SBI remained lower among virus-positive than virus-negative patients (4.2% (1/24) vs 21.8% (12/55);

Table 2 Investigation results and management of virus-negative and virus-positive patients

	Virus-negative (n=88)	Virus-positive (n=47)	P value
Initial investigations results, median (IQR)			
CBC			
Leucocytes, $\times 10^9$/L	14.0 (7.3–18.9)	8.0 (5.7–11.4)	<0.001
Neutrophils, $\times 10^9$/L	5.7 (2.3–10.1)	3.7 (2.1–5.4)	0.002
Unsegmented neutrophils, $\times 10^9$/L	0.2 (0.0–0.4)	0.1 (0.0–0.5)	0.737
Lymphocytes, $\times 10^9$/L	5.2 (3.1–6.9)	3.0 (2.0–5.0)	<0.001
CRP, mg/L	19.0 (6.0–74.3)	6.0 (2.0–31.0)	0.013
PCT, ng/L	0.3 (0.2–0.7)	0.2 (0.2–0.5)	0.165
Median anticipated risk of SBI (IQR)	40 (10–80)	20 (5–60)	0.029
Management			
Hospital admission, n (%)	52 (59.1)	16 (34.0)	0.006
Median duration, days (IQR)	5.0 (3.0–10.0)	3.0 (3.0–4.8)	0.048
Antibiotics, n (%)	60 (68.2)	30 (63.8)	0.702
Route			
Parenteral	58 (96.7)	21 (70.0)	0.001
Oral	2 (3.3)	9 (30.0)	
Median duration, days (IQR)	10.5 (4.0–15.0)	4.0 (3.0–8.0)	0.003
Final microbiological results			
Serious bacterial infection, n (%)*	18 (20.5)	2 (4.3)	0.011
Non-sterile blood culture, n (%)	3 (3.5)†	0 (0)	0.286
CSF, n (%)			
Bacterial meningitis	2 (8.7)‡	0	
Viral meningitis	2 (8.7)‡	8 (66.7)§	0.005
Abnormal CSF analysis, with negative cultures and PCR	3 (13.1)	0	
Urinary culture¶			
Sterile	36 (43.4)	28 (63.6)	
Contamination	22 (26.5)	14 (31.8)	0.044
Possible UTI	9 (10.8)	1 (2.3)	
Confirmed UTI	16 (19.3)	2 (4.5)	

*For this analysis, blood and urine cultures considered as contaminant were not considered as SBIs; cases with a possible UTI were not considered as SBIs.
†86 of 88 patients had blood culture performed, from which *Staphylococcus epidermidis*, *Streptococcus parasanguinis*/*Streptococcus salivarius* and *Haemophilus influenzae* were identified in one patient each.
‡23 of 88 patients had CSF analysis: *H. influenzae*, *Pseudomonas aeruginosa* and HEV were identified in 1, 1 and 2 patients, respectively.
§12 of 47 patients underwent CSF analysis performed: HEV and HPeV were identified in 7 and 1 patient, respectively.
¶83 of 88 virus-negative and 44 of 47 virus-positive patients had urinary culture performed.
CBC, complete blood count; CRP, C reactive protein;CSF, cerebrospinal fluid; HEV, human enterovirus; HPeV, human parechovirus; PCT, procalcitonin; SBI, serious bacterial infection; UTI, urinary tract infection.

p=0.045). The relative risk of an SBI was 5.24 among virus-positive compared with virus-negative patients (95% CI 0.72 to 38.04). Among patients with any virus detected, regardless of the anatomical compartment, the relative risk of an SBI was 8.61 among those with no virus detected (3.0% (1/33) vs 26.1% (12/46); p=0.006; 95% CI 1.18 to 63.00).

Discharge diagnosis

The discharge diagnosis of virus-positive as well as virus-negative patients with and without SBI is detailed in online supplementary table 2.

DISCUSSION

This study suggests that common viruses are detected in the blood of more than one-third of children <3 years old presenting with FWS, reflecting active infections. The high prevalence of viraemia confirmed previous findings by Colvin *et al*, who showed that the detection of HEV, HPeV, AdV and HHV-6 was frequent in children <3 years old with FWS,[6] although study designs were different on several points. First, Colvin *et al*[6] considered as FWS only patients without influenza or without definite or probable bacterial infection whereas we used the American Academy of Pediatrics' definition, which does not consider laboratory results.[1] Second, Colvin *et al*[6] tested for many other viruses, both in the blood and in the nasopharynx. In blood, they detected HPeV and HHV-6 in 7% and 17% of patients in their cohort, respectively,[6] which were slightly higher than our cohort (6% and 11%, respectively) and most likely explained by the fact that Colvin *et al*[6] did not include patients with SBI or influenza in the denominator. These authors did not clearly specify whether HEV and AdV were detected in each patient's blood and/or nasopharynx, which does not allow for a percentage comparison with our cohort. Similarly, a recent study found that infants with sepsis-like syndromes were positive in the plasma and/or the CSF for HEV and HPeV in 37% and 15% of cases, respectively, but without specifying which patients were positive in the plasma.[17] Other studies have shown that the HEV prevalence in the plasma of children <3 months old with fever was similar to our cohort, ranging from 14.5% to 16.7%,[18 19] or 24% after excluding patients with positive bacterial cultures.[20] Similarly, a recent multicentre study found that the prevalence of HEV in the blood of children <2 years old presenting with FWS,

Table 3 Comparison of study patients with FWS and controls

	Patients with FWS (n=135)	Controls (n=50)	P value
Demographics			
Median age, months (IQR)	2.4 (1.3–9.8)	19.0 (13.4–25.9)	<0.001
Male sex, n (%)	80 (59.3)	30 (60.0)	0.927
Season of enrolment			
Spring	27 (20.0)	6 (12.0)	<0.001
Summer	38 (28.1)	0	
Autumn	36 (26.7)	40 (80.0)	
Winter	34 (25.2)	4 (8.0)	
Virology results			
Any viraemia, n (%)	47 (34.8)	3 (6.0)	<0.001
Human enterovirus, n (%)	19 (14.1)	0	0.002
Median CT value (IQR)	28.7 (27.6–31.9)		
Median copies/mL (IQR)	9430 (1210–25 900)		
Human parechovirus, n (%)	8 (5.9)	2 (4.0)	0.462
Median CT value (IQR)	31.6 (26.8–34.7)	– (36.8–37.5)†	
Human herpesvirus type 6, n (%)	15 (11.1)	1 (2.0)	0.038
Median CT value (IQR)	32.6 (30.3–33.5)	39.7†	
Median copies/mL (IQR)*	61 900 (3438–90 200)	<250 (–)†	
Adenovirus, n (%)	7 (5.2)	0	0.106
Median CT value (IQR)	32.1 (31.0–35.8)		
Median copies/mL (IQR)*	14 050 (5190–25 325)		

*Leftover volume was insufficient to perform quantitative PCR in 3 patients for adenovirus and in 7 patients for human herpesvirus type 6.
†Median or IQR was not calculated given the number of positive cases.
CT, cycle threshold; FWS, fever without source.

sepsis or suspected meningitis, and enrolled between June and October, was 35.2%.[21] This was in line with our findings when considering that their enrolment only happened during the peak HEV season.

The high prevalence of virus detection in our study patients' plasma is unlikely to be an incidental finding, for several reasons. First, many patients with HEV and HPeV viraemia were also positive for the same virus in other anatomical compartments (CSF, stools, nasopharynx), confirming the high likelihood of a disseminated viral infection.[12 22] Second, the relative risk of an SBI was lower among patients with viraemia than among patients without documented viraemia. Third, coinfection involving more than one virus in the blood was rare in our cohort, as was coinfection with different viruses in other compartments, as previously shown for HEV-infected infants and neonates.[21] The prevalence of viraemia was also significantly lower in a cohort of children <3 years old who presented to dental or fracture clinic compared with our cohort of children with FWS. This confirms previous findings showing that viraemia is rare in afebrile children[6 23] and emphasises the fact that viraemia is not an incidental finding. Although viral shedding in stool or NPS is frequent, there is no evidence of shedding in the plasma after active infection, confirming that our findings reflect an active infection.[21 23–25]

Similarly, even though herpesvirus microRNAs can be detected in the plasma during latency,[26] there is no evidence that HHV-6 DNA can be detected in the absence of active infection.[27] Moreover, it is very unlikely that our patients with HHV-6 detected in the plasma had chromosomally integrated HHV-6 given their low viral load.[28]

One important finding in our study was that SBIs were approximately five times less common among virus-positive patients than among virus-negative patients. The relative risk of an SBI even dropped to eight times lower if any virus was detected, regardless of the anatomical site. This is in line with previous data showing that children infected with viruses are less likely to have an SBI,[6 29–33] although most data are for children infected with influenza and respiratory syncytial virus. In our cohort, the only SBIs identified among virus-positive patients were UTIs, which is in line with a recent multicentre study focused on HEV infection.[21] Interestingly, providing that physicians were blinded to viral plasma RT- PCR results, virus-positive patients were as likely as virus-negative patients to receive antibiotics despite a fivefold lower risk of SBI. One can therefore postulate that the availability of point-of-care viral PCR for plasma testing might reduce antibiotic use. Moreover, when analyses were restricted to patients ≤3 months old, the likelihood to be admitted did not differ between the two groups. Based on data showing that children with documented HEV meningitis or influenza infection were less likely to undergo invasive investigations, be admitted to hospital and receive antibiotics,[33 34] point-of-care viral PCR could also possibly reduce investigations, admission rates and length of stay.

This study also confirmed previous findings showing that clinical presentation alone does not help to discriminate patients with viraemia from patients without documented viraemia or with an SBI.[35 36] Therefore, in the absence of available viral plasma RT-PCR results, virus-positive patients were as likely as virus-negative patients to undergo invasive investigations. These data confirm the need for children with FWS to undergo thorough investigations, especially the younger ones, despite a relatively low prevalence of SBIs. Interestingly, virus-positive patients were less likely to be admitted, and when admitted they had shorter lengths of stay than virus-negative patients. Moreover, although the likelihood of antibiotic treatment was similar in both groups, virus-positive patients were less likely to receive parenteral antibiotics and had shorter durations of therapy than virus-negative patients.

Interestingly, the discharge diagnosis of virus-negative patients without SBI was strongly suggestive of viral infections, such as gastroenteritis and respiratory infection, with the exception of patients with a diagnosis of possible UTI. One cannot exclude that testing the plasma of these patients for other viruses might have found additional viral systemic infections.

The present study has several limitations. Because healthy children do not require blood tests, finding control patients was very challenging. Management of fractures is mostly conservative in our institution and many young patients have day surgery performed in private clinics. Consequently, the control group had to be enrolled in another country because we were logistically unable to enrol a reasonable number of healthy young children in our institution or nearby hospitals. However, both Canada and Switzerland have a continental climate with a seasonal circulation of HEV and HPeV,[7 8 17 22 37 38] whereas AdV and HHV-6 circulate throughout the year.[9 10] Also, the enrolment breakdown according to seasons differed between study patients and controls, with most controls enrolled during the autumn; however, a subanalysis including only

patients included in the autumn confirmed that viraemia was still significantly more frequent in study patients. The difficulty in enrolling very young, healthy control children also explains why they were older than study patients. However, as the rates of viraemia increased with age among study patients, one cannot exclude an overestimation in the rate of viraemia among controls. Some controls might also have been acutely infected when enrolled, overestimating the rates of viraemia. A final limitation is using controls' serum instead of plasma. The study design also did not include data collection for SBI risk factors. Other factors such as the cost of PCR testing and the relatively long turnaround time without availability of point-of-care PCR testing complicate the implementation of plasma PCR testing in clinical practice.

In conclusion, this study confirmed the high prevalence of common viruses in the plasma of young children with FWS. It also confirmed that clinical presentation does not help to discriminate these patients from those with an SBI, inferring the need for invasive investigations, hospital admission and antibiotics. Because of similar likelihood to receive antibiotics despite a lower risk of SBI in virus-positive patients, the availability of point-of-care viral PCR testing of their plasma could reduce antibiotic use and the associated antimicrobial resistance. This could also possibly reduce invasive investigations and costly hospital admissions, and therefore contribute to reducing healthcare-associated costs. This work warrants more studies to confirm its findings.

Contributors AGL conceived and designed the study, designed the data collection instruments, coordinated and supervised data collection, carried out the initial analyses, drafted the initial manuscript, and reviewed and revised the manuscript. CM designed the data collection instruments, coordinated and supervised data collection, carried out the initial analyses, critically reviewed the manuscript for important intellectual content, and reviewed and revised the manuscript. SC conceived and designed the study, designed the data collection instruments, carried out the initial analyses, drafted the initial manuscript, and reviewed and revised the manuscript. FL coordinated and supervised data collection, critically reviewed the manuscript for important intellectual content, and reviewed and revised the manuscript. SP designed the data collection instruments, coordinated and supervised data collection, critically reviewed the manuscript for important intellectual content, and reviewed and revised the manuscript. FH designed the data collection instruments, coordinated and supervised data collection, critically reviewed the manuscript for important intellectual content, and reviewed and revised the manuscript. LK conceived and designed the study, critically reviewed the manuscript for important intellectual content, and reviewed and revised the manuscript. AG conceived and designed the study, critically reviewed the manuscript for important intellectual content, and reviewed and revised the manuscript. KP-B conceived and designed the study, designed the data collection instruments, critically reviewed the manuscript for important intellectual content, and reviewed and revised the manuscript. AG-L conceived and designed the study, designed the data collection instruments, coordinated and supervised data collection, carried out the initial analyses, drafted the initial manuscript, and reviewed and revised the manuscript. All the authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Funding statement The study was supported by the Gertrude von Meissner Foundation, the Ernst and Lucie Schmidheiny Foundation and the Geneva University Hospitals' Research and Development Project Grant. The study sponsors had no role in study design, data collection, analysis and interpretation, writing of the manuscript, and decision to submit the manuscript for publication. No honorarium, grant or other form of payment was given to anyone to produce the manuscript.

Competing interests None declared.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

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