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Pathogen or Bystander: Clinical Significance of Detecting Human Herpesvirus 6 in Pediatric Cerebrospinal Fluid

Utsav Pandey,[a] Alexander L. Greninger,[b,c] Greg R. Levin,[b] Keith R. Jerome,[b,c] Vikram C. Anand,[d,e] Jennifer Dien Bard[a,e]

[a]Department of Pathology and Laboratory Medicine, Children's Hospital Los Angeles, Los Angeles, California, USA
[b]Department of Laboratory Medicine, University of Washington, Seattle, Washington, USA
[c]Fred Hutchinson Cancer Research Institute, Seattle, Washington, USA
[d]Division of Infectious Diseases, Department of Pediatrics, Children's Hospital Los Angeles, Los Angeles, California, USA
[e]Keck School of Medicine at the University of Southern California, Los Angeles, California, USA

ABSTRACT Human herpesvirus 6 (HHV-6) is an important cause of meningitis and meningoencephalitis. As testing for HHV-6 in cerebrospinal fluid (CSF) is more readily available using the FilmArray Meningitis/Encephalitis panel (FA-ME; BioFire Diagnostics, Salt Lake City, UT), we aimed to determine the clinical significance of detecting HHV-6 in order to identify true infections and to ensure appropriate antiviral initiation. Chart review on 25 patients positive for HHV-6 by FA-ME was performed to determine clinical presentation, comorbidity, treatment, and outcome. The presence of chromosomally integrated HHV-6 (ciHHV-6) DNA was also investigated. Of 1,005 children tested by FA-ME, HHV-6 was detected in 25 (2.5%). Five patients were diagnosed with either HHV-6 meningitis or meningoencephalitis based on HHV-6 detection in CSF, clinical presentation, and radiographic findings. Detection of HHV-6 by FA-ME led to discontinuation of acyclovir within 12.0 h in all 12 patients empirically treated with acyclovir. Six of the 12 patients were started on ganciclovir therapy within 6.8 h; 4 of these were treated specifically for HHV-6 infection, whereas therapy was discontinued in the remaining 2 patients. CSF parameters were not generally predictive of HHV-6 positivity. The presence of ciHHV-6 was confirmed in 3 of 18 patients who could be tested. Five of the 25 patients included in the study were diagnosed with HHV-6 meningitis/meningoencephalitis. FA-ME results led to discontinuation of empirical antiviral treatment in 12 patients and appropriate initiation of ganciclovir in 4 patients. In our institution, detection of HHV-6 using FA-ME led to faster establishment of disease etiology and optimization of antimicrobial therapy.

KEYWORDS meningoencephalitis, HHV-6, pediatric, molecular, FilmArray Meningitis/Encephalitis panel

Human herpesvirus 6 (HHV-6), which comprises HHV-6A and HHV-6B, belongs to the *Herpesviridae* family. Of the two viruses, HHV-6B is more commonly implicated in human diseases (1, 2). Infection with HHV-6 is ubiquitous, with seropositivity between 72% and 100% in adult populations (3). Furthermore, in approximately 1% of humans, HHV-6 viral DNA is integrated into their chromosomes. The clinical significance of chromosomally integrated version of HHV-6 (ciHHV-6) remains under investigation (4, 5). Infections caused by HHV-6 have been associated with an array of clinical manifestations, most typically with a self-limited febrile exanthematous illness termed roseola infantum but also with serious conditions such as hepatitis, hemophagocytic syndromes, and encephalitis (6, 7).

HHV-6 is recognized as a central nervous system (CNS) pathogen, and HHV-6 encephalitis is associated with high mortality and lifelong neurological sequalae,

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Address correspondence to Jennifer Dien Bard, jdienbard@chla.usc.edu.

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especially in immunocompromised patients (8). Recently, sample-to-answer platforms have opened new avenues for rapid detection of wide variety of pathogens based on clinical symptoms. The FilmArray Meningitis/Encephalitis (FA-ME) panel (BioFire Diagnostics, Salt Lake City, UT) is a multiplex PCR panel that rapidly detects 14 CNS pathogens (*Escherichia coli* K1, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, cytomegalovirus, enterovirus, herpes simplex virus 1 and 2, human parechovirus, varicella-zoster virus, *Cryptococcus neoformans/Cryptococcus gattii*, and HHV-6) with a specificity of 99.7% (9, 10). The primary concern is that increased detection of HHV-6, particularly of clinically irrelevant reactivation, self-limited infections, or ciHHV-6, may misguide clinicians and lead to inappropriate antiviral therapy (11). There is a paucity of data on the clinical utility of FA-ME in diagnosing true cases of HHV-6 CNS disease, particularly in children. Here, we sought to determine the clinical significance of detecting HHV-6 using the FA-ME panel at a tertiary care pediatric hospital after implementation of testing in June 2016 (12).

MATERIALS AND METHODS

Ethics statement. The study was conducted at Children's Hospital Los Angeles (CHLA), a free-standing tertiary care pediatric medical center, and was approved by the CHLA Institutional Review Board under IRB CHLA-16-00343. All patients who tested positive for HHV-6 by the FA-ME panel between 1 June 2016 and 31 May 2019 were included in the study.

Laboratory workflow. The FA-ME panel is available 24/7 to providers in all units to order on patients with signs and symptoms of meningitis or encephalitis (e.g., fever, headaches, seizures, altered mental status, and/or vomiting). Specimens approved for testing were cerebrospinal fluid (CSF) obtained from lumbar puncture. The microbiology laboratory staff confirm that every FA-ME panel order is accompanied by a bacterial CSF culture order. Positive results from the FA-ME assay are paged to the microbiologist on call, who reviews patient's overall clinical presentation and relevant laboratory findings before advising the laboratory staff to report the results in the electronic medical records (EMR). Positive results are considered critical and are called to the providers.

Data collection. Chart review was conducted on all patients who tested positive for HHV-6 DNA by the FA-ME panel during the established time frame. Relevant clinical information, which included clinical presentation, comorbidities, antimicrobial therapy, and radiographic findings, was obtained. Additional infectious disease testing on CSF and other clinical specimens collected within the encounter period was also conducted to determine alternate infectious etiology.

CSF parameters, which included white blood cell (WBC) counts and protein and glucose levels, were also collected. A ratio of 1:500 (WBC:red blood cells [RBC]) was used to correct WBC values when the CSF draw was traumatic (13). CSF samples with >5 WBC/μl were considered pleocytic (14). Protein values between 30 and 60 mg/dl and glucose values between 37 and 75 mg/dl were considered normal.

Droplet digital PCR analyses and genotyping. DNA was extracted from 200 μl of CSF or whole-blood specimen on a Magna Pure 96 instrument (Roche, Pleasanton, CA) and eluted into 100 μl DNA elution buffer. When 200 μl of specimen was not available, phosphate-buffered saline (PBS) was added to make the total volume 200 μl. For whole-blood specimens only, an additional HindIII digest step was performed on 5 μl of DNA, and HHV-6 droplet digital PCR (ddPCR) was performed as described previously (15, 16). The assay was run on a Bio-Rad QX100/200 system, and between 12,000 and 18,000 droplets were analyzed using QuantaSoft analysis software (Bio-Rad, Hercules, CA). The presence of ciHHV-6 was detected using an HHV-6 DNA to cell ratio of 1 ± 0.07.

HHV-6 quantitative PCR. Absolute quantification of HHV-6 DNA in blood and CSF was obtained using a lab-developed quantitative PCR (qPCR) test targeting a 150-bp region of the UL67 gene of HHV-6 (17). The assay was run on an ABI Prism 7500 instrument using TaqMan Universal PCR mastermix (Thermo Fisher Scientific, Lenexa, KS). The limit of detection for the assay was set at 280 copies/ml during clinical verification. The assay could reliably detect HHV-6A and HHV-6B.

Investigation for clinical significance. Final diagnosis for all 25 patients was determined using a combined approach. First, collection of International Classification of Diseases (ICD-10) codes and review of clinical notes was completed for each patient to determine the diagnosis per the primary attending and/or infectious disease (ID) consultation during the initial visit. Second, subsequent retrospective chart review for each patient was conducted by an ID attending, who was blind to the ciHHV-6 results, to determine whether HHV-6 was likely the primary cause of infection. Initial and follow-up diagnosis for each patient were required to be concordant in order to maintain diagnostic consistency for our patient cohort.

RESULTS

Between June 2016 and May 2019, 1,005 CSF samples obtained via lumbar puncture were tested on the FA-ME panel at our institution as part of the standard of care, with a median turnaround time of 3.33 h. Data for patients with HHV-6 DNA detected in their CSF is summarized in Table 1. A total of 25 (2.5%) patients were positive for HHV-6 by

TABLE 1 Summary of clinical findings for patients testing positive for HHV-6 by FA-ME panel

Patient	Gender[f]	Age (yrs)	Length of stay (days)	Previously healthy[g]	Presented symptoms[i]	CSF[h] results for:			Empirical antiviral treatment	Antiviral treatment after FA-ME
						WBC[a] (cells/mm^3)	Glucose (mg/dl)	Protein (mg/dl)		
1[b]	M	1.22	Expired	Yes	Fever, emesis, altered mental status	0.89	83	426	Acyclovir	Ganciclovir
2	M	0.02	4.7	Yes	Fever, irritability	6	38	141	None	None
3	F	0.55	2.6	Yes	Fever, emesis, loose stool, episodic limpness	0	77	26	None	None
4	F	1.21	1.1	Yes	Fever, seizures, diarrhea	0	62	28	Acyclovir	None
5	M	0.04	5.3	Yes	Fever, lethargy	5.996	35	98	Acyclovir	None
6[c]	M	0.91	13.8	Yes	Fever, emesis, diarrhea, seizures	6,141	25	166	Acyclovir	None
7	F	1.32	Expired	No (brain neoplasm, on chemotherapy)	Brain neoplasm, hydrocephalus	0	Not done	Not done	None	None
8	F	6.01	11.4	Yes	Fever, headache	138.976	46	356	Acyclovir	Ganciclovir
9	F	17.01	7.6	Yes	Sensory loss, weakness, fever, abnormal gait, headache	843.99	45	101	Acyclovir	Ganciclovir, then oral valganciclovir
10[b]	M	0.01	2.7	Yes	Rash	13	46	104	Acyclovir	None
11	F	0.43	1.3	Yes	Fever, irritability, bulging fontanelles	0.2	61	38	None	None
12[d]	M	0.08	2.7	Yes	Fever, cough	947.7	40	81	None	None
13	M	0.07	7.6	Yes	Fever	2.962	37	56	Acyclovir	None
14	M	0.04	9.9	Yes	Fever, jaundice, congestion, cough	0	36	195	None	None
15	F	0.12	0.2	Yes	Fever, diarrhea	0	49	47	None	None
16	M	0.07	1.3	Yes	Fever	5.932	37	108	Acyclovir	None
17	M	1.87	12.7	No (microcephaly)	Microcephaly, seizures, vomiting, constipation, fussy	0	60	32	None	None
18	M	12.87	1.3	Yes	Fever, stiff neck, vomiting, sensitivity to light	Not done	44	102	Acyclovir	None
19	M	0.54	0.8	Yes	Fever, bulging fontanelles	0	67	618	None	None
20	M	0.54	0.1	Yes	Fever, fussiness, bulging fontanelles	0	58	32	None	None
21[b]	M	12.59	25.8	No (HSCT)	HSCT, altered mental status, acute GVHD, hypertension, diarrhea, seizures	0	77	26	Cidofovir	Foscarnet
22	M	0.84	2.6	Yes	Fever, irritability, cough, rash	0.89	83	426	Acyclovir	Ganciclovir
23	M	0.15	3.8	Yes	Fever	1	115	93	None	None
24	F	0.76	18.2	Yes	Fever, emesis, altered mental status, seizure, left foot twitching	0	Not done	Not done	Acyclovir	Ganciclovir
25[e]	M	0.67	1.6	Yes	Fever, bulging fontanelles	2	63	25	None	None

[a]A ratio of 1:500 (WBC:RBC) was used to correct WBC values.
[b]Patients were admitted to intensive care units.
[c]Coinfection with HHV-6 and *Streptococcus pneumoniae*.
[d]Coinfection with HHV-6 and enterovirus.
[e]HHV-6 IgM negative.
[f]M, male; F, female.
[g]HSCT, hematopoietic stem cell transplant.
[h]CSF, cerebrospinal fluid.
[i]GVHD, graft versus host disease.
[j]ciHHV-6, chromosomally integrated HHV-6.

FA-ME; HHV-6 was the sole pathogen detected in the CSF of 23 patients, and the remaining two patients had HHV-6 codetection alongside *Streptococcus pneumoniae* and enterovirus, respectively, from their CSF. Patients positive for HHV-6 had a median age of 0.55 years (range, 3 days to 17 years), and 22 (88%) of patients were previously healthy.

Fever (21/25, 80%) was the most common symptom described in the HHV-6 positive patients, followed by emesis and seizure. Radiographic evaluation was conducted in

TABLE 1 (Continued)

ciHHV-6[j] present	HHV-6 genotype	Whole-blood HHV-6 qPCR value (no. of genomes/ml)	Positive laboratory finding(s) other than FA-ME[k]	Radiographic finding(s)[l]	ID consult[m]	Diagnosis
No	B	3,371	None	Infarctions in the bilateral putamen and in the medial thalami; cerebral edema	Yes	HHV-6 meningoencephalitis
Not done	Not done	Not done	None	Not done	No	Presumptive primary HHV-6 infection
Not done	Not done	Not done	None	Not done	No	Presumptive primary HHV-6 infection
No	B	33,174	Urine positive for *Enterococcus faecalis*	Unremarkable appearance of the brain	Yes	Presumptive primary HHV-6 infection
Not done	Not done	Not done	Urine positive for *Klebsiella pneumoniae*	Not done	No	Presumptive primary HHV-6 infection
Not done	Not done	Not done	None	Leptomeningeal enhancement, most prominent along the bilateral frontal and parietal regions	Yes	Presumptive primary HHV-6 infection
Yes	B	89,844	Blood positive for *Streptococcus mitis*; urine positive for *Proteus mirabilis*; lower back drainage positive for *Pseudomonas aeruginosa* and *E. faecalis*	T2 hyperintense mass	Yes	Probable reactivation
No	B	<280	EBV DNA detected from CSF	Numerous multifocal areas of signal abnormality within the white matter	Yes	HHV-6 meningoencephalitis
No	Not done	<280	None	Multiple nonenhancing scattered T2/FLAIR hyperintense foci within subcortical and deep white matter	Yes	Probable reactivation
No	B	508,427	Urine positive for *Escherichia coli*	Not done	Yes	Presumptive primary HHV-6 infection
Not done	Not done	Not done	None	Small extra-axial fluid collections, probably developmental; no evidence of intracranial mass or hemorrhage	No	Probable HHV-6 meningoencephalitis/ meningitis
No	Not done	Not done	None	Not done	No	Presumptive primary HHV-6 infection
Not done	Not done	Not done	NP swab positive for rhinovirus/enterovirus	Not done	Yes	Presumptive primary HHV-6 infection
Yes	A	2,855,447	Blood positive for *Streptococcus vestibularis*; NP swab positive for RSV	Not done	Yes	Significance unknown
No	B	Not done	None	Not done	No	Presumptive primary HHV-6 infection
Yes	B	2,346,517	None	Not done	No	Significance unknown
No	B	Not done	None	Not done	No	Probable reactivation
No	Not done	Not done	None	Tiny focus of nonspecific T2/FLAIR hyperintensity in the left frontal white matter; no evidence of abnormalities	No	HHV-6 meningitis
No	B	Not done	None	Not done	No	Probable HHV-6 meningoencephalitis/ meningitis
No	Not done	Not done	None	No evidence of abnormalities other than mild prominence of the extra-axial spaces is noted without significant associated mass effect	No	Probable HHV-6 meningoencephalitis/ meningitis
No	B	271,518	Adenovirus and EBV DNA detected from blood; NP swab positive for rhinovirus/enterovirus and adenovirus	Mild/moderately enlarged ventricles, with FLAIR hyperintensity; new areas of diffusion restriction seen suggesting an acute ischemic insult to the brain	Yes	HHV-6 meningoencephalitis
No	B	296,494	None	Not done	Yes	Presumptive primary HHV-6 infection
No	B	Not done	Urine positive for *E. faecalis*	Not done	Yes	Presumptive primary HHV-6 infection
No	B	2,549	CSF positive for HHV-6 by mNGS; stool positive for norovirus	Innumerable small, scattered foci of diffusion restriction with corresponding T2 hyperintensity	Yes	HHV-6 meningoencephalitis
Not done	Not done	Not done	None	Not done	No	Probable HHV-6 meningoencephalitis/ meningitis

[k]NP, nasopharyngeal; EBV, Epstein-Barr virus; RSV, respiratory syncytial virus; mNGS, metagenomic next-generation sequencing.
[l]FLAIR, fluid-attenuated inversion recovery; T2, transverse relaxation time.
[m]ID, infectious disease.

11/25 (44%) patients, and ID consultation was requested in 13/25 (52%) patients. For the 23 patients that were admitted, the median length of stay was 2.7 days (range, 0.1 to 28.1 days). Using the criterion of a WBC count of ≥5 cells/mm^3, 8/25 (32.0%) HHV-6 positive patients exhibited pleocytosis. CSF glucose and protein levels were abnormal in 6/24 (25.0%) and 14/24 (58.33%), respectively, of HHV-6 positive patients.

Significance of HHV-6 in CSF. A total of five patients (20%) were diagnosed with HHV-6 meningitis ($n = 1$) or meningoencephalitis ($n = 4$) based on ICD-10 codes and/or notes in the medical chart that specifically indicated HHV-6 CNS infection as a primary cause (Table 1, patients 1, 8, 18, 21, and 24). Thorough chart review conducted by an ID attending corroborated the original reports. All five patients had radiographic abnormalities (magnetic resonance imaging [MRI] or computed tomography [CT] scan)

consistent with CNS disease, including multifocal areas of signal abnormalities, infarctions, and cerebral edema. There was a total of two deaths related to HHV-6 CNS infections; patient 1 was a previously healthy 14-month-old who presented with fever, emesis, and altered mental status who progressed to coma and then death 6 days from symptom onset and 3 days from admission to our hospital. Likewise, patient 21 was a hematopoietic stem cell transplant (HSCT) recipient who, in addition to the HHV-6 infection, developed graft-versus-host disease and expired. All four meningoencephalitis patients were treated with ganciclovir ($n = 3$) or foscarnet ($n = 1$), as opposed to the one patient with HHV-6 meningitis, who recovered in the absence of antiviral therapy.

The remaining 20 patients had HHV-6 detected in the CNS but did not have clinical findings indicative of meningitis/meningoencephalitis. Four patients (patients 11, 19, 20, and 25) presented with notably bulging fontanelles in the absence of other CSF abnormalities or significant imaging abnormalities. The bulging in all cases was self-limited and resolved at the time of discharge. No other infectious etiology was identified for any of the four patients. Review of the medical records revealed that 11/20 patients had presumptive primary HHV-6 infection without clinically significant CNS disease. Finally, detection of HHV-6 DNA in the remaining five patients was considered to indicate reactivation or to be clinically insignificant by the providers.

Whole blood and CSF molecular testing. Quantitative CSF and whole-blood test results may be useful in cases of HHV-6 encephalitis to monitor viral loads and antiviral efficacy. Whole-blood HHV-6 PCR was performed on 11/25 patients as part of the standard of care, and 9 (81.8%) were positive, with viral loads ranging from 3,371 to 2,855,447 copies/ml. Patient 21 had CSF qPCR values as high as 1,200,000 copies/ml but was consistently negative (reported as $<$280 copies/ml) for HHV-6 DNA in whole blood, indicating that HHV-6 meningoencephalitis might occur in the absence of viremia. Four of the five patients diagnosed with HHV-6 meningoencephalitis/meningitis had whole-blood HHV-6 viral load ordered as part of the standard of care, out of which three (75%) were positive for HHV-6. In one patient (patient 24) with HHV-6 meningoencephalitis, testing by metagenomic next-generation sequencing was also positive for HHV-6.

Further investigation for ciHHV-6 by droplet digital PCR was performed on 18 patients with remnant samples available. We confirmed the presence of ciHHV-6 DNA in three (16.7%) (Table 1, patients 7, 14, and 16) patients tested using either whole blood ($n = 7$), CSF ($n = 7$), or both ($n = 4$). Among the patients with ciHHV-6, none were diagnosed with HHV-6 meningoencephalitis. All three patients were not treated for HHV-6 infection. Patients with confirmed ciHHV-6 DNA (mean, 1,763,936 copies/ml) had significantly (*P* value, 0.0004) greater HHV-6 DNA load in their whole blood compared to that in patients without ciHHV-6 (mean, 120,654 copies/ml). For patients without ciHHV-6, viral loads in whole blood ranged widely, from 508,427 copies/ml to 2,549 copies/ml. HHV-6 genotyping was successfully performed on 14 patient samples. HHV-6B was detected in 13 patients, including all four patients with HHV-6 meningoencephalitis. One of the three patients with ciHHV-6 was positive for HHV-6A.

Impact of FA-ME on antimicrobial therapy. In total, 22/25 (88%) patients were started on empirical antimicrobial therapy prior to availability of FA-ME result, as follows: antibiotics plus acyclovir (11 patients), antibiotic only (8 patients), acyclovir only (1 patient), and antibiotic and antifungal (2 patients). Testing of CSF by FA-ME prompted discontinuation of acyclovir in all 12 patients within a median of 12 h due to negative herpes simplex virus 1 (HSV-1) and HSV-2 results. Six patients in total were started on ganciclovir ($n = 5$) or foscarnet ($n = 1$) based on FA-ME results within a median of 6.8 h, including the four patients with meningoencephalitis. In the remaining two patients, one (patient 9) was diagnosed with CNS demyelination and HHV-6 roseola infantum and was treated with intravenous (i.v.) ganciclovir for 24.5 h, followed by an oral valganciclovir course for 12 days. The final patient (patient 22) was diagnosed with primary HHV-6 infection (exanthema subitum and rash), and i.v. ganciclovir was discontinued in 12.4 h, as it was considered unnecessary.

Clinical significance of other laboratory findings. Nine out of 25 (36%) patients had positive laboratory findings other than FA-ME, 3 of which were in patients diagnosed with HHV-6 meningoencephalitis (Table 1). In one of these patients (patient 8), Epstein-Barr virus (EBV) was also detected in the CSF but was interpreted as likely to be episomal, since serological investigation revealed a positive Epstein-Barr nuclear antigen (EBNA) index and viral capsid antigen IgG, but negative viral capsid antigen IgM. Despite only HHV-6 being detected from CSF, patient 21 had multiple viruses detected from other sources, which included EBV and adenovirus from blood and rhinovirus/enterovirus and adenovirus from a nasopharyngeal (NP) swab. The patient was diagnosed with adenovirus viremia alongside HHV-6 meningoencephalitis. Patient 24, who was diagnosed with HHV-6 meningoencephalitis, also had norovirus detected from a diarrheal stool specimen, which resolved and did not explain the neurological findings.

In the remaining six patients with additional positives, HHV-6 detection was considered insignificant or presumptive primary infection. Three patients (patients 4, 5, and 23) had <50,000 CFU/ml of potential uropathogens recovered from urine, but findings were deemed insignificant in the setting of negative urinalysis. Blood cultures were positive in two patients (patients 7 and 14) considered to have HHV-6 reactivation in the setting of sepsis, as previously described (18). Patient 7 also had positive urine and wound cultures that were considered significant, and patient 14 was also positive for rhinovirus/enterovirus from an NP swab, which correlated with the presence of cough and congestion. Rhinovirus/enterovirus was also detected from an NP swab in patient 13.

DISCUSSION

Contrary to recent studies that reported low incidences of meningoencephalitis despite detection of HHV-6 DNA in CSF (11, 19), we report CNS infections in 20% of all HHV-6 positive patients in this cohort. Furthermore, the presence of HHV-6 DNA in the CNS during primary HHV-6 infection in infants and young children may be a common feature, even in the absence of significant inflammation. Four infants had presumptive primary HHV-6 infection presenting with bulging fontanelles despite a lack of significant CNS inflammation, all of which self-resolved.

Although considered primarily a pathogen in immunocompromised individuals and HSCT recipients (8, 20, 21), HHV-6 CNS infection has been documented in immunocompromised and immunocompetent patients (7, 22). Our results demonstrate that HHV-6 infection of the CNS can occur in patients that are not immunocompromised, as only one of these patients had a history of HSCT. Therefore, testing for HHV-6 should not be based solely on immune status. Our findings contrast those of a recent study in which the only patient diagnosed with HHV-6 encephalitis had a history of HSCT (11). This difference could be attributed to the ages of the patients included in the studies. In immunocompetent pediatric patients, meningoencephalitis following exanthem subitum is well documented, whereas CNS infection in immunocompetent adults is a rare occurrence (23). Abnormal radiological findings were strong predictors of HHV-6 meningitis/meningoencephalitis, as 5 out of 5 patients with meningitis/meningoencephalitis had abnormal findings.

One of the major concerns regarding widespread use of the FA-ME panel has revolved around the relevance of HHV-6 detection and its effects on antimicrobial management (24, 25). As the majority of primary HHV-6 infections in immunocompetent hosts are self-limiting, and unnecessary exposure to ganciclovir and foscarnet can lead to bone marrow suppression and renal insufficiency (26), routine antiviral therapy is not recommended. The efficacy of antivirals in HHV-6 encephalitis in immunocompetent hosts is also unknown, as data are limited to case reports and small case series with mixed results (27). The issue was raised in a recent study of predominantly adult patients (12/15), in which detection of HHV-6 DNA in the CSF resulted in unwarranted ganciclovir or foscarnet therapy (undisclosed amount of time) in 40% ($n = 6$) of patients (11). Another study of 19 patients (10 adults) reported inappropriate ganciclovir treat-

ment in 15.8% ($n = 3$) patients (19). The authors did report that empirical antibiotic therapy was promptly discontinued in 8 pediatric patients found to have primary HHV-6 infection, demonstrating potential antimicrobial stewardship benefit. Although unnecessary exposure to antiviral agents were not prevalent in our cohort of 25 patients, in which only 2/6 patients treated with either ganciclovir or foscarnet did not have CNS involvement, it is important to emphasize that this is a potential risk for targets with lower clinical specificity, particularly in the setting of sample-to-answer testing offered in the clinical laboratories.

In our patient cohort, negative HSV-1/2 results by FA-ME led to discontinuation of all empirical acyclovir therapy. This is in line with a previous study reported from our institution and elsewhere, which demonstrated that rapid testing and reporting of negative HSV-1/2 results allows for prompt discontinuation of acyclovir (28, 29). Despite previous studies that raised concerns about the analytical sensitivity for HSV-1/2 of the FA-ME panel (30), data within our institution have shown 100% correlation between HSV-1/2 results compared to an alternate FDA-cleared PCR assay (data not shown). A recent meta-analysis examining the overall sensitivity and specificity of the FA-ME panel determined that the negative predictive value of HSV-1/2 was >99% (31). Furthermore, detection of viral targets in general using the FA-ME panel have been associated with decreases in unnecessary antibiotics, length of hospital stays, and hospital costs (29, 32).

Presence of ciHHV-6 is one of the confounding factors in understanding the clinical significance of detecting HHV-6 in the CSF. Clinically, ciHHV-6 has the potential to reactivate and cause disease in hosts and is an important consideration during HSCT (33). Diagnostically, in patients with ciHHV-6, differentiating active versus latent infection in the presence of CSF pleocytosis can be challenging. We analyzed samples from 18 patients for the presence of ciHHV-6, out of which 3 were positive. The mean genome copy number in patients with ciHHV-6 (1,763,936 copies/ml) was much higher than that in patients without ciHHV-6 (mean, 120,654 copies/ml), generally consistent with previous work indicating that levels of HHV-6 above 300,000 copies/ml of whole blood are highly suggestive of ciHHV-6 (33, 34). Importantly, detection of ciHHV-6 by FA-ME did not lead to patients being diagnosed with HHV-6 meningoencephalitis or to unnecessary antiviral treatment in our patients. It is worth noting that testing for ciHHV-6 can lead to increased cost for the patient, and the test availability is limited to a few reference laboratories. Thus, a viral load of >300,000 in peripheral blood. along with persistently high viral load while on therapy, may be used to predict the likelihood of ciHHV-6.

Abnormal CSF parameters values for glucose, protein, and pleocytosis are often used as an indicator of CNS infection, but the utility of these parameters in pediatric setting has been dubious (12, 35, 36). In our study, we found that in majority of the cases of HHV-6 infection of the CNS occurred in the presence of normal CSF parameters. Among the five patients diagnosed with meningoencephalitis or meningitis, WBC pleocytosis was present in only one patient (patient 8). This finding is not too surprising, as pleocytosis is often absent or minimal during primary infection-associated HHV-6 encephalitis (37). Thus, CSF parameters should not be used to rule out HHV-6 CNS infections in pediatric patients. In a larger study of 1,025 samples from 948 patients, we reported that CSF parameters were poor predictors of FA-ME positivity with viral pathogens, including HHV-6 (36).

There are several limitations in this study that warrant discussion. First, this is a single-center study in a tertiary care pediatric medical center on 25 patients, and our findings may not reflect findings and practices in other institutions. Although the patient cohort of 25 HHV-6 positive patients is small, it represents the largest set of cases detected by FA-ME compared to previously published studies (11, 19). Nevertheless, future multicenter studies involving larger patient populations may allow better assessment of treatment and patient outcomes. Second, the lack of serological data in our patient population is another limitation of this study, as ordering of HHV-6 IgM and IgG serologies is not part of the routine standard of practice in our institution.

Serologies were ordered on one patient (patient 25) only. Thus, in the absence of serological data, determination of primary HHV-6 infection was based upon a patient's past medical history, CNS findings, and overall clinical picture. That said, interpretation of IgG and IgM results can be problematic in our patient population (median age, 0.55 years) due to the presence of maternal IgG, and IgM itself has been shown to have poor sensitivity and specificity (38). Last, the FA-ME panel does not provide quantitative PCR values on CSF samples, and alternate qualitative HHV-6 PCR was only performed on CSF samples from three patients (patients 21, 24, and 25) alongside the FA-ME panel. HHV-6 detection was confirmed in all three cases at semiquantitative values ranging from 280 to 2×10^6 copies/ml. In addition, we previously published on our experience with the FA-ME panel, and of the 7 HHV-6 cases with samples available for confirmation, 6 were confirmed to be HHV-6 positive by alternate PCR testing of CSF ($n = 5$) or blood ($n = 1$) (12). Despite a high level of analytical specificity (9), the clinical specificity of FA-ME is lower, and further testing for quantitative viral load value may assist in determining how meaningful a positive FA-ME result is and also in monitoring viral load in cases of true infection.

Compared to other herpesviruses, HHV-6 CNS infection is not commonly part of the differential diagnosis for meningitis/encephalitis, despite it being a common primary infection in infants and young children. We found that availability of HHV-6 as one of the targets in the FA-ME panel led to faster and definitive diagnosis of HHV-6 meningoencephalitis in our patients. As with all diagnostic tests, correlation between detection of HHV-6 in the CSF and the overall clinical picture of the patient is paramount. Radiographic imaging is imperative, and investigation for ciHHV-6 can provide further insights into whether HHV-6 is truly the causative agent. Likewise, other CNS pathogens not targeted within the FA-ME panel should also be investigated when appropriate. Rapid detection of HHV-6 in CSF has led to quicker establishment of disease etiology, making it an integral part of the algorithm to establish a cause of meningitis/encephalitis in our institution.

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U.P. and J.D.B. designed the study. U.P., J.D.B., V.C.A., and A.L.G. wrote the manuscript. A.L.G., K.R.J., and G.R.L. conducted testing for ciHHV-6. U.P., J.D.B., and V.C.A. conducted patient chart review.

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