Gastroenteritis, Hepatitis, Encephalopathy, and Human Herpesvirus 6 Detection in an Immunocompetent Child: Benefits and Risks of Syndromic Multiplex Molecular Panel Testing

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An immunocompetent toddler came to medication attention with gastroenteritis, complicated by encephalopathy and hepatitis. Multiplexed testing using a polymerase chain reaction meningitis panel was positive for human herpesvirus 6 (HHV-6). Clinical correlation, quantitative HHV-6 polymerase chain reaction, and metagenomic next-generation sequencing supported a likely diagnosis of primary HHV-6B infection. *(J Pediatr 2019;212:228-31).*

Human herpesvirus 6 (HHV-6) infects 90% of children by 2 years of age[1] and accounts for 10% of visits to the emergency department for febrile illness in the first 3 years.[2] The classic presentation is a self-limited high fever followed by a rash, the illness known as roseola infantum or sixth disease.[3] The rapid temperature rise also can be associated with seizures, accounting for one-third of febrile seizures before 2 years of age.[2] In immunocompromised patients, HHV-6 is a well-recognized cause of severe disease.[4-6] In clinical practice, however, the frequency and potential for severe HHV-6 infection in immunocompetent children is likely under-recognized because of a lack of routine clinical testing.

HHV-6 has been included in a multiplex polymerase chain reaction (PCR) meningitis/encephalitis panel for pathogen detection from cerebrospinal fluid (CSF) (BioFire FilmArray Meningitis/Encephalitis (ME) panel; BioMérieux).[7] Multiplexed testing has the potential to identify a broader clinical spectrum of acute infection in immunocompetent children, as testing for HHV-6 alone is rarely pursued. Results must be interpreted cautiously, however, because qualitative DNA detection alone is insufficient evidence for active infection.[8] Clinical judgment and consideration of secondary quantitative DNA testing (in the CSF, serum and/or whole blood) is required when HHV-6 is detected in CSF on a syndromic multiplex PCR panel. We describe a previously healthy child with severe gastroenteritis, hepatitis, and encephalopathy because of probable primary HHV-6B infection, a constellation of symptoms predominantly described in immunocompromised patients.

Case

A 13-month-old male child came to medical attention with vomiting, diarrhea, and dehydration. His past medical history was unremarkable. There were no prior hospitalizations, unusual infections, or concerns about growth. A sibling was ill with similar but milder symptoms, and there was no concerning travel, food, or animal exposure history.

Following hospitalization with a diagnosis of viral gastroenteritis and failure of oral fluid challenge, he developed fever and prolonged somnolence but no meningeal signs, seizures, or focal neurologic deficits. He also failed several trials off intravenous fluids, with associated deterioration in perfusion and alteration in mental status.

A blood gas, comprehensive serum metabolic panel, complete blood count, and abdominal radiographs were performed on hospital day 2, and all results were unremarkable. Because of ongoing symptoms, the laboratory tests were repeated on hospital day 5; in the interim, his white blood count had doubled, platelet count had dropped, and serum hepatic transaminases, lactate dehydrogenase, and ferritin had increased markedly. Testing for erythrocyte sedimentation rate, C-reactive protein, liver function, acetaminophen level, lactate and triglyceride levels, inborn errors of metabolism, cortisol, and antinuclear antibodies were normal, as was an echocardiogram and brain magnetic resonance imaging. Peripheral blood smears showed no evidence of malignancy or hemophagocytosis (**Table**).

A stool PCR panel (BioFire FilmArray gastrointestinal panel; BioMérieux) was positive for *Clostridium difficile*, but testing of nasal washings for respiratory pathogens (BioFire FilmArray respiratory pathogen panel), serum for

CSF	Cerebrospinal fluid
HHV-6	Human herpesvirus 6
mNGS	Metagenomic next-generation sequencing
PCR	Polymerase chain reaction

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Table 1. Selected laboratory testing

Laboratory studies	Hospital day 2 result	Hospital day 5 result	Reference values/units
White blood cell count	6.9	14.2	7.7-13.7 × 10^3/uL
Neutrophils	46	38	%
Bands	22	0.4	%
Lymphocytes	21	56	%
Hemoglobin	11.2	11.2	10.3-13.8 g/dL
Platelet count	278	97	150-500 × 10^3/uL
Erythrocyte sedimentation rate		1	0-15 mm/h
C-reactive protein		<0.5	0-0.9 mg/dL
Ferritin		1920	10-60 ng/mL
Lactate dehydrogenase		3896	500-920 U/L
Total bilirubin	0.2	0.2	0.2-1.2 mg/dL
Alkaline phosphatase	131	163	129-291 U/L
Aspartate aminotransferase	32	1053	20-60 U/L
Alanine aminotransferase	21	482	5-45 U/L
Stool PCR panel			
Clostridium difficile detected*			
Respiratory PCR panel			
No target detected†			
CSF PCR panel			
HHV-6 detected‡			
HHV-6 quantitative PCR tests			
CSF: 21 630 copies/mL (no reference range)			
Serum: 146 340 copies/mL (no reference range)			

**Cryptosporidium, Cyclospora cayetanensis, Entamoeba histolytica, Giardia lamblia*, adenovirus, astrovirus, norovirus, rotavirus, sapovirus, *Campylobacter spp, Escherichia coli spp, Shigella spp, Salmonella spp, Yersinia enterocolitica, Vibrio spp* not detected.
†Adenovirus, coronavirus, human metapneumovirus, rhinovirus, enterovirus, influenza, parainfluenza, respiratory syncytial virus, pertussis, *Mycoplasma pneumoniae, Chlamydophila pneumoniae* not detected.
‡*Escherichia coli, Haemophilus influenzae, Listeria monocytogenes, Neisseria meningitidis, Streptococcus agalactiae, Streptococcus pneumoniae, Cryptococcus spp*, cytomegalovirus, enterovirus, herpes simplex virus, parechovirus not detected.

infection because of Epstein-Barr virus viral capsid antigen IgG/IgM, hepatitis A virus IgM, hepatitis E virus IgM, and parvovirus IgM, as well as blood, urine, and CSF bacterial cultures were negative.

A CSF PCR panel (BioFire FilmArray ME panel, BioMérieux) obtained on hospital day 6, performed on a CSF specimen with 3 white blood cells/μL, 0 red blood cells/μL, and normal protein and glucose, was positive for HHV-6. All other panel targets, including enterovirus, parechovirus, and herpes simplex virus, were negative. Follow-up quantitative HHV-6 PCR testing on the CSF and serum were remarkable for HHV-6 viral loads of 21 630 copies/mL and 146 340 copies/mL, respectively (**Table**). Clinical metagenomic next-generation sequencing (mNGS) of CSF was performed, which detected 4517 sequence reads spanning 67% of the genome of HHV-6, confirmed as subtype B (HHV-6B) by phylogenetic analysis (**Figure**); no other bacterial, viral, parasitic, or fungal pathogen was detected by mNGS. The patient developed a viral exanthem on hospital day 7 with defervescence, further supporting a diagnosis of primary HHV-6 infection. His state of alertness and well-being, examination, and laboratory abnormalities improved with supportive care, and he was discharged on hospital day 13.

Discussion

HHV-6 generally is considered a benign infection in immunocompetent children, but this case suggests that HHV-6 should be considered in otherwise healthy patients with clinical manifestations of more severe illness, such as gastroenteritis, hepatitis, and/or encephalopathy. Though not commonly associated with these clinical conditions, studies show that children with HHV-6 are more likely to have diarrhea than age-matched controls (26% vs 11% $P = .05$),[1] and hepatitis has been associated with HHV-6 in infants[10,11] and stem-cell and solid organ transplant patients.[12,13]

This case also demonstrates that liver and central nervous system involvement may not be present initially, warranting repeated laboratory testing if symptoms persist beyond a few days. Although this patient lacked features for an encephalitis diagnosis according to International Encephalitis Consortium criteria (seizures, focal neurologic abnormalities, pleocytosis, neuroimaging changes),[14] the presence of HHV-6 DNA in the CSF was associated with encephalopathy without an alternative explanation (broad-based CSF multiplex PCR and mNGS testing were negative for other pathogens). Normal CSF white blood cell counts are well-described in HHV-6 central nervous system infection, which supports a role for HHV-6 testing in the setting of encephalopathy regardless of pleocytosis.[6]

Qualitative detection of HHV-6 DNA alone does not establish the virus as the cause of an illness. Chromosomal integration of HHV-6 is present in 1% of the population,[15] often passed through the germline in newborns. With chromosomal integration, HHV-6 DNA is detectable in all nucleated cells, often present in CSF, persistent over time,[16] and present in blood at high viral loads. HHV-6 also can establish latency in monocytes and macrophages following primary infection. Although detection of reactivated herpesviruses can represent serious illness in immunocompromised

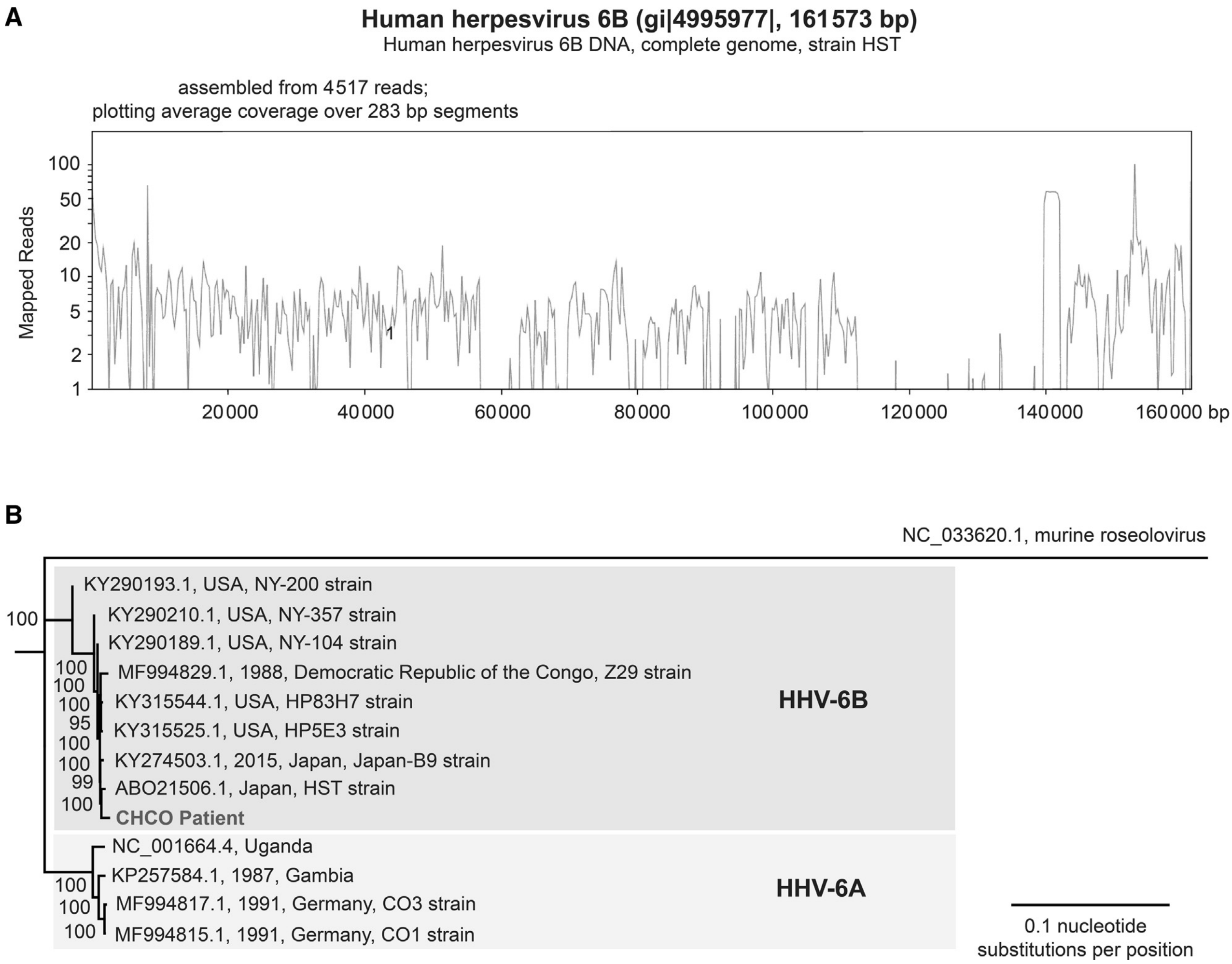

Figure. mNGS and phylogenetic analysis of HHV-6 from patient's CSF. **A,** Coverage map. A total of 4517 HHV-6 reads obtained by CSF mNGS were mapped to the most closely matched viral genome in the National Center for Biotechnology Information GenBank database using the SURPI+ pipeline (Naccache et al[9]). There is approximately 68% coverage of the HHV-6 genome. **B,** Phylogenetic tree. The patient's assembled consensus genome was aligned in parallel with 12 representative HHV-6 genomes and the genome of murine roseolovirus (as an outgroup) from National Center for Biotechnology Information GenBank using MAFFT software (Kazutaka Katoh, Computational Biology Research Center, National Institute of Advanced Industrial Science and Technology, Tokyo, Japan) at default settings, followed by tree construction using PHYML software (Stephane Guindon, Montpellier Bioinformatics, Montpellier, France). By phylogenetic analysis, the genotype corresponding to the patient's strain is HHV-6B.

patients,[17] their detection usually is unrelated to the primary cause of disease in immunocompetent hosts.

Additional laboratory studies are available to distinguish chromosomally integrated HHV-6 DNA from active or reactivated infection. For example, a whole blood quantitative PCR result greater than 5.5 log copies/mL of blood[18] or serial tests showing persistently high levels of HHV-6 DNA over time support a diagnosis of chromosomal integration. Acute and convalescent serologic testing is another method to distinguish active infection from reactivation or chromosomally integrated DNA. However, serial testing may be unavailable or impractical for use in the clinical setting, particularly when a timely diagnosis is needed. This patient's young age made reactivation unlikely, and his eventual development of a characteristic roseola-type exanthem with self-resolution of fever was highly consistent with primary HHV-6 infection. As a result, definitive laboratory confirmation was not performed.

Besides describing a unique constellation of symptoms in an immunocompetent patient with likely primary HHV-6 infection, this case demonstrates both the advantages and challenges associated with the clinical use of multiplexed testing such as syndromic multiplex PCR panels or mNGS. Detection of nucleic acid from a pathogen consistent with the clinical presentation, such as HHV-6 in this case, can support a diagnosis that informs management and limits unnecessary testing or treatment. However, nonpathogenic organism detection may lead to incorrect assumptions of cause-and-effect, increase utilization of ineffectual and potentially harmful therapies, and impede evaluation and management of the true diagnosis. This patient's positive *Clostridium difficile* PCR result most likely represented colonization based on his age,[19] and lack of diarrhea with blood or mucous in stools or recent antibiotic use. Multiplex test results should be interpreted in the clinical context with consideration of the pre-test probability for each potential pathogen detected. ■

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