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BRIEF REPORT

Human Herpesvirus-6 Detection in Cerebrospinal Fluid on the BioFire FilmArray Meningitis/Encephalitis Panel in a High Human Immunodeficiency Virus-Prevalence African Setting

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The prevalence and clinical relevance of human herpesvirus-6 (HHV-6) detection in cerebrospinal fluid (CSF) using multiplex polymerase chain reaction (PCR) testing in patients with suspected meningoencephalitis in high human immunodeficiency virus-prevalence African settings are not known. We describe the clinical and laboratory characteristics of 13 patients with HHV-6 CSF PCR positivity in Botswana.

Keywords. cerebrospinal fluid; encephalitis; HIV; human herpesvirus-6; meningitis.

Human herpesvirus-6 (HHV-6) is a common cause of infantile fever and roseola and has been implicated as a central nervous system (CNS) pathogen causing meningitis or meningoencephalitis, predominantly in immunocompromised hosts [1]. Because multiplexed cerebrospinal fluid (CSF) polymerase chain reaction (PCR) testing is becoming more widely used in the diagnosis of suspected meningoencephalitis [2], HHV-6 PCR positivity in the CSF is increasingly reported [2, 3]. However, its clinical significance remains unclear [4, 5]. Prior reports from United States and Germany have shown that HHV-6 comprises between 10.7% and 31.3% of all positive CSF BioFire FilmArray Meningitis/Encephalitis (FilmArray-ME) panel results among patients with suspected meningoencephalitis [2, 3]. The majority of these HHV-6-positive results were in children [2], and overall HHV-6 was the second most frequently encountered viral pathogen after enterovirus [2, 3]. To date, there are very limited data on the prevalence and clinical correlates of CSF HHV-6 detection in sub-Saharan Africa [6, 7] where the etiology of meningoencephalitis differs markedly from Europe and North America [8], and a large proportion of individuals presenting with meningoencephalitis have immune suppression due to human immunodeficiency virus (HIV), and culture-negative lymphocytic meningitis is common and associated with high mortality [9]. As part of a the prospective Botswana National Meningitis Survey [9], we implemented multiplex PCR CSF testing using the FilmArray-ME Panel at the main referral hospital in Gaborone. Human herpesvirus-6 was the second most frequently detected virus after cytomegalovirus. We describe the clinical and laboratory characteristics of the 13 patients with HHV-6 detected on CSF analysis.

METHODS

Cerebrospinal fluid samples from 690 sequential patients presenting to Princess Marina Hospital, Gaborone, between April 2017 and November 2018, were analyzed using the BioFire FilmArray Meningitis/Encephalitis multiplex PCR panel (bioMérieux, Grenoble, Rhone-Alpes, France) (Appendix 1). Clinical and laboratory data and in-hospital outcomes were captured using REDCap. A consensus diagnosis was attributed to each patient, and the relevance of HHV-6 detection in the CSF on FilmArray-ME and its role as a CNS pathogen was classified as "likely", "possible", or "unlikely" (adapted from previously described criteria, outlined in Table 1 [4], following detailed records review by 2 independent clinicians [J.M. and C.G.W.], with arbitration by a third [J.N.J.]). Ethical approvals for the study were provided by the Botswana Health Research and Development Committee, the University of Pennsylvania, and the London School of Hygiene and Tropical Medicine.

RESULTS

Of the 690 patients with CSF multiplex PCR analysis, at least 1 organism was identified on PCR in 124 (18%). Human herpesvirus-6 was detected in the CSF of 13 patients, comprising 11% of all positive results (Table 1). Six of the HHV-6-positive individuals were infants under 1 year of age (of a total of 225 tested with FilmArray-ME), 1 was a 12-year-old child (of 45 children

Received 08 April 2022; editorial decision 15 April 2022; accepted 16 May 2022; published online 17 May 2022

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https://doi.org/10.1093/ofid/ofac229

Table 1. Characteristics of Patients With HHV-6 Detections in CSF[a]

Patient	Age and Sex	Presenting Complaint/Clinical Syndrome	HIV Status	CD4 Count	CSF Cell Count (Leucocytes/mm^3) and Differential[b]	Antiretroviral Therapy	CSF Protein (mg/mL)[c]	CSF Glucose (mmol/L)[d]	Hemoglobin (g/dL)[e]	Peripheral WCC (10^9/L)[f]	Objective ever on Admission	Antiviral Treatment	Additional Clinically Relevant Microbiological Results	Final Diagnosis	HHV-6 Meningoencephalitis (Unlikely/Possible/Likely)	Outcome at Discharge
1	2 months male	Vomiting	Unexposed	n/a	2	n/a	0.29	2.53	10	13.74	No	Nil	Nil	Gastroenteritis possible primary HHV-6 infection	Unlikely	Alive
	2-month-old infant with normal birth weight range presented with 4-day history of fever, vomiting, and irritability. On examination, the anterior fontanelle was flat and the child was moving all 4 limbs. An LP was performed as part of the diagnostic work-up to investigate for a source of sepsis. Treated empirically with ampicillin and gentamicin. Clinically improved and was discharged after 4 days.															
2	2 months Female	Tachypnoea	Exposed	n/a	2	n/a	0.22	3.44	7.0	10.70	Yes	Nil	Nil	Pneumonia Possible primary HHV-6 infection	Unlikely	Alive
	2-month-old HIV-exposed infant with normal birth weight presented with 1-day history of fever and tachypnoea. Treated empirically with ceftriaxone and vancomycin for suspected meningitis until LP results known. Discharged after 2 days.															
3	5 months male	Diarrhea and vomiting	Unexposed	n/a	2	n/a	0.31	3.90	9.8	5.50	Yes	Nil	Unidentified Gram-negative organism in blood culture	Gram-negative sepsis	Unlikely	Alive
	5-month-old infant admitted with 1-day history of fever, diarrhea, and vomiting. Bulging fontanelle on examination. Treated empirically with cefotaxime and vancomycin for suspected meningitis until LP results known. Developed rash that was attributed to vancomycin. Unidentified Gram-negative rod seen on blood culture. Discharged after 3 days when clinically improved.															
4	7 months female	Fever, seizure, lethargy, and diarrhea	Unexposed	n/a	2	n/a	0.23	4.14	11.3	4.07	No	Nil	Nil	Pneumonia complicated by febrile seizure Possible primary HHV-6 infection	Unlikely	Alive
	7-month-old infant with 3-day history of diarrhea, fever, shortness of breath, lethargy, and seizures. Treated empirically with ceftriaxone for suspected meningitis. Switched to oral coamoxiclav after LP results known for pneumonia complicated by a febrile seizure and discharged after 1 day.															
5	7 month male	Fever, seizure, and URT symptoms	Negative	n/a	2	n/a	0.16	3.53	5.6	13.9	No	Nil	Nil	Viral respiratory tract infection complicated by febrile seizure Possible primary HHV-6 infection	Unlikely	Alive
	7-month-old infant admitted with 1-day history of fever, seizure, and lethargy. Received a single dose of intramuscular ceftriaxone before lumbar puncture but no other antimicrobials. Discharged with diagnosis of URT infection complicated by febrile seizures.															
6	10 months male	Fever, seizure, and cough	Negative	n/a	3	n/a	0.19	4.27	11.4	5.09	Yes	Nil	Nil	Possible primary HHV-6 infection	Possible	Alive
	10-month-old admitted with 1-day history of cough, fever, and seizures. Did not receive any antimicrobials during admission and was discharged after 1 day.															
7	12 years female	Headache, neck stiffness, seizure, and left-sided weakness	Unknown	n/a	10 (differential not performed)	n/a	0.51	2.1	12.3	6.93	No	Nil	Coagulase-negative staphylococcus cultured in CSF	Hydrocephalus	Unlikely	Alive
	Transferred from local hospital with a history of headache, vomiting, seizure, neck stiffness, and fever. On examination the patient had weakness in left arm and left leg. Treated empirically with cefotaxime for suspected meningitis. CT scan of the brain showed hydrocephalus and a V-P shunt was inserted. Lumbar puncture performed on day 21 of admission that grew a coagulase-negative staphylococcus.															
8	34 years male	Altered mental status	Positive	40	2	On ARVs	0.45	3.29	9.7	3.07	No	Nil	Nil	Psychiatric disorder	Unlikely	Alive
	Presented with a history of abnormal behaviour with a background of an uncharacterized previous psychiatric illness. The duration of symptoms was unknown. No antibiotics were administered. A CT scan was performed and the patient was discharged after unremarkable CSF analysis.															
9	35 years female	Cough, weight loss, and night sweat	Positive	Unknown	3	Naive	2.05	5.95	8.3	22.42	Yes	Nil	Nil	Pneumonia	Unlikely	Alive
	Presented with a 5-day history of cough, fever, and night sweats. HIV was diagnosed on admission and the patient was treated for community-acquired pneumonia with ceftriaxone and subsequently chloramphenicol.															
10	40 years female	Left-sided weakness and slurred speech	Positive	111	9 (99% lymphocytes/1% polymorphs)	Defaulted	0.81	3.18	11	9.37	No	Aciclovir	VZV	VZV encephalitis	Unlikely	Died during admission
	Presented with 1-day history of left-sided weakness and slurred speech. Left-sided facial weakness with forehead sparing was noted on examination. Defaulted from ARV therapy and CD4 was 111. VZV PCR was positive on BioFire and MRI findings were consistent with encephalitis. The patient was treated for VZV encephalitis with aciclovir and also treated empirically for TB. The patient passed away 3 days after admission.															
11	49 years male	Headache, vomiting, neck	Positive	Unknown	945 (99% lymphocytes/1% polymorphs)	On ARVs	Not performed	Not performed	9.7	1.44	Not recorded	Nil	Positive CrAg in CSF	Cryptococcal meningitis	Unlikely	Alive

Table 1. Continued

Patient	Age and Sex	Presenting Complaint/Clinical Syndrome	HIV Status	CD4 Count	CSF Cell Count (Leucocytes/mm^3) and Differential[b]	Antiretroviral Therapy	CSF Protein (mg/mL)[c]	CSF Glucose (mmol/L)[d]	Hemoglobin (g/dL)[e]	Peripheral WCC (10^9/L)[f]	Objective ever on Admission	Antiviral Treatment	Additional Clinically Relevant Microbiological Results	Final Diagnosis	HHV-6 Meningoencephalitis (Unlikely/Possible/Likely)	Outcome at Discharge
		stiffness, and photophobia														
	Presented with 7-day history of headache, vomiting, and neck stiffness. On examination they were photophobic with oral thrush with signs of malnourishment. Opening pressure on lumbar puncture was 48 cm CSF with a positive CrAg on CSF analysis. The patient was treated for cryptococcal meningitis and also treated empirically for TB.															
12	27 years male	Diarrhea, altered mental status, and neck stiffness	Positive	107	250 (98% lymphocytes/ 2% polymorphs)	Defaulted	0.79	1.95	8.8	6.49	No	Aciclovir	Nil	Possible tuberculous meningitis	Possible	Alive
	Presented with 1-month history of diarrhea, altered mental status, and neck stiffness. There was a history of advanced HIV disease having previously defaulted treatment and a recent diagnosis of pulmonary TB 3 months before presentation but defaulted after approximately 4 weeks therapy. Treated for tuberculous meningitis and received ceftriaxone for 9 days for possible bacterial meningitis. Aciclovir started after positive HHV-6 result (valganciclovir unavailable).															
13	40 years male	Cough, headache, visual hallucination, and altered mental status	Positive	9	3	Previously defaulted— recently restarted on ARVs 2 weeks before presentation	1.15	1.15	Not performed	Not performed	Yes	Valganciclovir	Nil	Possible tuberculous meningitis	Possible	Alive
	Presented with 2-week history of headache, hallucinations, fever, and cough. No features of meningitis on examination. Previously defaulted ARV treatment. Started on ATT and restarted on ARVs 2 weeks before admission when presented with symptoms suggestive of TB meningitis. Presented this admission with similar symptoms and ARVs held due to suspected IRIS but were restarted on discharge. Treated with valganciclovir for possible HHV-6 meningoencephalitis.															

Abbreviations: ARV, antiretroviral drugs; ATT, antituberculosis therapy; CrAg, cryptococcal antigen; CSF, cerebrospinal fluid; CT, computed tomography; HIV, human immunodeficiency virus; HHV-6, human herpesvirus-6; IRIS, immune reconstitution inflammatory syndrome; LP, lumbar puncture; MRI, magnetic resonance imaging; n/a, not applicable; PCR, polymerase chain reaction; TB, tuberculosis; URT, upper respiratory tract; V-P, ventriculoperitoneal; VZV, varicella zoster virus; WCC, white cell count.

[a]Likely HHV-6 meningoencephalitis was defined as HHV-6 detected on FilmArray, no alternative diagnosis identified, supportive surrogate investigations, and the exclusion of chromosomally integrated HHV-6, although testing for chromosomally integrated HHV-6 (ciHHV-6) was not available in Botswana at the time. Possible infection was defined as HHV-6 detected on FilmArray with a clinical presentation compatible with central nervous system infection and no alternative diagnosis identified, and Unlikely was defined as HHV-6 detected on FilmArray with a clear alternative diagnosis. Diagnostic structure adapted from Green DA, Pereira M, Miko B, Radmard S, Whittier S, Thakur K. Clinical significance of human herpesvirus 6 positivity on the filmarray meningitis/encephalitis panel. Clin Infect Dis. 2018;67:1125–28.

[b]Reference range: children aged under 1 month 0–15 cells/μL; children aged 1–3 months 0–9 cells/μL; adults and children over 3 months 0–5 cells/μL.

[c]Reference range: 0.15–0.60 mg/mL.

[d]Reference range: 2.5–4.4 mmol/L.

[e]Reference range: children aged 0–1 month 13.4–19.9 g/dL; children aged 1–2 months 10.7–17.1 g/dL; children aged 3–12 months 11.3–14.1 g/dL; men aged over 1 year 14.0–17.5 g/dL; women aged over 1 year 12.3–15.3 g/dL.

[f]Reference range: children aged 0–1 month 9.0–30.0 cells/mL; children aged 1–3 months 5.0–19.5 cells/mL; children aged 3–12 months 6.0–17.5 cells/mL; adults and children aged over 1 year 4.5–11 cells/mL.

tested), and 6 were adults (of 420 adults tested; 334 adults were HIV positive and 86 HIV negative). In 652 patients, the indication for lumbar puncture was suspected central nervous system infection (CNSI), 8 patients did not have suspected CNSI, and 28 patients had complications related to a CNS device. The indication was unknown in 2 patients.

All 6 infants presented with a history of fever, 3 had seizures, 3 had respiratory symptoms, and 2 had diarrhea. No infants were HIV positive and none had a CSF pleocytosis. In 5 of the 6 infants, clear alternative clinical diagnoses were made during their admission, and they were treated for a condition that was not a CNSI. Human herpesvirus-6 meningoencephalitis was classed as unlikely to be contributing to the CNS pathology in these 5 cases. The other infant had no alternative confirmed diagnosis during admission, with possible HHV-6 encephalitis on the differential diagnosis, but had unremarkable CSF white cell count (WCC) and protein levels and recovered without receiving antimicrobial therapy. Due to the inability to perform serum HHV-6 PCR in those infants without an alternative microbiological diagnoses, we were unable to determine whether clinical presentation and CSF HHV-6 PCR detection were the result of primary systemic HHV-6 infection in these cases. All were discharged home alive.

The 12-year-old female presented to a secondary hospital with headache, neck stiffness, fever, and left-sided weakness. She was treated empirically for presumed meningoencephalitis with intravenous cefotaxime. Hydrocephalus was demonstrated on computed tomography (CT) of the brain and she was transferred to Princess Marina Hospital for ventriculoperitoneal shunt insertion. On day 21 of admission a lumbar puncture was performed for suspected shunt infection and HHV-6 was detected in the CSF. She did not receive antiviral treatment, made a clinical recovery, and was discharged home alive; HHV-6 meningoencephalitis classification was unlikely.

Among HIV-positive adults, 6 of 194 with stage 4 disease or CD4 <200 cells/mL had HHV-6 detected on FilmArray-ME compared with 0 of 140 without stage 4 disease or known CD4 counts >200 cells/μL; 0 of 86 HIV-negative adults were HHV-6 positive. Of the 6 adults with HHV-6 detected in CSF, 1 presented with a respiratory illness and was diagnosed with community-acquired pneumonia, and HHV-6 meningoencephalitis was classified as unlikely. One had a history of a known psychiatric disorder and was diagnosed with an acute psychotic episode after normal CSF WCC and protein results, normal peripheral WCC, and CT head scan; they were treated with haloperidol and discharged without receiving antiviral therapy, and HHV-6 meningoencephalitis was classified as unlikely. The remaining 4 were all determined to have CNS infections. Two had other pathogens detected in CSF in addition to HHV-6: 1 had a positive cryptococcal antigen and was treated for cryptococcal meningitis, and 1 had varicella zoster virus (VZV) detected on PCR and compatible brain magnetic resonance imaging treated with intravenous aciclovir; HHV-6 meningoencephalitis was classified as unlikely in both cases. Two had no other pathogen isolated but a presumptive diagnosis of tuberculous (TB) meningitis based on clinical presentation and laboratory findings (Table 1). Both were treated with anti-TB therapy plus antivirals for possible HHV-6 meningoencephalitis.

DISCUSSION

Human herpesvirus-6 was the second most commonly detected virus in the CSF of patients undergoing multiplex PCR testing for suspected CNSI in Gaborone, Botswana. Positive HHV-6 PCR results were identified in 6 infants and 1 child, none of whom were known to be HIV positive, and in 6 adults, all with advanced HIV disease. Human herpesvirus-6 was classified as being unlikely to be causing CNS pathology in 10 of 13 cases with clear alternative diagnoses, and possibly contributing to a meningoencephalitis in 3 of 13 cases, with no cases in which HHV-6 meningoencephalitis was considered the most likely diagnosis.

Primary HHV-6 infection in infants is common and typically self-limiting, and it has been associated with up to 20% of childhood admissions with fever and one third of febrile seizures in the United States [10] and Zambia [11]. With the increased availability of multiplex PCR panels, HHV-6 detection in CSF has been more frequently reported during febrile childhood illnesses [3, 5], although the clinical interpretation of these results is challenging. Human herpesvirus-6 detection in CSF has been described in children with self-limiting primary HHV-6 infection, subclinical reactivation of latent infection, chromosomally integrated HHV-6 ([ciHHV-6] occurring in approximately 1% of patients after primary HHV-6 infection), and HHV-6 meningoencephalitis. One American study reported 25 HHV-6 detections in CSF from 1005 children screened with FilmArray-ME and after review of medical records, radiological findings, and ciHHV-6, it attributed 5 of these HHV-6 detections to HHV-6 meningoencephalitis [5].

In European adult populations, HHV-6 seropositivity estimates range between 78% and 92%, but there are limited data from sub-Saharan Africa. Periodically, HHV-6 can subclinically reactivate from a latent reservoir in mononuclear cells, particularly during times of host stress; detection of HHV-6 deoxyribonucleic acid (DNA) in this setting is not necessarily clinically relevant [4, 5]. However, serious infections such as encephalitis, hepatitis, uveitis, and pneumonitis can occur either as primary infection or through reactivation, predominantly in immunocompromised patients [1].

In our cohort, all 6 adults with HHV-6 DNA detected in their CSF had advanced HIV disease. There is a paucity of data on CNS HHV-6 infection in people with HIV, particularly in populations from sub-Saharan Africa. Only 18 CSF HHV-6 detections using the FilmArray-ME panel have been reported from Africa, 5 from pediatric patients with suspected meningitis in

Ethiopia where individual HIV statuses were not reported [12], and in 13 HIV-positive adult patients with suspected meningitis from 2 Ugandan studies [6, 7]. Accurately assessing the contribution of HHV-6 to disease in this population is challenging due to limited access to healthcare resources needed to support a diagnosis of HHV-6 meningoencephalitis, including neuroimaging, and the diagnostics required to reliably exclude TB meningitis or ciHHV-6. These limitations, including the lack of quantitative HHV-6 PCR in Botswana to help to exclude ciHHV-6 or primary HHV-6 infection and a lack of long-term follow-up data, restricted our ability to definitively diagnose or exclude HHV-6 meningoencephalitis.

Further complicating the clinical interpretation of HHV-6 detection in CSF in our context was the presence of coinfections, with an additional pathogen detected in the CSF of 2 adults in our study: 1 with VZV and 1 with cryptococcal meningitis. Human herpesvirus-6 was not believed to be a pathogen in either patient. Codetections of HHV-6 and other pathogens have been reported previously in sub-Saharan Africa with a 2021 Ugandan study describing 7 of 12 patients with HHV-6 detected in the CSF also diagnosed with cryptococcal meningitis [6].

CONCLUSIONS

With the expanding availability of multiplex PCR diagnostics, clinicians will increasingly have to interpret the clinical relevance of HHV-6 detection in CSF in high HIV-prevalence African settings. Our data suggest that in the majority of cases, HHV-6 detection is incidental and not contributory to CNS pathology; however, the potential role of HHV-6 as a CNS pathogen in patients with advanced HIV requires further investigation.

Acknowledgments

Disclaimer. The views expressed in this publication are those of the author(s) and not necessarily those of the National Institute for Health Research (NIHR) or the United Kingdom (UK) Department of Health and Social Care. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Financial support. J. N. J. is funded by the NIHR through a Global Health Research Professorship (RP-2017-08-ST2-012; to J. N. J.) using United Kingdom aid from the UK Government to support global health research. This work was funded by bioMérieux who provided materials and financial and technical support for the study.

Potential conflicts of interest. J. M., D. M. G., and J. N. J. have received investigator-initiated funding from bioMérieux. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Appendix 1

Pathogens detected on BioFire FilmArray-ME Panel

Viruses	Bacteria	Yeast
Cytomegalovirus (CMV)	*Escherichia coli K1*	*Cryptococcus neoformans/ Cryptococcus gattii*
Enterovirus	*Haemophilus influenzae*	
Epstein-Barr virus (EBV) *This was not reported to treating clinicians results were only given to the study team retrospectively from bioMérieux after their review*	*Listeria monocytogenes*	
Herpes simplex virus 1 (HSV-1)	*Neisseria meningitidis*	
Herpes simplex virus 2 (HSV-2)	*Streptococcus agalactiae*	
Human Herpes Virus 6 (HHV-6)	*Streptococcus pneumoniae*	
Human parechovirus		
Varicella Zoster Virus (VZV)		