Fatal Subacute Myocarditis Associated with Human Bocavirus 2 in a 13-Month-Old Child

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Human bocavirus has rarely been incriminated in fatal or life-threatening respiratory infections. We report a case of fatal disseminated infection with subacute lymphocytic myocarditis in a 13-month-old child. The human bocavirus 2 genome was detected by PCR analysis in nasal swab, plasma, urine, ascitic fluid, and mesenteric node, skeletal muscle, and lung tissue specimens.

CASE REPORT

A 13-month-old female child with respiratory distress was admitted to the emergency department of the General Hospital of Vichy, Vichy, France, in April 2013. She was born at full term and had never been previously hospitalized. Her further medical history was unremarkable. At this time she had received all the vaccinations required by the French Public Health Council. When questioned, the mother reported no cases of infectious disease in close family members in the immediate past.

Twelve hours before admission, the child had vomited and lost her appetite. During admission, she developed respiratory grunting, which led to medical consultation. At presentation, the child was pink, reactive, and a pyrexic and had moderate grunting at the peak of each inspiration. The preliminary diagnosis was asthma, and she was given inhaled terbutaline sulfate and salbutamol 1.5 h later. Two hours after admission, she had a C-reactive protein concentration of 31.8 mg/liter (normal level, <7.5 mg/liter). A peak of each inspiration. The preliminary diagnosis was asthma, and she was given inhaled terbutaline sulfate and salbutamol 1.5 h later. Two hours after admission, she had a C-reactive protein concentration of 31.8 mg/liter (normal level, <7.5 mg/liter). A complete blood count was impossible because of the presence of a clot in the EDTA whole-blood sample. Capillary gas sampling showed a pH of 7.30 with a decreased level of bicarbonates (9.5 mmol/liter; normal level, 21.2 to 27.0 mmol/liter) and an elevated anion gap. Respiratory compensation of metabolic acidosis led to a partial CO2 concentration of 19.2 mm Hg.

Four hours after blood analysis, the child was pale and still a pyrexic with a decreased level of consciousness. Her vital signs were as follows: pulse rate, 122 beats/min with ectopic heartbeats; blood pressure, 84/74 mm Hg; respiratory rate, 98 breaths/min; and capillary O2 saturation, 89%, for which she received oxygen therapy at 2 liters/min. The child’s condition rapidly deteriorated to hemodynamic collapse secondary to paroxysmal supraventricular tachycardia. Despite cardiopulmonary resuscitation with chest compression, repeated intravenous adenosine injection, and electrolyte treatment, she died 8 h after admission.

On autopsy, macroscopic examination showed no cardiac anatomical abnormality. Moderate ascites and significant lymph node hypertrophy (mesentery and mediastinum) were observed. Tissue specimens from all major organs were routinely processed, formalin fixed, paraffin embedded, and stained with hematoxylin-eosin. Histological examination revealed lymphoid follicular hyperplasia of whole lymphoid tissue, abundant infiltration of the myocardium by mononuclear cells in the interstitium, and moderate edema. In the interventricular septum, intense lymphocytic infiltration, atrophy of the myocytes, and areas with fibrosis were observed (Fig. 1). Immunohistochemical analysis of sections from the myocardium was negative for parvovirus B19 capsid antigens. Analysis of lung tissue showed mild lymphocytic infiltration. Postmortem examination determined that the child had died from heart failure caused by cardiac tissue injury that may have been triggered by a viral infection. Septal tissue damage had probably led to cardiac rhythm disorders.

A nasal swab specimen was collected a few hours after death and immediately sent in universal viral transport medium to be screened for respiratory viruses. Nucleic acids were extracted on a NucliSENs EasyMAG automated system (bioMérieux, Marcy l’Etoile, France). The specimen was screened by real-time PCR (RT-PCR) for influenza viruses A and B, respiratory syncytial viruses (RSVs) A and B, human metapneumovirus, adenovirus (ADV), human bocavirus (HBoV) types 1 to 4, and the two picornaviruses human rhinovirus (HRV) and enterovirus (EV) with respiratory multiwell system (MWS) R-gene (bioMérieux) kits. Each RT-PCR included positive and negative controls. The genomes of three respiratory viruses were detected in the nasal swab specimen: RSV (cycle threshold [C T] value at 35.7 cycles), HBoV (C T value at 21.1 cycles), and picornavirus HRV/EV (C T value at 38.7 cycles). Specific EV genome detection in nasal swab and plasma specimens by nucleic acid sequence-based amplification using a NucliSens EasyQ enterovirus kit (bioMérieux) was negative. It was thus concluded that the viral genome detected in the nasal swab with a picornavirus RT-PCR was that of a rhinovirus, although genotyping, based on 1A/1B genomic region sequencing (1), failed.

A sample of plasma collected a few hours before the child’s death and various body fluid and tissue samples collected during...
autopsy were screened for the eight respiratory virus genomes as described above. For frozen stored samples of urine, ascitic fluid, and psoas, liver, and mesenteric node tissue, nucleic acid extraction was performed with an EasyMAG system. For paraffin-embedded myocardial and lung tissue, nucleic acid extraction was performed, after removal of paraffin with xylene, using a QIAamp DNA blood extraction kit (Qiagen, France), as previously described (2). The HBoV genome was detected in all samples, except in liver and myocardial tissue. No other viral genome was detected. The ADV/HBoV MWS R-gene (bioMérieux) assay does not contain an internal control. An amplification reaction inhibitor was detected by testing the amplification of a cellular gene with the HRV-EV/Cc MWS R-gene (bioMérieux) kit.

To type the detected HBoV strain, we performed PCR targeting the partial VP1/VP2 region with previously described primers AK-VP-F2 and AK-VP-R2 (3) on nasal swab, urine, and psoas, liver, and mesenteric node tissue nucleic acid extracts. The purified PCR products were subjected to direct sequencing using a BigDye Terminator (v1.1) kit (Applied Biosystems, France) with the forward and reverse primers used in the PCR assay. VP1/VP2 partial sequences were identical in all samples. Phylogenetic analysis based on a 457-nucleotide VP1/VP2 segment was performed using the MEGA (v5) program (neighbor joining; bootstrap value, 1,000). The clinical strain was assigned to HBoV species 2 (HBoV2). We also performed specific HBoV2 nested PCR targeting the partial NS1 region with the previously described primers HBoV2-sf1/2 and HBoV2-sr1/2 (4) on nasal swab, urine, and psoas, liver, and mesenteric node tissue samples. The specific HBoV2 PCR was positive for all samples. The purified PCR products were subjected to direct sequencing using inner primers.

Various investigations were made to diagnose an alternative viral infection. PCRs were performed according to the manufacturer’s recommendation, including monitoring of inhibition using an internal control. Plasma, urine, and tissue samples were screened by PCR for herpes simplex viruses 1 and 2, cytomegalovirus (CMV), Epstein-Barr virus, and human herpesvirus 6, performed with R-gene real-time PCR kits (bioMérieux). We detected urinary excretion only of CMV, Bordetella pertussis genome detection, performed by real-time PCR with an R-gene kit (bioMérieux), was negative in a nasopharyngeal aspiration sample. Serological tests for CMV (Cobas; Roche Diagnostics) and parvovirus B19 (Biotrin) were positive for IgG but negative for IgM. A serological test for HIV (Cobas, Roche Diagnostics) was negative. Serological tests for Mycoplasma pneumoniae (Serion Classic enzyme immunoassay IgG [Virion/Serion] and Medac IgM [Medac]) were negative. Blood, urine, and pericardial fluid bacteriological examinations were negative. Urine and pericardial fluid underwent microscopic examination after Gram staining. Each sample was cultured. Samples were processed in accordance with the recommendations of the European Society for Clinical Microbiology and Infectious Diseases.

No disorder of amino acid metabolism was detected by liquid chromatography-mass spectrometry (LC-MS) of plasma, urine, and cerebrospinal fluid specimens. The assay of the plasma acylcarnitine profile by LC-MS revealed no mitochondrial fatty acid oxidation disorders. The assay of very-long-chain fatty acids by gas chromatography-mass spectrometry ruled out the possibility of peroxisomal disorders. Chromosomal analyses performed on fibroblasts using conventional GTG and RHG banding showed a normal female karyotype.

Human bocaviruses, members of the Parvoviridae family, were first identified in 2005 in clinical specimens from infants and children with respiratory tract illness (5). Four species (HBoV1 to HBoV4) have been identified worldwide. To date, the pathogenesis and host range of human bocaviruses are unknown, because the virus can be cultured only in differentiated human airway epithelial cells and no animal model is available.

Human bocaviruses have been associated with a broad spectrum of symptoms and are often codetected with other viruses. Far more clinical data are available for HBoV1 than for the three other species. HBoV1 is mainly detected in children aged 6 to 24 months with upper or lower respiratory tract disease, while HBoV2 to HBoV4 are detected mainly in stools and rarely in the respiratory tract. HBoV2, the most common species, and HBoV3 are associated with gastroenteritis (6).

The human bocavirus genome is commonly detected in respiratory or stool samples in childhood, but severe cases are rare. Three cases of life-threatening lower respiratory tract infection associated with HBoV1 have been observed in previously healthy children aged from 8 months to 4 years old (7–9). A case of fatal lower respiratory tract infection associated with HBoV1 was recently reported in an immunosuppressed 61-year-old man (10). Fatal pneumonia with myocarditis has been reported in an immunocompetent child and was found to be associated with ADV type 7 and HBoV infection, on the basis of throat swab specimen culture and isolation of ADV and HBoV (11). HBoV2 has never been reported in life-threatening infections. To our knowledge, there is no documented case of fatal disseminated infection or myocarditis associated with HBoV with strong clinical relevance.

We describe a case of fatal disseminated HBoV2 infection in a child presumed to be immunocompetent. In postmortem investigations, HBoV was detected by PCR in tissues and body fluids. RSV and HRV were also codetected with HBoV in a nasal swab specimen. Detection of one or more respiratory viruses by PCR is frequent in children, even in those who are asymptomatic, with a prevalence of 24% (12). HBoV was the only microorganism detected in blood or tissue samples, which is strongly suggestive of its clinical relevance. Cardiac histological examination revealed subacute myocarditis with areas of fibrosis, which usually appears

FIG 1 Histological examination of interventricular septum with subacute myocarditis by hematoxylin-eosin stain. Atrophy of the myocytes, lymphocytic infiltration (yellow arrow), and areas with fibrosis (black arrow) can be seen. Magnification, ×20.
several days after the acute phase of virus-induced myocarditis (13). Although HBoV DNA was detected in numerous body samples, it was not detected in the fragment of paraffin-embedded myocardial tissue studied. As the ADV/HBoV MWS R-gene assay (bioMérieux) does not include an internal control and since the cellular gene signal obtained with the HRV-EV/Cc MWS R-gene assay (bioMérieux) was lower in the myocardial nucleic acid extract than in other samples, the myocardial nucleic acid extract may contain inhibitors. The low cell density due to myocyte atrophy fibrosis may also explain the low cellular gene signal obtained with the HRV-EV/Cc MWS R-gene assay (bioMérieux). Moreover, pretreatment with paraffin and xylene may have reduced the sensitivity of the HBoV PCR. Unfortunately, no frozen stored sample of myocardial tissue was available to check this possibility. Alternatively, the result could be explained by the pathophysiology of viral myocarditis. The subacute phase of viral myocarditis is characterized by immune reaction processes, and during this phase, it is possible that the virus genome is no longer detectable in the myocardium by PCR (13).

The present clinical case suggests that HBoV should be considered in cases of sudden infant death syndrome and in viral myocarditis in childhood. When a viral etiology remains undetermined after first-level investigations, molecular testing of blood for HBoV should be performed.

Nucleotide sequence accession numbers. The VP1/VP2 partial sequences and the sequence of the NS1 region of HBoV2 recovered from the patient can be found in the GenBank database under accession numbers KF905229 and KF956066, respectively.

ACKNOWLEDGMENTS

We thank Claire Dauphin for her cardiologic expertise and Jeffrey Watts for help with preparation of the English manuscript. We also thank Audrey Mirand and Martine Chambon for critical reading of the manuscript. We are grateful to Jean-Louis Kémény and his laboratory personnel for providing technical support to process paraffin-embedded tissue.

REFERENCES


