Possible Involvement of Human Bocavirus 1 in the Death of a Middle-Aged Immunosuppressed Patient

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An immunosuppressed 61-year-old man went to the hospital with fever, nonproductive cough, and increasing shortness of breath. The subject died 8 days later of respiratory complications. PCR of respiratory samples as well as a blood sample revealed exceptionally high DNA levels of the emerging pathogen, human bocavirus 1 (HBoV1), a recently found pathogen associated with respiratory symptoms in young children. We describe the clinical progression of the case and discuss the potential role of HBoV1 in the outcome.

CASE REPORT

On 16 July 2011, a 61-year-old man presented to his local emergency department with a 4-week history of shortness of breath on exertion associated with cough productive of white sputum. He had an extensive medical history that included relapsed chronic lymphocytic leukemia and secondary hypogammaglobulinemia, therapy-related myelodysplastic syndrome, ischemic heart disease, and emphysema. He had been treated with monthly azacitidine since June 2009 and was also taking a prophylactic dose of co-trimoxazole. He had received monthly prophylactic intravenous immunoglobulin from October 2009 to March 2011. A previously heavy smoker, he had quit 2 weeks earlier. Examination of the chest showed normal results, as did chest radiograph. The C-reactive protein (CRP) level was higher (77 mg/liter). He was started on an oral corticosteroid in view of his history of chronic obstructive pulmonary disease.

The man returned to the hospital 6 days later (22 July 2011) and was admitted. He complained of fever, nonproductive cough, and increasing shortness of breath. His body temperature was 36.3°C, pulse 92/min, and blood pressure 99/60 mm Hg. His oxygen saturation at rest measured 93% on room air; however, it fell to 83% on mild exertion. The pharynx appeared erythematous. Bibasilar crackles were noted on chest examination. There was no lymphadenopathy or rash.

A complete blood count showed a hemoglobin concentration of 9 g/dl (normal range, 13.0 to 18.0 g/dl), white cell count of $51.1 \times 10^9/\text{liter}$ (4.0×10^9 to $10.0 \times 10^9/\text{liter}$), platelet count of $50 \times 10^9/\text{liter}$ (150×10^9 to $450 \times 10^9/\text{liter}$), neutrophil count of $8.07 \times 10^9/\text{liter}$ (2.0×10^9 to $7.5 \times 10^9/\text{liter}$), and lymphocyte count of $41.4 \times 10^9/\text{liter}$ (1.0×10^9 to $3.5 \times 10^9/\text{liter}$). Renal function was modestly impaired (estimated glomerular filtration rate [GFR] of 56 ml/min). The serum aminotransferase (alanine transaminase [ALT]) level was chronically raised at three times the upper limit of normal. The C-reactive protein level had risen further to 93 mg/liter. Chest radiograph now showed multifocal areas of consolidation in both lung fields, most prominent in the left middle and lower zones. A reticulonodular infiltrate was also present in the perihilar regions. The differential diagnosis included bacterial pneumonia and an atypical chest infection.

Oxygen saturation improved to 96% with nasal cannula oxygen delivery at 4 liters/min. Intravenous clarithromycin and piperacillin with tazobactam were commenced. Fluconazole was given to treat presumed pharyngeal thrush. Prophylactic intravenous immunoglobulin was restarted.

The patient's symptoms improved over the next 48 h, but his condition then began to deteriorate. The fever returned (38 to 39°C) 48 h after admission to the hospital (24 July 2011), and his cough became productive with large amounts of purulent sputum. Examination of the chest revealed bilateral crackles from the lung bases to the middle zones. Chest radiograph showed progressive consolidation. The piperacillin with tazobactam treatment was discontinued. Meropenem and teicoplanin were commenced. High-dose co-trimoxazole was introduced to cover possible *Pneumocystis jirovecii* pneumonia. Clarithromycin and fluconazole were continued as before. Over the next 6 days, he developed progressive tachypnea (respiratory rate up to 24/min), hypoxia (oxygen saturation of 88% with face mask oxygen delivery at 15 liters/min), and tachycardia. The C-reactive protein level rose to a maximum of 346 mg/liter. Computed tomography (CT) scan of chest showed widespread ground glass changes and granular opacification in both lungs. The patient's condition continued to deteriorate. He was transferred to intensive care on 29 July 2011 and died the following day.

Fungal cultures from the patient's throat swabs and sputum cultures yielded heavy growth of *Candida albicans*. Detection of pathogenic bacteria by PCR from sputum samples, tracheal secretions, and a blood specimen collected were negative (Table 1). However, the samples were obtained after initiation of antibiotic therapy.

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TABLE 1 Results from PCR testing of sputum specimens, tracheal secretions, and a blood sample for known or potential respiratory pathogens^a

Microbe ^b	Diagnostic method ^c	PCR test result ^d on the following type of sample:		
		Sputum	Tracheal secretion	Blood
Influenza A virus	In-house multiplex PCR	N	N	NT
Influenza B virus	In-house multiplex PCR	N	N	NT
RSV	In-house multiplex PCR	N	N	NT
Metapneumovirus	In-house multiplex PCR	N	N	NT
PIV1 to PIV3	In-house multiplex PCR	N	N	NT
Coronavirus	In-house multiplex PCR	N	N	NT
Adenovirus	In-house PCR	N	N	NT
Rhinovirus	In-house PCR	P	N	NT
CMV	In-house qPCR	N	N	NT
EBV	In-house qPCR	3.2×10^6	4.9×10^3	NT
HSV1	In-house multiplex PCR	N	N	NT
<i>Coxiella burnetii</i>	In-house PCR	N	N	NT
<i>Mycoplasma pneumoniae</i>	In-house PCR	N	N	NT
<i>Chlamydia pneumoniae</i>	In-house PCR	N	N	NT
<i>Legionella pneumophila</i>	In-house PCR	N	N	NT
<i>Streptococcus pneumoniae</i>	In-house PCR	N	N	NT
<i>Haemophilus influenzae</i>	In-house PCR	N	N	NT
<i>Moraxella catarrhalis</i>	In-house PCR	N	N	NT
<i>Staphylococcus aureus</i>	In-house PCR	N	N	NT
<i>Pseudomonas aeruginosa</i>	In-house PCR	N	N	NT
<i>Bordetella pertussis</i>	In-house PCR	N	N	NT
<i>Pneumocystis jirovecii</i>	In-house PCR	N	N	NT
HBoV1	In-house multiplex qPCR	4.3×10^9	4.8×10^{10}	1.5×10^4
HBoV2 to HBoV4	In-house multiplex qPCR	N	N	N
WUPyV	In-house nested PCR	N	N	N
KIPyV	In-house nested PCR	N	N	N
MCPyV	In-house singleplex qPCR	N	N	N
TSPyV	In-house singleplex qPCR	N	N	N

^a Blood, tracheal aspirate, and sputum samples were collected on days 5, 8, and 9 after admission to the hospital, respectively. The patient died on day 8 after admission to the hospital.

^b RSV, respiratory syncytial virus; PIV1 to PIV3, parainfluenza virus types 1 to 3; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HSV1, herpes simplex virus 1; HBoV1, human bocavirus 1; WUPyV, Washington University polyomavirus; KIPyV, Karolinska Institutet polyomavirus; MCPyV, Merkel cell polyomavirus; TSPyV, trichodysplasia spinulosa-associated polyomavirus.

^c PCR, qualitative PCR; qPCR, quantitative PCR.

^d N, negative; P, positive; NT, not tested. HBoV, rhinovirus, and EBV were detected in the samples. The copy numbers of EBV and HBoV1 are shown.

Nineteen viruses, including 11 respiratory disease-causing pathogens, four newly discovered human polyomaviruses (Karolinska Institutet polyomavirus [KIPyV], Washington University polyomavirus [WUPyV], Merkel cell polyomavirus [MCPyV], and trichodysplasia spinulosa-associated polyomavirus [TSPyV]), and four human bocaviruses (human bocavirus 1 [HBoV1] to HBoV4), were investigated by PCR/quantitative PCR (qPCR) in sputum samples, tracheal secretions, and a blood sample. A blood sample was obtained on the day the patient died (30 July 2011). Tracheal aspirate and sputum samples were obtained postmortem on 31 July 2011 and 1 August 2011, respectively.

HBoV was present in very high copy numbers in tracheal aspirate (4.8×10^{10} copies/ml) and sputum (4.3×10^9 copies/ml) and also occurred in blood plasma (1.5×10^4 copies/ml) (1). In addition, rhinovirus and Epstein-Barr virus (EBV) were detected in the sputum and tracheal samples. No other pathogens were detected in these samples.

Almost the entire HBoV1 genome sequence was determined by cloning the viral genome from the sputum by high-fidelity PCR. The 5,146-nucleotide consensus sequence (GenBank accession no. [KC823115](#)) was established by aligning sequences from four separate clones. Phylogenetic analysis demonstrated that the HBoV1 identi-

fied in our study shared 98 to 100% nucleotide identity with published strains of HBoV1. We found no viral mutations that would suggest altered cell tropism or viral gene expression.

The patient's single existing sample of serum was tested for IgG and IgM antibodies against HBoV1 to HBoV4, with a competition enzyme immunoassay (EIA) based on HBoV1 to HBoV4 virus-like particles (2–6). Neither IgM nor IgG antibodies were detected against any of the four HBoVs.

HBoV1 was discovered in 2005 by Allander et al. in respiratory secretions (7). This virus is increasingly recognized as a cause of respiratory infections worldwide, and numerous studies have investigated its prevalence rates, basic epidemiology, genome variability, and possible pathogenicity (8). Recently, Söderlund-Venemo et al. and Meriluoto et al. disclosed that HBoV1 infection is significantly associated with respiratory disease in young children, characteristically at 6 to 48 months of age (4, 5). More than 94% of adults have antibodies against the HBoVs (5, 9, 10); however, as a portion of these IgG results is due to the enteric HBoV2 to HBoV4, the seroprevalence of HBoV1-specific IgG approaches 60% (2).

HBoV1 infections seem rare among immunocompromised adults (11–13).

While not all disease associations of HBoV1 have been clarified, recent studies provide evidence that HBoV1 may, in children, cause encephalitis and life-threatening complications (14–17). For instance, a well-documented case of a fatal HBoV1/adenovirus coinfection in an immunocompetent child with pneumonia and myocarditis has been reported (18). As in the present case, the respiratory condition resulted in a lethal outcome.

Our patient was admitted to the hospital with typical symptoms of a viral respiratory tract infection. Despite supportive treatment, he eventually succumbed. In light of his extensive medical background and cooccurrence of EBV and rhinovirus infections, the relative significance of HBoV1 infection as a cause of death in this case is not easy to determine. However, viral DNA of exceptionally high copy number was observed both in the trachea and nasopharynx and also occurred in blood, pointing to acute HBoV1 infection. It is tempting to speculate that the high viral load reflects the unusually complicated clinical course.

The exceptionally high HBoV1 copy number and the adverse outcome are unlikely to be connected to the virulence of this particular HBoV1 strain, since its genome was virtually indistinguishable from commonly circulating HBoV1 strains. A more likely explanation for the high HBoV1 titer and the lack of anti-HBoV1 IgG and IgM is the patient's secondary hypogammaglobulinemia. The apparent lack of anti-HBoV1 antibodies may also be due to serum collection before the initiation of antibody response.

A correlation between the high HBoV1 DNA levels in the nasopharynx and respiratory illness has been observed (3, 4, 19, 20). In general, acute HBoV1 infection is accompanied by the presence of viral DNA in blood (3, 4). An interesting possibility and topic for forthcoming research are to what extent HBoV1 DNA levels at various sites are associated with virulence or adverse clinical outcome.

Recently, Streiter et al. (21) reported HBoV1 infection in a 14-month child with a known history of a myelosarcoma affecting the bone marrow, and secondary immunodeficiency. In agreement with Streiter et al., our case is in line with the notion that the clinical effects of HBoV1 infection might be influenced by coinfections with other viruses. HBoV DNA-positive subjects have a high coinfection rate up to 83% in respiratory samples (8).

Accumulating evidence indicates that HBoV1 is an important respiratory pathogen and can be life-threatening. Further studies are needed to determine to what extent exposure to this virus in general and in coinfections can lead to severe disease or death during immunosuppression or advanced age.

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We declare that we have no conflicts of interest.

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