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CASE REPORT

Detection of bocavirus DNA in nasopharyngeal aspirates of a child with bronchiolitis

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Summary We describe a case of bronchiolitis associated with the newly detected human bocavirus (hBoV) in a child with a suspected Noonan syndrome. This is the first report of a bronchiolitis probably linked to hBoV that required intensive care while being accompanied by a congenital heart disease and a history of several episodes of severe respiratory symptoms.
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Human bocavirus (hBoV),¹ a parvovirus newly detected in October 2005, seems to be a worldwide^{1–3,5,10,11} distributed respiratory pathogen. The prevalence of this newly identified agent ranges between 1.5%⁵ and 10.3%.⁸ A recent

study by the Australian group from the Queensland Paediatric Infectious Diseases Laboratory demonstrated that hBoV is one of the most frequent pathogens responsible for respiratory tract infections in hospitalized children, thus being directly behind picornaviruses, adenoviruses and human metapneumovirus.¹⁰ Clinical symptoms most frequently associated with human bocavirus infection include cough, rhinorrhoea, and fever, but also diarrhoea and paroxysmal cough may be symptoms of interest.⁷ However, the risk of severe clinical complications following bocavirus infection

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in infants remains to be elucidated. We have recently described a case report of a 28-year-old female (adult) patient with a non-Hodgkin lymphoma who suffered from an atypical pneumonia that was associated with hBoV.⁶ Based on this clinical experience and in continuation of our study of new viral respiratory infections in pediatric patients we also started to investigate respiratory specimens from a series of severe lower respiratory tract infections treated in primary care pediatric hospitals in our region. In nasopharyngeal aspirates of one patient suffering from an acute bronchiolitis and negative results for other respiratory viruses hBoV was detected by PCR.

Case report

The patient, a 2-year-old fully immunocompetent mature male, suffered from respiratory distress immediately after birth and had been mechanically ventilated for 7 days. Echocardiographic studies showed a hypertrophic cardiomyopathy with trivial subvalvular obstruction and a mild pulmonary stenosis. The patient had clinical signs of Noonan Syndrome (hypertelorism, antimongoloid slant of the palpebral fissures, ptosis, low posterior hair line, low set ears, cubitus valgus, and cryptorchidism). A molecular genetic test revealed a polymorphism (IVS7-21T > C), but no mutation of the PTPN11-gene on chromosome 12q24.1.

At the age of 1 year and 10 months, the boy was treated for RSV-negative bronchiolitis (as defined earlier⁹) of unclear aetiology. He developed severe respiratory failure, and was mechanically ventilated for 18 days. No causative agent was found in bronchial lavage fluid or serological tests. After dismissal no signs of chronic lung disease were present.

On admission of the most recent episode of respiratory distress that coincided with our generalized search for hBoV, the patient suffered from severe dyspnea, tachypnea, and cyanosis. Oxygen saturation measured by pulse oxymetry was 70% while breathing ambient air, respiratory rate was 46 breaths/min, pulse rate 145 beats/min, body temperature 38.0 °C (100.4 °F), and blood pressure 115/51 mmHg. A capillary blood gas analysis revealed a global respiratory insufficiency with a pH of 7.11 and a severe hypercapnia (pCO₂ 84 mmHg). The boy was treated with oxygen, salbutamol and ipratropium bromide by inhalation, systemic steroids, chloral hydrate, and theophylline. Severe dyspnea persisted, thus inhalative adrenaline was administered, which leads to a decrease in the severity of the respiratory symptoms. Oxygen was given for additional 2 days, the boy was dismissed from hospital after 8 days.

The chest X-ray showed hyperinflation and a cardiomegaly, but no pneumonic infiltrates were observed. Blood cultures were sterile, C-reactive protein was negative, the white cell count was $24.7 \times 10^9/L$, and the hematocrit was 38.1. Flow cytometry revealed increased CD56 NK-cells, normal numbers of CD3-T cells, CD4 helper cells, CD8 suppressor cells, and CD19 B-cells. IgG was moderately increased (12 g/L), IgA, IgM, and complement factors were normal.

Nasopharyngeal aspirates obtained during the aggravation of the pulmonary symptoms were tested for *Mycobacterium tuberculosis*, *Chlamydia pneumoniae*, *Pneumocystis*

jirovecii, *Aspergillus* sp., *Candida* sp., *Cryptococcus neoformans*, HSV, VZV by PCR and culture with negative results. Negative results for CMV, EBV, HBV, HCV, Parainfluenza viruses, picornaviruses, and adenoviruses were obtained by serology.

Laboratory testing for influenza viruses, RSV, and adenoviruses was immediately performed in the first virological laboratory (Institute for Virology, University of Cologne). As these tests were negative for any of the investigated respiratory viruses, the specimen was sent to the neighbour university (University of Bonn, Institute for Medical Microbiology, Immunology, and Parasitology), in which a project on the epidemiology of newly detected respiratory viruses was already established including PCR assays for newly detected coronaviruses (NL63, HKU1, and SARS), human metapneumovirus, and also hBoV. A second nasopharyngeal aspirate was directly sent to the University of Bonn and was tested by PCR for the "new" viruses as well.

PCR was negative for human metapneumovirus, influenza viruses A and B, coronaviruses (pan-corona-PCR, detailed protocol on request), RSV, and adenoviruses. Blood cultures and respiratory fluids were tested negative for bacteria by culture. Surprisingly, nasopharyngeal aspirates were repeatedly tested positive for the recently detected human bocavirus by two PCR protocols using two different sets of primers. Sequence analysis of the PCR amplicates revealed that the amplified DNA was identical to the strain hBoV-ST2. Laboratory contamination by the control plasmid was excluded as the control plasmid displayed a one-nucleotide deletion.

The first set contained primers described by Allander et al.,¹ the second set consisted of the newly designed primers OS1 (5'-CCCAAGAAACGTCGCTAAC) and OS2 (5'-GTGTTGACTGAATACAGTGT), which were derived from the bocavirus genome from subtype 2 (accession no: DQ000496). However, pharyngeal washes, equivalent to the nasopharyngeal aspirates from children, from 15 healthy adult volunteers not suffering from any respiratory symptoms were tested negative for the human bocavirus, suggesting and supporting the assumption that the virus indeed is associated with a disease.

The detection of hBoV in the nasopharyngeal aspirate of this high-risk pediatric patient with acute bronchiolitis suggests that the pulmonary symptoms were caused by this agent. In infants the newly detected human metapneumovirus (hMPV) and also hBoV seem to be important pathogens in acute lower respiratory tract infection. HBoV DNA has been detected in 3.1–10.3% of children less than 3 years of age^{1–3,5,7,8} and has also been observed as a respiratory pathogen in adult high-risk patients.^{4,6}

Taking into account the clinical observations by Foulongne and co-workers¹¹ who described bronchiolitis as the major diagnosis in hBoV infected patients with the major symptoms of dyspnea, respiratory distress, and cough, the clinical picture described here seems to be typical for an hBoV infection. Most frequent symptoms observed in the largest study on hBoV epidemiology and clinical symptoms were cough, throat, rhinorrhea, and fever, thus presenting as the common cold.^{5,7} Arnold and co-workers⁷ found that most patients (63%) were below

12 months of age, thus it cannot be excluded that the patient described here (2 years of age) suffered from a reinfection. In contrast to the recent study by Weissbrich et al.,⁸ who observed a high rate of coinfections (39%), no accompanying and hitherto known pathogen was detected in the recent case. Thus, although it remained still impossible to fulfil Koch's postulates, and although a coinfection with another respiratory pathogen cannot be fully excluded, it seems probable that the current episode was indeed induced by hBoV.

Of note, although the detection of hBoV in the specimen derived from the child's respiratory tract did not result in a specific antiviral therapy, it avoided an additional administration of intravenous antibacterials, thus avoiding potential side-effects, while also reducing (unnecessary) costs (for antibacterials).

To our knowledge, this is the first prospective report of a respiratory tract infection associated with hBoV in a young patient with congenital heart disease that required intensive care. The observations support the assumptions by others^{1–3,5,7,8,10,11} that hBoV is an important emerging pathogen.

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