Severe human bocavirus 1 respiratory tract infection in an immunodeficient child with fatal outcome

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Abstract

We report a case of lower respiratory tract infection with human bocavirus 1 (HBoV1) in an immunodeficient 6-month-old boy leading to respiratory failure with fatal outcome. PCR of serum/tracheal secretions revealed exceptionally high HBoV1-DNA levels and immunoassays showed seroconversion indicating an acute primary HBoV1 infection. All assays for other pathogens were negative, strongly suggesting that HBoV1 was the causative agent in this case.

Background

Human bocavirus (HBoV) 1 is a recently identified viral agent that belongs to the family of *Parvoviridae* and comprises a non-enveloped capsid with a linear single-stranded DNA genome [1]. Viral DNA has been detected mainly in nasopharyngeal secretions, and in serum or blood samples of younger children with upper or lower respiratory tract infections (RTI). Besides HBoV1, which is predominantly detected in the respiratory tract, three other bocaviruses, HBoV2 – 4, are mainly found in stool.

Detection of HBoV1 DNA in airway samples of children with RTI is frequently combined with other viruses or bacteria because HBoV1 persists and is shed for a prolonged period. This makes interpretation of a PCR-positive test result difficult. The high detection rate of multiple respiratory viruses in an airway specimen, and the presence of HBoV1 DNA in asymptomatic children, thereby complicate the diagnosis of acute HBoV1 infections [2]. In addition to detection of HBoV1 DNA in airway samples, other diagnostic methods, such as serology, should be used. With accurate diagnostic methods, acute HBoV1 infection has been shown to cause mild to life-threatening RTIs. Due to the lack of an animal model, the Koch's postulates have not been formally fulfilled, thus proving clinical relevance is challenging.

Life-threatening infections are rare. Here we present a case of HBoV1 lower RTI, diagnosed by both PCR and serology, leading to severe respiratory failure with fatal outcome in an immunodeficient child.

Case presentation

A six-month-old boy, the first child of consanguineous parents, was transferred from Dubai, United Arab Emirates, to the University Hospital Heidelberg, Germany, for further diagnostic workup of an unspecified syndrome including failure to thrive, distinct psychomotor retardation, multiple osseous malformations, microcephaly due to cerebral atrophy, blindness, and symptoms of an acute hemolytic uremic syndrome. Shortly after admission, a T-cell defect was diagnosed and whole-exon screening identified a homozygous mutation in the *NIN* gene which codes for Ninein, a protein crucial for mitosis. The *NIN* gene mutation was regarded as the cause of the complex syndrome. The cause of the immunodeficiency found in our patient was an impaired cytokine response in combination with insufficient formation of antigen-presenting cells to T lymphocyte synapses leading to a functional T-cell deficiency. Because of feeding difficulties and significant dysphagia, a percutaneous gastrostomy tube as well as a Hickman line were placed. The hemolytic uremic syndrome was treated with eculizumab; however, proteinuria and oliguria persisted and required high doses of diuretics. About ten days after admission, the boy developed pneumonia and was transferred to the intensive care unit where he had to be intubated, and was mechanically ventilated. He developed an acute respiratory distress syndrome (ARDS) with multiple critical deteriorations. One week after admission, an initial radiograph was performed in the context of the surgery for a Hickman line and a gastrostomy tube. He did not show signs of a preceding lung injury (Fig. 1). Mechanical ventilation was difficult with an FiO_2 of 1.0, high inspiratory pressures up to 40/11 mbar, and intermittent use of high frequency oscillation and

inhaled nitric oxide. Tracheostomy was performed after six weeks of mechanical ventilation. Because of atelectasis, pulmonary secretion, and bronchospasm, the mechanical ventilation had to be intensified several times. Elevated CRP values (average 92.4 mg/l, peak 221.7 mg/l, normal <5 mg/l) were first noted one week prior to intubation and mechanical ventilation, and did not normalize over the next four months. This prompted antibiotic treatment with different combinations of cefotaxime, meropenem, ciprofloxacin, teicoplanin, linezolid, and erythromycin with no clinical effect. Apart from *Staphylococcus epidermidis* detected in a single blood culture, bacterial as well as fungal cultures and PCR for atypical microorganisms in tracheal secretions were always negative. Tracheal secretions were also tested in a real time multiplex respiratory PCR (Fast Track Diagnostics respiratory pathogens 21, Luxembourg) for influenza A virus including H1N1, influenza B virus, rhinovirus, respiratory syncytial virus, bocavirus, adenovirus, parainfluenza virus 1 through 4, four coronaviruses (NL63, 229E, OC43, HKU1), parechovirus, enterovirus, human metapneumovirus A/B and *Mycoplasma pneumoniae*. Additionally, these samples were also tested for herpes simplex virus and varicella zoster virus DNA by in-house PCR and for bacteria and fungi by culture. Human bocavirus 1 (HBoV1) was the only pathogen detected in tracheal secretions. All serial tracheal secretions were positive for HBoV1 DNA, which was present in high copy numbers in some samples (Fig. 2A). The highest viral load in respiratory samples of 3.1×10^9 copies/ml was detected about day 20 of hospitalisation, one week after pneumonia was diagnosed and mechanical ventilation initiated. The viral load decreased slowly in respiratory samples but DNA was detectable for several months. Blood was taken from the patient once per week and HBoV1 DNA was detectable in serum for 50 days with a peak of 2.0×10^3 copies/ml. The specificity of the real-time PCR was confirmed with a qPCR and an in-house PCR followed by DNA sequence analysis of the amplified product. The qPCR assays were performed using the 1xSensiMix SYBR No-ROX Kit (Bioline Reagents Ltd, London, UK)

with HBoV1-specific primers (forward primer *5′-CCTATATAACCTGCTGCACTTCCT-3′*, reverse primer *5′- AAGCCATAGTAGACTCACCACAAG-3′*).

The complete *VP1* gene (2016 bp) of the HBoV1 genome was amplified by an in-house PCR assay including the HBoV1-specific primers (forward primer *5′-GTTACGTCTCGAAGATTACAACACTTTATTGATGTTTG-3′*, reverse primer *5′-*GTTACGTCTCAGCAGATGCCTCCAATTAAGAGACA-*3′*). The PCR product was purified and subsequently sequenced. The sequence (accession no. MG680946) was then aligned with different HBoV strains reported in GenBank and subjected to a phylogenetic analysis (Fig. 3). This confirmed a 99% identity of the study sample with HBoV1, thus verifying the specificity of the multiplex respiratory PCR, the qPCR and the immunoassays. Moreover, the phylogenetic analysis revealed a close relationship to a previously reported HBoV1 isolate from Egypt [3] (GB accession no. KU557404.1, as shown in Fig. 3). By following the course of infection over a period of four months, we detected the emergence and persistence of a mutation at amino acid position 590 (VP1 numbering) that results in an amino-acid change from threonine to serine.

HBoV1-specific IgG and IgM were measured by highly sensitive and specific competition immunoassays based on HBoV1-like particles [4, 5]. Both IgG and IgM antibodies against HBoV1 were detected and seroconversion was observed (Fig. 2B), indicating an acute HBoV1 infection. Thus, HBoV1 was considered the most likely cause of ARDS. The patient died of multi-organ failure following four months of mechanical ventilation.

Discussion and conclusions

Human bocavirus 1 (HBoV1) was discovered in 2005 by Allander et al. in respiratory secretions [1] and is increasingly recognized as a cause of pediatric respiratory tract infections

worldwide [2, 6, 7]. By PCR of airway samples, HBoV1 DNA has been detected in 2-20% of children with respiratory tract infection, whereas 40-75% of the HBoV1 DNA-positive patients show co-detections with other respiratory pathogens [8, 9].

However, it is important to acknowledge that almost all routine testing and published studies of HBoV1 infections rely on only PCR testing of respiratory secretions. HBoV1 DNA can by sensitive PCRs be detected for months or even up to a year after acute infection, leading to co-detections and false clinical diagnoses — and thereby, inaccurate disease associations [10 - 13]. Mere qualitative PCR is therefore not an adequate method for diagnosing acute HBoV1 infections, instead a combination of other diagnostic means including qPCR of respiratory samples and serum, as well as serology should be applied [4, 14].

By utilizing accurate diagnostics, increasing evidence has been gathered of HBoV1 being the cause of mild to severe upper and lower respiratory tract infections in children over 6 months of age [6, 7]. HBoV1 may cause also life-threatening complications of lower respiratory tract infection including emphysema, pneumomediastinum, pneumothorax and acute respiratory failure [15 - 18]. In addition, both Sadeghi et al. [19] and Krakau et al. [20] described immunocompromised adult patients suffering from an advanced myelodysplastic syndrome with severe HBoV1 pneumonia with fatal outcome. Further fatal cases associated with HBoV1 infection were described in an adult and a pediatric patient with underlying lung diseases [21, 22]. The need for ventilator support for four months reflecting the extensive lung damage is perfectly explained by the severe ARDS leading to lung fibrosis, impaired gas exchange and eventually death of the patient. Most of the damage to the lungs of patients that do not recover from ARDS is caused by pulmonary inflammation and interstitial fibrosis. It has been shown that HBoV1 infection of in vitro airway epithelium cultures inhibits apoptosis and induces pyroptotic cell death, resulting in tissue injury and inflammation [23, 24]. Persistent HBoV1 infection of the lungs in immunocompromised children may thus lead to

146 lung tissue injury. It can therefore be hypothesized that persistent HBoV1 infection in this
147 child directly damaged the alveolar tissue.

148 Nevertheless, the significance of HBoV1 infection as a cause of death, as described in this
149 case of an immunodeficient child, is not easy to determine. However, viral DNA of
150 exceptionally high copy numbers of $5x10^9$ copies/ml was observed in tracheal secretions at
151 the same time as it occurred in serum, pointing to an acute HBoV1 infection. In general, acute
152 HBoV1 infection is accompanied by the presence of viral DNA in serum [4]. After atypical
153 hemolytic-uremic syndrome had been diagnosed at the age of three months, eculizumab was
154 given five times with approximately three weeks between each application. The last dose was
155 applied 18 days before the respiratory decompensation. It is tempting to speculate that the
156 very high viral load reflects the unusually complicated clinical course and the
157 immunocompromised status of the patient. High viral loads of HBoV1 are associated with
158 respiratory symptoms whereas low viral loads mostly indicate longitudinal asymptomatic
159 shedding [2, 4, 6, 14, 25, 26]. Detection of HBoV1 DNA in serum has further been more
160 tightly linked to symptoms than DNA in respiratory samples. The functional T-cell defect in
161 our case is regularly found in Schimke immuno-osseous dysplasia that was initially suspected
162 but ruled out genetically. Other cases with severe HBoV1 infection in patients with T-cell
163 defect or immunodeficiency have been reported previously [10, 19, 27, 28].

164 High HBoV1 DNA copy numbers in airway samples, short viremic phase, detection of
165 HBoV1-specific IgM and seroconversion of IgG antibodies have been shown to be accurate
166 diagnostic markers in children with acute HBoV1-induced respiratory illness and can thereby
167 separate acute infection from asymptomatic virus shedding [4]. The usefulness of the applied
168 serology has been documented in studies of children with acute wheezing or with community-
169 aquired pneumonia [4, 29, 30]. HBoV1 IgM positivity correlates with both HBoV1 viremia
170 and seroconversion of IgG in paired serum samples, whereas healthy subjects are generally

IgM negative with stable IgG absorbance levels [4]. While in this case, multiplex PCR for 19 other respiratory viruses and mycoplasma was negative, the course of the disease perfectly matched a primary infection by HBoV1, supported by serology and detection of HBoV1 DNA in serum.

The emergence and persistence of a mutation at amino acid position 590 that resulted in an amino-acid change from threonine to serine reflects either (i) a mixed primary infection with two HBoV1 variants, where one dominates over time, (ii) a secondary infection with a different HBoV1 variant, as hypothesized in Martin et al. [12], or (iii) the occurrence of a *de novo* mutation that fostered clonal selection. Further time-course studies with more patients and the use of deep sequencing approaches are required to unanimously resolve these possibilities.

This report illustrates that blood sampling is important for linking HBoV1 with disease, and it indicates that HBoV1 should be considered in severe respiratory tract disease in children. HBoV1 is the most probable cause of respiratory tract disease if the patient has a high viral load in respiratory samples accompanied by viremia, if HBoV1 is the only pathogen detected, and if an acute primary HBoV1 infection is diagnosed by serological testing [4, 8]. We detected HBoV1 DNA in both respiratory and serum samples. Moreover, the serologic results indicate that this child had an acute primary HBoV1 infection. The dramatic increase of HBoV1 load in tracheal secretions and viral dissemination most likely resulted from a progressive impairment of cellular immunity. The observation that all other viral and microbiological assays were negative, strongly suggest that HBoV1 was the causative agent of respiratory failure and death in the present case.

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Availability of data and materials

The data supporting the conclusions of this article are included within this article. The sequence generated and analysed for this report is available in GenBank under the accession number MG680946.

Author's contributions

Study design: JT, PS; Clinical evaluation: JT, JM, PS; Laboratory testing: JF, K-PL, MX; Data analysis and manuscript preparation: JT, JF, K-PL, MS-V, DG, PS. All authors reviewed and approved the final manuscript.

Ethics approval

The study was approved by the Ethical Research Board of the University Hospital Heidelberg, Germany (S-547/2015). All samples and medical information included in this study were obtained during routine medical care.

Competing interests

The authors declare that they have no competing interests.

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Fig. 1.

Chest X-ray showing bilateral opacities as sign of acute respiraotory distress syndrome (ARDS).

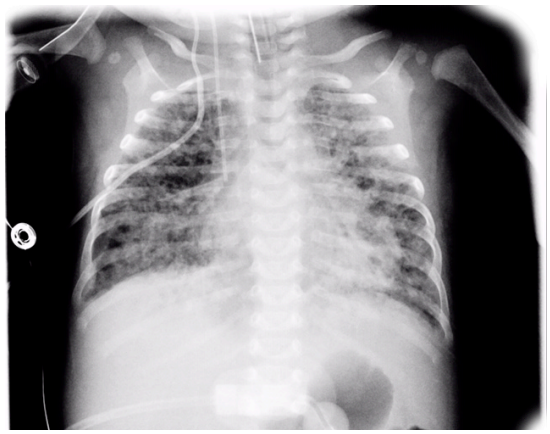

Fig. 2.

HBoV1 diagnostic findings. A) Detection of HBoV1 DNA in tracheal secretions and serum. B) Detection of anti-HBoV1 IgG and IgM antibodies. OD, optical density; Days, days after symptom onset.

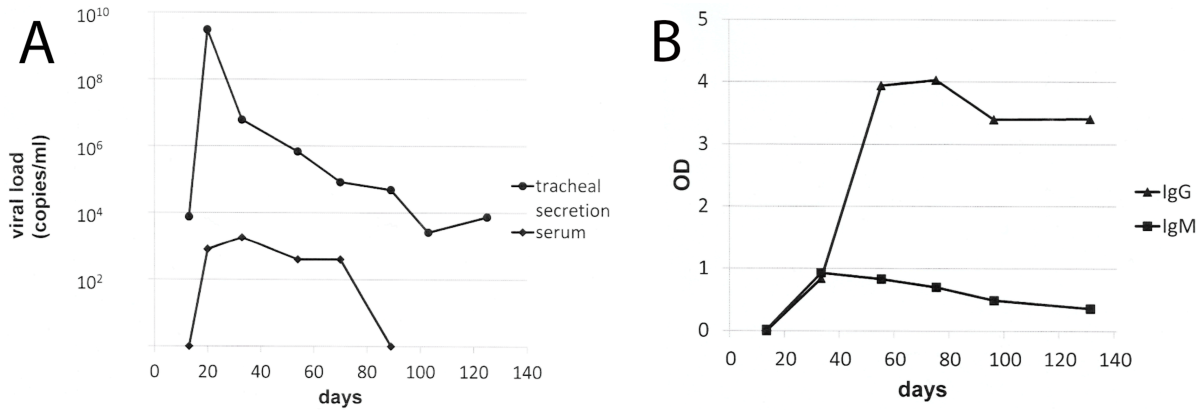

Fig. 3.

Maximum likelihood phylogenetic consensus tree for the *VP* ORF nucleotide sequence of the HBoV1 isolate (V1445149) studied here. The numbers next to the nodes indicate the value of 500 bootstrap analyses. To root the tree, an outgroup of the indicated closely related members of the genus *Bocaparvovirus* was defined. Only bootstrap values higher than 50% are presented. Codon positions included were 1st+2nd+3rd+noncoding. Evolutionary analyses were conducted in MEGA 7.0.26.