Fatal HBoV-1 infection in adult female cystic fibrosis patient

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ABSTRACT

A clinical case of fatal HBoV infection in an adult cystic-fibrosis patient awaiting lung transplantation is reported. The case is important as the genetic background of the underlying disease is congruent with the background of the sole permissive permanent cell culture CuFi-8 which originates also from a CF patient donor.

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The human bocavirus (HBoV) is a parvovirus that is associated with acute and chronic infections of the upper and lower respiratory tract, persists in some tissues and solid cancers and putatively may play an aetiologic role in the development of idiopathic lung fibrosis [1–8]. The viral infection can switch between long term periods of viral shedding and silent phases of latency [9]. To date, no animal model exists, thus studies on the pathology of HBoV infections are limited to clinical studies, case descriptions, and air-liquid interface cell culture models that have been shown to mimic some important steps of the infection cycle [1,7,10–12]. The latter infections models are based on either primary cells that can be differentiated into organ like tissues from primary cells [1,7,11–13] or CuFi-8 cells [1,10,13]. CuFi-8 is a cell line derived from a 24 year old female patient suffering from cystic fibrosis; the cell line carries the mutation pattern ΔF508/ΔF508 (the most common CF associated mutation) and is an immortalized human epithelial airway cell line from the bronchus of the patient; the immortalization was performed with Weinberg hTERT and HPV-16 E6/E7 [10,14].

This information might be relevant for a current clinical case that was treated in our hospital in Cologne. The patient was a 24 year old non-smoking Caucasian cystic fibrosis patient. She was regularly treated in our cystic fibrosis outclinics and was deemed to be eligible for lung transplantation shortly before the fatal clinical episode started. In advance to the lung transplantation, a microbiological check-up revealed

colonization of the lung with low titres of *Aspergillus* and a multi-resistant *Pseudomonas aeruginosa* strain, combined with allergic asthma, pancreatic insufficiency, and malnutrition (BMI 14–15 kg/m^2). In order to overcome the malnutrition, percutaneous endoscopic gastrostomy (PEG) was required and initiated in our hospital. The patient started to recover well, episodically required non-invasive ventilation (NIV), but was in a condition stable enough to continue NIV at home. The night before the patient should have left the hospital she developed an ARDS unexpectedly and was shifted to our intensive care unit. During intensive care treatment, despite expected obstipation, the patient developed massive diarrhea. In order to identify a pathogen causative for the ARDS, a BAL was performed and analysed for respiratory bacteria and viruses. An all-embracing molecular and microbiological diagnostic algorithm was performed as described earlier [15–17], including molecular screening for human herpesviruses 1–8, *Parechoviruses*, *Rhino-* and *Enteroviruses*, *Adenoviruses*, Influenza A and B virus including H1N1, *Human metapneumovirus*, respiratory syncytial virus A and B, *Coronaviruses* 229E, OC43, HKU-1, NL63, parainfluenzaviruses 1–4, mumps and measles, and human bocavirus, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Legionella pneumophila*, and *Bordetella pertussis*, but revealed human bocavirus as the sole pathogen detectable in the BAL during the fatal clinical flare of the ARDS. Detailed protocols for the diagnostic procedures were published previously [1,9,16–18]. Moreover, any attempts to culture further respiratory pathogens including Mycobacterium tuberculosis and atypical mycobacteria revealed negative results.

Although the clinical course could have been triggered by the *Pseudomonas aeruginosa* or *Aspergillus* colonization and no *Pseudomonas cepacia* infection was differentially excluded, the most likely explanation for the fatal outcome remains the sole infection with human bocavirus. In this context it is important to note, that the patient was

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homozygous for the cystic fibrosis genotype ΔF508/ΔF508, and as the donor of the CuFi-8 cell line, at the time point of death was 24 years of age. It is important to mention, that the BAL was characterized by serious bleeding, which may have contributed to the clinical outcome. This bleeding is another parallel to the CuFi-8 cells: The cells are permissive for HBoV if they are seeded to filter membranes and differentiated to pseudostratified air-liquid interface cultures; thereby, they fall dry and develop a mucus layer on top, while receiving nutrition from the basal medium. If those air-liquid interface cultures are infected with HBoV they develop a serious cytopathic effect and may become "leaky", i.e. the basal cell culture medium passes the membrane, which can be compared to the clinical bleeding. The fact that age, sex, and genetic phenotype for the patient and the permissive CuFi-8 cell culture were equal solicits the cautious conclusion that the patient was highly permissive for the HBoV infection and died because of the serious damages the virus initiated. This hypothesis is further supported by the fact that the patient suffered from diarrhea, although cystic fibrosis in concert with pancreatic insufficiency is generally accompanied by obstipation. In this context it is important to mention that HBoV-1 is not generally associated with diarrhea but is observed in HBoV-2 and 3 infections [19–22]; however, HBoV-1 can be shed via the gastrointestinal route after swallowing and was repeatedly found in colorectal malignancies [23,24]. Unfortunately, due to the fact that the clinical course progresses so rapidly, and that the clinical colleagues were not aware of the parallels between the genetic background of the CuFi-8 cells and the patient, no further clinical specimen were collected for scientific purposes, thus no further laboratory investigations could be performed, although this would not have changed the clinical decision making. This is a considerable limitation of our report, which in our view still contains important hints regarding the pathogenesis of HBoV.

Taking into account the fact that it remains formally difficult to attribute clinical causalities to human bocavirus as previously discussed by Martin [25] and Byington [26], a permissive animal model for studying HBoV pathology would be a great advantage. However, although exclusively investigating a clinical cohort, Byington [26] and coworkers have unintendedly confirmed the assumption that HBoV is indeed a true pathogen rather than a blind and silent passenger [27], which was further confirmed by our recent translation study in which we have shown that HBoV-1 induces a profibrotic and procancerogenic cytokines expression in vivo and in vitro [1].

We also have to critically discuss the putative link between the CF genotype of the patient and the cell culture and its relation to the severe outcome of the HBoV infection. So far it remains a matter of speculation if there is a causality between these co-incidences or if the clinical case was just a rare event that was congruent to our observations in cell culture. However, it remains possible that such a causative link exists and therefore this clinical case presentation is intended to trigger further research in this direction.

As a consequence, more detailed clinical studies that focus on the high risk group of cystic fibrosis patients suffering from HBoV infections are required, and it needs to be tested in vitro if cystic fibrosis specific factors contribute to a more severe clinical course of the HBoV infection of those patients.

Ethical approval

All procedures described in this case report were performed with approval from the Ethic Committee of the University of Witten (vote 73/2012).

Conflict of interest declaration

The authors declare that no conflict of interest apply according to the ICMJE definitions.

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