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Case report

Detection of HBoV DNA in idiopathic lung fibrosis, Cologne, Germany

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ABSTRACT

We report two confirmed cases of usual interstitial pneumonia (UIP) associated with infection of the human bocavirus (HBoV). In one case HBoV was identified in the bronchoalveolar lavage (BAL) during an acute exacerbation as well as post mortem in different tissues giving rise to the hypothesis that HBoV infections trigger UIP or could be a causative agent and be a systemic component in UIP.

In the other case, the UIP was confirmed by radiological methods and HBoV was detected in the BAL during an acute exacerbation. Both cases give rise to the hypothesis that HBoV could be a causative agent of UIP or could contribute to its development and/or acute exacerbations.

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1. Case report

1.1. Why are these cases important?

The human bocavirus was initially discovered in 2005 [1] and has been associated with serious infections of the respiratory tract and gastrointestinal disease [2]. Although Koch’s postulates could not be fulfilled for human bocavirus so far, there is increasing evidence that it is a true pathogen rather than an innocent bystander in airway infection (reviewed by [3–4]). Recently, we and others have shown that acute HBoV infection can lead to a persistent infection [5–7] leading to covalently closed circular HBoV genomes in infected cells [6]. It becomes a likely hypothesis that chronic HBoV infection could lead to ongoing, maybe subclinical inflammation processes that finally lead to fibrosis of the chronically infected tissue, as observed for other DNA viruses like hepatitis B virus infections [8]. It might also be speculated that persistent infection HBoV could be responsible for chronic idiopathic interstitial lung diseases such as usual interstitial pneumonia (UIP).

2. Case descriptions

2.1. Case 1

Based on the above mentioned background knowledge, we observed the clinical case of a 62 year old Caucasian male who came to our hospital with an acute dyspnoea based on a chronic interstitial lung disease. By history, the patient, formerly smoker but non-smoker since several years, was suffering from non-productive cough and chronic dyspnoea, which severe symptomatic progression over the last year. Past medical history revealed a coronary heart disease with a history of myocardial infarction and bypass surgery nine years ago. Associated risk factors included arterial hypertension, diabetes mellitus, hyperlipidemia, and a history of smoking until 1999 with a cumulative dosage of 30 pack years.

The current computed tomography (CT scan) of the thorax revealed reticular abnormalities, traction bronchiectasis and honeycombing with subpleural and basal predominance consistent with a UIP pattern. The radiological findings were consistent with a progressive UIP. Other known causes of interstitial lung diseases ILD such as domestic and occupational environmental exposures, connective tissue disease, and drug toxicity were not evident.

Blood gas analyses revealed pH of 7.46, a PaCO2 of 32 mmHg, a PaO2 of 57 mmHg and a bicarbonate level of 24 mmol/l. Lung function testing using spirometry and bodyplethysmography demonstrated a restrictive pattern with a forced vital capacity (FVC) of 2.1 L (44% predicted), a forced expiratory volume in 1 s (FEV1) of 1.91 (51% predicted), a FEV1/FVC ratio of 90%, a residual volume (RV)
of 1.61 (64% predicted), and a total lung capacity (TLC) of 3.81 (50% predicted); thereby, the diffusion capacity was seriously impaired with a Krogh factor of 0.8 mmol/kPa/min (58% predicted). Based on these findings and current recommendations the patient was diagnosed to have a UIP [9]. The patient received antibiotic therapy with sulacinilcin, antifungal therapy with fluconazole and amphotericin B, and steroid therapy with prednisolone combined with antifibrotic therapy with azathioprine.

On cytology the bronchoalveolar lavage (BAL) as gained by flexible bronchoscopy displayed 89% macrophages, 10% lymphocytes, and 10% neutrophils with a slight elevated overall cell count of 65 million cells per l with a non-smoker status since 9 years. The number of neutrophils thereby counts at the upper border of the normal range, which is also compatible with a UIP. The histopathological investigation of the airways revealed signs of a mild chronic bronchitis, but transbronchial biopsies were not performed.

Microbiological and virological investigations remained negative by culture and molecular methods except a distinct positive signal for HBoV by Luminex RVP® (Luminex, TX, USA) [10]. Pathogens screened for were Influenza viruses H3N2 and H1N1, HMPV, RSV, PIV 1–4, HBoV, Coronavirus NL63, HKU1, OC43, and 229E, Adenoviruses, Entero/Rhinoviruses (by xTAG RVP), HSV 1 and 2, CMV, EBV, VZV (by Artus/Qiagen LC-PCR assays), Mycobacterium tuberculosis and atypical mycobacteria (by CHIPRON MYCO-Direct 1.7 assay), and respiratory bacteria Bordetella pertussis (PCR), Haemophilus influenzae (culture), Legionella pneumophila (antigen test), Mycoplasma pneumoniae (PCR), Chlamydia phila pneumoniae (PCR), Streptococcus (culture), and Staphylococci (culture). Screening for bacteria was performed by an external laboratory providing this service for our hospital. Candida albicans was found in the mouth of the patient, but no fungi were detected in the BAL after cytospin preparation and routine stainings (Haematoxylin–eosin-staining, May–Grünwald–Giemsa, and Periodic acid–Schiff staining) followed by microscopic investigations. Thus, in this case, only HBoV was detected in the BAL. Half a year later, the patient presented with a progress of UIP which was confirmed by radiographic means and was listed for lung transplantation.

2.2. Case 2

The second case was a non-smoking 69 year old Caucasian male. The patient was transferred from an external hospital for treatment of respiratory failure. An interstitial lung disease was known for several months, but the establishment of a defined diagnosis has not been sought for before. The CT scan revealed reticular abnormalities and diffuse honeycombing, but also severe ground glass opacities, while the patient was thought to suffer from respiratory infection. However, sequential antibiotic and antiviral treatment with Meropenem and Ceftazidime/Ciprofloxaxin and ganciclovir was not successful. Here, initial white blood cell count (WBC) was 4.9/l, but increased to a maximum of 15.8/l, while the C-reactive protein reached values of 169 mg/l. Because of worsening respiratory failure the patient was transferred to the Intensive Care Unit. Here, after 12 days of intermittent non-invasive ventilation the patient was intubated and received invasive mechanical ventilation.

During invasive ventilation flexible bronchoscopy was performed. Thereby, a BAL was obtained demonstrating an enhanced cell count of 86 mio. cells/ml (norm for a non-smoker: 50 mio. Cells/ml) and a differential cytology of 35% macrophages, 19% lymphocytes, and 46% neutrophilic granulocytes. Microscopic investigation of the BAL revealed numerous erythrocytes and alveolar macrophages, but no hints for a fungal infection such as Pneumocystis jiroveci. Single cells with an enlarged nucleus were observed, compatible with a viral cytopathic effect but not in the typical form of owl-eye cells known for CMV infections. The granulocytosis is compatible with a broncho-alveolar infection of bacterial origin, the lymphocytosis in concert with the observed vireocytosis are a sign for a viral infection, finally superinfected with bacteria. The CD4/CD8 ration of 1.3 was normal and the CD1a count was slightly elevated but not typical for a histiocytosis X, which in turn was excluded.

The BAL was also tested positive by PCR for CMV and human Bovacuvirus (HBoV) by the CMV-PCR by Qiagen (Hilden, Germany) and the Luminex RVP assay (Luminex, Austin, TX), respectively. Although no P. jirovecii pneumonia was suspected, this was tested by PCR but remained negative. No further pathogens were identified by PCR or microbiological screenings.

However, the clinical condition of the patient rapidly deteriorated, and subsequently, the patient died. Post mortem tissue samples collected during the autopsy were formalin fixed and paraffin embedded and tested for CMV and HBoV. Of the tested tissue samples lung tissue from the right lower lobe, the heart’s sep- tum, the pancreas, and the thyroid gland became positive for CMV. Surprisingly, in addition, both right and left ventricle myocard was tested positive for HBoV. Other Formalin Fixed Paraffin embedded tissues (liver, spleen, kidney left and right, bone marrow, testis, lymph node, adrenal gland, and prostate gland remained negative for both viruses. Finally, tissue samples of the lungs revealed an interstitial pulmonary fibrosis with scarring and honeycomb change, which was consistent with UIP [9]. Based on these finding it cannot be fully excluded that the clinical course could be a reactivation of CMV as previously described [11]; however, CMV was detected in some but not all tissue sample, the latter being compatible with the assumption of local persistence of the virus rather than a ubiquitous reactivation by immunosuppression.

Unfortunately in both cases it was impossible to perform serological HBoV screening as no blood samples were available for further analyses. Quantification of HBoV was not performed in both cases as a quantification of HBoV in both the BAL or the tissue would have required a cell count in the tissue as a reference parameter, which was impossible to count. Moreover, quantification of HBoV loads in the BAL is implicate as the instillation volume strongly dilutes the HBoV titre in the infected tissue or the tissue mucus and thus was not performed. In addition, head-to-tail PCRs were positive for both BALs, indicating the presence of cccDNA in both patients.

2.3. Other similar cases in the literature

To our surprise, although bocavirus has been shown to persist, there is no information available in the literature if HBoV could be associated with chronic inflammatory diseases or that it could contribute to chronic diseases. We assume that the respective research is either ongoing or neglected. A reason for this may be that as it was postulated by some researchers that HBoV is an innocent bystander rather than a serious pathogen.

3. Discussion

This is the first report of UIP patients, in whom HBoV was diagnosed by PCR by the use of a BAL. In the two patients UIP was diagnosed according to established recommendations [9]. Recently, acute HBoV infection has been shown to potentially lead to a persistent infection [5–7]. Thereby, HBoV was shown by us and others to persist as cccDNA [6,12,13]. A cccDNA is also known as the persisting genome of the human hepatitis virus infections in which the chronic persistence may lead to fibrosis and cirrhosis after years in which the infections was clinically silent [14,15]. In addition, several studies have addressed the possible role of chronic viral
infection in the aetiology of idiopathic pulmonary fibrosis with most research having focused on Epstein–Barr virus [9]. Based on these observations and on the present findings the current authors would raise the possibility that HBoV playing a significant role in the aetiology of UIP.

In addition in one case, in which autopsy was feasible, HBoV was detected in the ventricular myocard. Although the role of this observation still needs to be elucidated, and although it remains unclear when the primary HBoV occurred in the reported cases and how it contributed to the onset of fibrosis, the current report has identified HBoV as a potential agent causing UIP and possibly even heart disease. This clearly warrants further systematic studies on HBoV an UIP, which should include systematic testing for the shedding period of the virus [16]. Although we are aware of the bias of our case reports that a corresponding serology was missing we are convinced that the presented cases are important to trigger further clinical studies that take HBoV into account as a pathogen that may be responsible for the development of fibrotic diseases of the lung.

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Competing interests

None of the authors has any competing interest relevant to this report.

Ethical approval

All procedures were performed in congruence to the declaration of Helsinki and according to a vote from the Ethical Committee of the Private University of Witten-Herdecke (vote no. 73/2012).

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