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Case report

Acute human bocavirus infection in MDS patient, Cologne, Germany



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ARTICLE INFO

Article history:

Received 10 April 2015

Received in revised form 22 May 2015

Accepted 30 May 2015

Keywords:

Human bocavirus infection in adult

Myelodysplastic syndrome

Pneumonia

Why is this case important?

The human bocavirus (HBoV) was discovered in 2005 in respiratory secretions of children suffering from clinical symptoms of viral respiratory infections [1]. Since then the virus has been clinically associated with respiratory infections worldwide and cannot be distinguished from other respiratory viruses on clinical observations alone. The situation becomes more complicated as the virus is frequently detected as one of two or more pathogens and thus it remains unclear if a distinct clinical course is indeed caused by HBoV or whether HBoV is only a bystander [2–4]. This latter statement must in full consequence lead to the conclusion that also other viruses that are detected in concert with other pathogens are only blind passengers rather than true pathogens, but for HBoV the modified Koch's postulates have not been formally fulfilled due to the lack of an animal model [2–4]. The co-detections originate on the one hand from the fact that modern diagnostics more and more rely on rapid multiplex assays which detect more pathogens in parallel than ever before. Thus the more questions are being asked, the more answers are produced by a single assay, and in earlier times the multiple infections have simply not been detected due to technical limitations [5–8]. Second, we and others have previously shown that HBoV is likely to persist and therefore could be

shed even without being the leading pathogen in an acute infection setting [8–12].

The virus is mainly detected in children, adult cases have been described occasionally [13–17], although the virus is frequently detected in the BAL of adult patients suffering from serious clinical respiratory symptoms [14–15,17–18]. However, life threatening infections are rare and appear to depend on the patient's underlying disease status. Here we describe a clinical case of severe HBoV pneumonia in an immunocompromised female patient suffering from an advanced stage of myelodysplastic syndrome (MDS). This is in contrast to our earlier study in which we have shown that MDS is not associated with HBoV infections [19].

2. Case description

We describe the case of an immunocompromised 74 year old Caucasian woman suffering from acute pneumonia and severe acute respiratory distress syndrome (ARDS) according to the Berlin classification [20] with previously diagnosed MDS. On admission to our hospital, the haematological parameters were haemoglobin level 7.4 g/dl, leukocytes 3.7 G/l, thrombocytes 65 G/l; smear analyses revealed 0% pro-erythroblasts, 0% erythroblasts, 1% normoblasts, 0% myeloblasts, 0% pro-myelocytes, 0% metamyelocytes, 17% stab cells, 1% eosinophilic cells, 1% basophilic cells, 14% monocytes, 26% lymphocytes, 0% plasma cells, and 41% segmented neutrophils. The erythropoiesis was accompanied by rouleaux formation, dysplastic erythrocytes, and poikilocytosis, the granulopoiesis was maturing with signs of dysplasia. The blood analyses match with a lack of vitamin B12 and the underlying MDS, while

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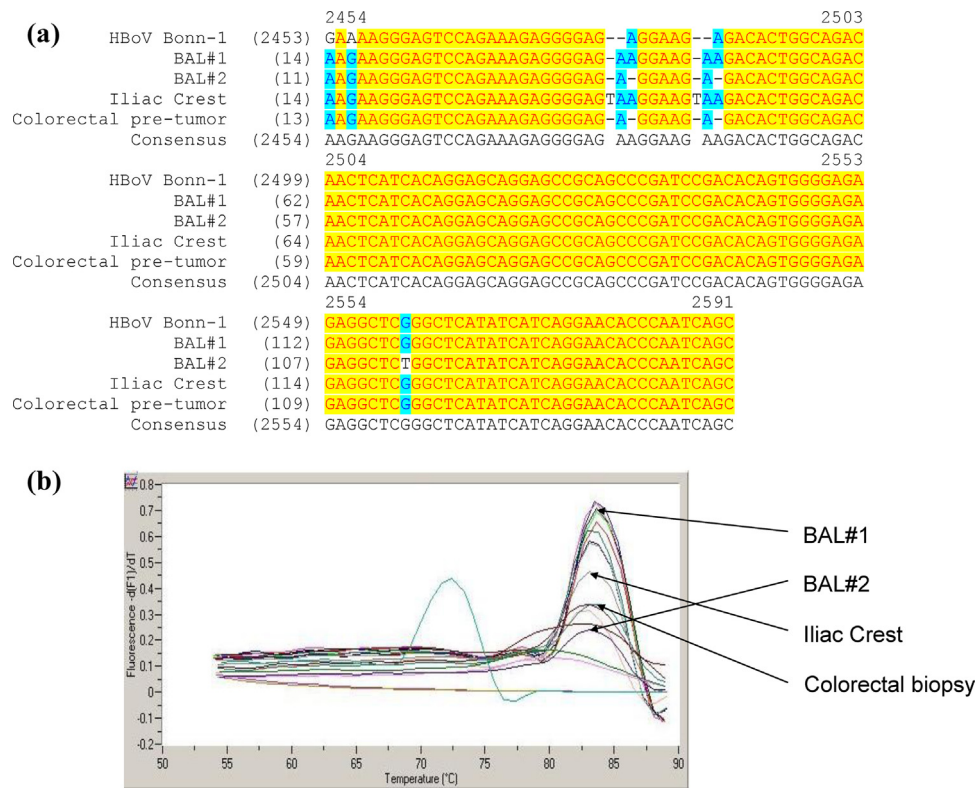


Fig. 1. (a) The figure shows an alignment of the qPCR product sequences compared to the German prototype HBoV-1 strain Bonn-1 [29]. A partial sequence of the NP1 gene was used for the analyses. (b) Screenshot of the qPCR melting curve in which the iliac crest sample, the BALs and the colorectal sample were tested. Melting peaks were nearly identical; the similarity of the strains was confirmed by sequencing (Fig. 1a).

the also observed reactive monocytosis could have matched to a viral infection.

On admission to the hospital the patient in addition had radiological signs of pneumonia, developed an ARDS and required mechanical ventilation. Further a mild pleural effusion evolved later in the clinical course.

Laboratory testing included routine bacteriological screening for respiratory pathogen by culturing. On laboratory investigations no further facultative or obligate respiratory pathogens (including viruses, fungi, and bacteria) were detected by Respifinder Smart 22 and Meningofinder Custom Assays (Pathofinder, Maastricht, The Netherlands), *P. jirovecii* PCR, nor by conventional microbiological screening methods. In detail, the patient's bronchoalveolar lavage was negative for influenza viruses, parainfluenzaviruses 1–4, RSV, HMPV, coronaviruses NL63, OC43, 229E, and HKU-1, adenoviruses, *Mycoplasma pneumoniae*, mumps, measles, humans herpesviruses 1–8, parechoviruses, rhinoviruses and enteroviruses, *Legionella pneumoniae*, *Chlamydia pneumoniae*, *Pneumocystis jirovecii*, *Aspergillus*, and *Bordetella pertussis* by molecular assays and also negative by culturing. *Mycobacteria* tested negative by the MYCO-Direct 1.7 assay (Chipron, Berlin, Germany). The Respifinder assay as well as the Meningofinder assay were previously described as suitable and sensitive tools by our group and others [21–25]. Routine culturing and Gram stainings were performed by our (outsourced) microbiology laboratory with negative results.

In serial bronchoalveolar lavages human bocavirus and herpes simplex virus DNA were repeatedly detected by the Respifinder Smart 22 Assay (Pathofinder, Maastricht, The Netherlands), and the Meningofinder Assay (modified RUO version including mumps and measles, Pathofinder, Maastricht, The Netherlands), respectively. The corresponding serum tested negative for both viruses, which normally would exclude an active general HBoV replication. Sur-

prisingly, HBoV was detected in a corresponding iliac crest biopsy, as well as in an archived rectal tubular adenoma that was surgically removed 4 years ago. The sequencing of the PCR products of the qPCR and the melting curve analyses showed only minor differences in the HBoV DNA detected in the BALs, the iliac crest, and the colorectal sample, supporting the assumption that the same subtype was present in the entire patient history, which in turn supports the hypothesis that the virus persisted in the patient (Fig. 1a and b).

The viral load in the biopsy samples was low compared to the active viremia of acute infection in which 10^6 and more genome copies can be observed per ml blood; in the serial BALs, the viral load was 3.5 copies per ml and 2.99 copies per ml BAL fluid, while in the iliac crest biopsy 7.4 copies per 0.3 mm^3 tissue sample and in the gut sample 3.3 copies per 0.3 mm^3 were detected.

Both, the detection of HBoV in the iliac crest biopsy and the detection of HBoV in a non-malignant tumour precursor are novel findings in context of the HBoV infection and complement the model of the HBoV life cycle. Furthermore, based on this case and earlier observations, it appears possible that herpes viruses trigger a local and/or focal replication of the virus in lung tissues, which could be a reactivation of the virus that in turn was encouraged by MDS-related immunosuppression.

Because of pancytopenia of unknown cause a iliac crest biopsy was performed and bone marrow examination revealed a myelodysplastic syndrome. In the first 34 days of treatment no bacteria or fungi could be detected in blood, lungs or urine. After that time different *Candida* species were found in urine and BAL.

The patient developed multi-organ-failure including acute kidney injury and secondary sclerosing cholangitis. On day38 of their treatment she died after her family had asked for a palliative therapy goal.

3. Other similar and contrasting cases in the literature

Hitherto, there is only limited information about the adult HBoV infection available. Earlier, our group described the clinical case of a young adult cancer patient suffering from a severe HBoV pneumonia [26]. Also in this earlier patient a severe pneumonia was observed, which is in line with the current observations, and haematological malignancies with immunosuppression appear to be a serious risk factor for HBoV pneumonia. We have also described two cases in which in parallel to HBoV a human herpesvirus was detected, which led us to the hypothesis that HBoV replication can be triggered or influenced by helperviruses of the herpesvirus family [27–28]. HBoV detections in the BAL of adult patients are frequent [21] but in none of the previous cases the virus was found in the iliac crest. In an earlier study we have analysed the DNA extracted from bone marrow of 20 MDS patients and none of those samples was positive for HBoV DNA. However, the most surprising fact is that in the present case an archived tissue sample, i.e. an FFPE biopsy of a rectal adenoma, was available, that was tested positive for HBoV. This on the one hand confirms our earlier assumptions that the virus may be able to trigger tumour development or could replicate in the growing (micro-) environment of the tumour or its precursors.

4. Discussion and references

The presented clinical case adds two important and novel pieces of knowledge to the puzzle of HBoV infections. First, it shows that the virus can occur in iliac crest of MDS patients suffering from HBoV associated pneumonia, an observation that to the best of our knowledge was not yet described. It remains, however, to be investigated if HBoV displays any effects on this tissue; earlier observations of our group in contrast have shown that the virus is not generally present in the bone marrow of MDS patients, thus this detection could be a hint for active replication although a viremia was absent. Anyway, it has to be noted that in this earlier study none of the MDS patients suffered from an acute pneumonia as the current patient.

Second, we detected HBoV DNA in a colorectal sample taken from a benign adenoma four years ago, giving rise to the assumption that the virus may have persisted for this time span and confirming our previous study that HBoV is associated with lung- and colorectal tumours [12]. Due to our restrictive laboratory procedures we can exclude any cross-contamination of the samples with HBoV DNA, and all negative controls and also further patients' samples that were routinely screened in parallel for HBoV DNA were negative. However, the copy numbers in the tissue and the BAL appear low, on the one hand putatively indicating the persistence of the viral DNA in the tissues, on the other hand being accounted to the fact that HBoV replicates in foci [29] and those replikative foci have not been explicitly isolated from the tissue samples nor were exclusively covered by the BAL sampling, which cannot differentiate between healthy tissue and replicative foci.

Moreover, the repeated co-detection of a herpes virus in the HBoV positive BAL gives rise to the hypothesis that herpesviruses could trigger the local, organ-specific HBoV replication or even reactivation without inducing a viremia that is a typical sign of the active primary HBoV infection.

Conflict of interest

None.

Ethical approval

Publication of this case report is in accordance with a vote from the local ethical committee of the university of Witten/Herdecke (votes 73/2012 and 75/2013).

Acknowledgement

This report was partially supported by a grant from the Else Kröner-Fresenius Stiftung, Germany.

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