Case Report

HBoV-1 in pleura of an adult patient in Cologne, Germany

Jessica Lüsebrink,¹ Xuemeng Li,^{2,3} Maria Söderlund-Venermo,² Klaus Hedman,² Michael Brockmann,¹ Soumaya Khalfaoui,¹ Verena Schildgen,¹ Oliver Schildgen¹ and Wolfram Windisch⁴

Correspondence

Oliver Schildgen
schildgeno@kliniken-koeln.de

¹Kliniken der Stadt Köln, Klinikum der Privaten Universität Witten/Herdecke, Institut für Pathologie, Germany

²Haartman Institute, Helsinki University, Department of Virology and Helsinki University Central Hospital Laboratory Division, Helsinki, Finland

³College of Veterinary Medicine, Northeast Agricultural University, Harbin, PR China

⁴Kliniken der Stadt Köln, Klinikum der Privaten Universität Witten/Herdecke, Lungenklinik, Germany

Introduction: The human bocavirus is associated with respiratory tract infections and has been shown to infect virtually all age groups. However, the majority of HBoV infections is observed in children, on the one hand because this group of patients receives a broader diagnostics, on the other hand because up to the age of five years all people have passed the primary infection and developed antibodies. Thus, there is limited knowledge about the HBoV infection in adults.

Case presentation: A case of persistent pleural effusion associated with an infection with human bocavirus (HBoV) is reported. To the best of our knowledge, this is the first case in which HBoV has been found in the pleura during an active infection in an adult patient.

Conclusion: HBoV infection can lead to serious disease in adults and can affect also the pleura.

Keywords: adult patient; HBoV; pleura; respiratory infection.

Received 8 July 2014

Accepted 1 September 2014

Introduction

In 2005, human bocavirus type 1 (HboV1) was identified for the first time by Allander *et al.* (2005). HBoV1 has since been discovered in patients suffering from respiratory infections and gastrointestinal diseases (Jartti *et al.*, 2012; Kantola *et al.*, 2011).

HBoV1 infections are frequently accompanied by co-pathogens. The rate of HBoV1 coinfections has been found to be significantly higher than that of other viruses, and it was postulated that HBoV1 is only a passenger and not a pathogen in airway infections, although it causes productive infections with viral shedding, viraemia and putative persistence (Huang *et al.*, 2012; Jartti *et al.*, 2012; Kapoor *et al.*, 2011). In 2009, Dijkman *et al.* (2009) reported the first successful approach towards cultivating HBoV1 and it was classified as an autonomous parvovirus. Nevertheless, the possibility that HBoV1 infections depend on helper viruses still remains. For example, the replication of another representative group of the family *Parvoviridae*, the dependoviruses, requires the help of another virus,

mostly herpesvirus or adenovirus (Geoffroy & Salvetti, 2005).

Case report

The patient was a 38-year-old female in a good general state of health and in an obese nutritional condition (body mass index of 34 kg m⁻²). The patient was a smoker with 20 pack years, and no immunosuppression was reported or detected.

Since January 2013, the patient had complained of a dry cough and a decrease in stamina. In February 2013, the patient was treated for a right-sided pneumonia externally, but we did not receive information about the aetiology of this earlier event. Thus, we were informed that the patient presented with right-sided pneumonia of the right upper lobe. Pulmonary embolism was excluded this time by a contrast computed tomography (CT) scan. The patient received penicillin with the addition of penicillinase inhibitors plus macrolides (clarithromycin). However, the patient was not treated in our hospital at that time. Therefore, other investigations aimed at defining the aetiology of the pneumonia were not performed. In particular, bronchoscopy was not performed, and there

Abbreviations: CT, computed tomography; EBV, Epstein-Barr virus; HBoV, human bocavirus; VLP, heterologous virus-like particle.

was also no effort to establish microbiological results. A CT scan of the thorax revealed a slight pleural effusion on the right side of the lung. A second CT scan in October due to persistent discomfort after cholecystectomy revealed a distended pleural effusion. Externally, cholecystectomy was performed in July 2013 due to cholecystitis based on cholecystolithiasis. There were reportedly no complications related to surgery, and the pleural effusion still existed several months after surgery. Therefore, any pathology of the gall bladder did not seem to be responsible for the long-lasting pleural effusion.

Pleural effusion was not sparse but was not significant enough to impact negatively on respiratory function. Therefore, repeating punctures, drainages or even pleurodesis were not necessary. The patient also did not receive diuretics. Therefore, primarily diagnostic procedures were performed, while treatment of the pleural effusion itself was not performed, despite the fact that moxifloxacin was probatorily given in February 2014 for a duration of 10 days (400 mg day^{-1}), even though there were again no established microbiological results.

In November 2013, the patient was sent to our clinic for further clarification of the persistent pleural effusion. Pathological and anatomical analysis of the pleuramesothelium revealed a fibrin-rich, chronic pleurisy. There was no sign of a tuberculous pleurisy, and mycobacterial PCR testing was negative. Fresh and formalin-fixed paraffin-embedded pleural tissue and bronchoalveolar lavage samples tested negative for influenza viruses A and B, respiratory syncytial virus, coronavirus (NL63, OC43, 229E, and HKU1), parainfluenzaviruses 1–4, human metapneumovirus, enterovirus, rhinovirus, adenovirus, *Bordetella pertussis*, *Chlamydia pneumoniae*, *Legionella pneumophila*, *Mycoplasma pneumoniae*, herpes simplex viruses 1 and 2, cytomegalovirus, parechovirus and varicella-zoster virus by RespiFinder SMART 22 Fast and MeningoFinder SMART 7 (both from PathoFinder). However, the tested tissue samples did contain Epstein–Barr virus (EBV) and HBoV1 DNA; HBoV1 but not EBV DNA was also found repeatedly in the serum, indicating an ongoing HBoV1 infection. In order to confirm the RespiFinder results, an end-point PCR was performed, followed by sequencing, confirming the detection of HBoV1 (Simon *et al.*, 2007). Neither pleura nor serum was positive for HBoV2, -3 or -4 DNA (Kantola *et al.*, 2010).

The serum was further investigated for HBoV1–4, EBV and parvovirus B19 antibodies, using HBoV enzyme immunoassays (EIAs) as described previously (Kantola *et al.*, 2011), and two other EIAs at the Helsinki University Central Hospital Laboratory. HBoV1–4 IgM was not detected. IgG reactivities for all four HBoVs were observed in a standard (non-blocking) EIA. After blocking with heterologous virus-like particles (VLPs), HBoV1 and -4 IgGs were absent. HBoV2 and -3 IgGs were detectable but only when HBoV1 VLPs alone were used as competitor. If HBoV1 and -3 VLPs were used together as competitors, the HBoV2 IgG EIA was negative. The same result was

observed for the HBoV3 IgG EIA when HBoV1 and -2 VLPs were used as competitors (Table 1). In addition to the HBoV serology, the serum was screened for EBV and parvovirus B19 antibodies. EBV IgG could be detected, but B19 IgG could not. IgM for EBV and B19 was, similarly to HBoV1 IgM, absent, not pointing to acute primary infections. Moreover, on cytological investigation, no signs for non-infectious lung disease were observed in the bronchoalveolar lavage, nor were there any hints of an immune disorder identified by analyses of the circulating immune cells in the peripheral blood.

Discussion

HBoV1 has frequently been associated with upper and lower respiratory diseases. It is detectable mainly in children aged 6–24 months and less frequently in other age groups including adults. The case reported here was interesting, as pleural effusion associated with HBoV1 infection has only rarely been described in children and never in adults. The pleural effusion may have been a result of the HBoV infection or a synergistic interaction between EBV and HBoV, such as HBoV reactivating latent EBV.

EBV infects >90 % of the population worldwide. Like other herpesviruses, it establishes a lifelong latent infection (Maeda *et al.*, 2009). EBV may play a role in the pathogenesis of pleural effusion (Thijssen *et al.*, 2005). In one study, EBV was detected in 59 % of the pleural fluids of patients with unexplained pleural effusions, with 93 % positivity in the serum as well. As EBV reactivation in pleural fluid is a frequent event, the absence of an alternative diagnosis may point to an involvement of EBV in the development of pleural effusion. In our patient, EBV DNA was detected in the pleural tissue along with HBoV1 but not in the serum. Serological investigations revealed that there was no primary EBV infection at this time, although a possible transient viral infection prior to the signs of pleural symptoms could not be ruled out (Thijssen *et al.*, 2005). Parvovirus B19 has been associated with pleural effusion, but in our patient no B19 virus was detected in the pleural tissue or serum and she was B19V seronegative, ruling out this aetiology (Castagna *et al.*, 2011; Seishima *et al.*, 2010).

HBoV1 was the only other pathogen detected in the samples from the patient, in both the pleural biopsy and the serum. The occurrence of HBoV1 in serum, regardless of the low load, indicates an active infection, or could be residual virus circulating after a primary infection. No HBoV IgM against any of the four known HBoV strains could be detected in serum. This could, however, be due to the length of time (several months) between the onset of symptoms and blood sampling. Interestingly, taking into account the prolonged clinical course, no HBoV1-specific IgG antibodies were detectable, although they should have had time to develop. It may be that the patient was infected by HBoV2 and/or -3 in the past and by HBoV1 more

Table 1. Overview of the HBoV serology testing

Detection antigen	Blocking antigen	Absorbance (492 nm)
HBoV1	–	++
	HBoV1	–
	HBoV2	–
	HBoV2 and -3	–
HBoV2	–	+++
	HBoV2	–
	HBoV1	+
	HBoV1 and -3	–
HBoV3	–	+++
	HBoV3	–
	HBoV1	+
	HBoV1 and -2	–
HBoV4	–	++
	HBoV4	–
	HBoV2	+
	HBoV1, -2 and -3	–

recently – perhaps too recently for the antibodies to develop. In this case, the virus would not be the cause of the persistent pleural signs, although viral DNA was detected in pleural tissue. However, due to the phenomenon of ‘original antigenic sin’, an immune response against the current HBoV1 may not have emerged due to an earlier infection by the cross-reactive HBoV2 or -3 (Francis, 1960). Instead a secondary immune response against the priming HBoV2 or -3 virus would occur. No or only weak antibody reactivity specific to the second infecting virus would be produced, perhaps leading to a more severe course of infection. This phenomenon has been described for infections with dengue virus, influenza virus and human immunodeficiency virus (Kim *et al.*, 2009; Muller, 2004; Zompi & Harris, 2013), and has also been implied for human bocaviruses.

Acknowledgement

This work was supported by grants from the Private University of Witten/Herdecke (no. 11930 Grossprojekt Schildgen) and the Else Kröner-Fresenius Stiftung, Germany. All procedures were carried out in accordance with a vote from the Ethical Committee of the Private University of Witten/Herdecke (vote no. 75/2013). The funders did not have any financial interest in the study. None of the authors has any conflict of interest related to this manuscript.

References

Allander, T., Tammi, M. T., Eriksson, M., Bjerkner, A., Tiveljung-Lindell, A. & Andersson, B. (2005). Cloning of a human parvovirus

by molecular screening of respiratory tract samples. *Proc Natl Acad Sci U S A* **102**, 12891–12896.

Castagna, L., Furst, S., El Cheikh, J., Faucher, C. & Blaise, D. (2011). Parvovirus B19 as an etiological agent of acute pleuro-pericarditis. *Bone Marrow Transplant* **46**, 317–318.

Dijkman, R., Koekkoek, S. M., Molenkamp, R., Schildgen, O. & van der Hoek, L. (2009). Human bocavirus can be cultured in differentiated human airway epithelial cells. *J Virol* **83**, 7739–7748.

Francis, T. J. (1960). On the doctrine of original antigenic sin. *Proc Am Philos Soc* **104**, 572–578.

Geoffroy, M. C. & Salvetti, A. (2005). Helper functions required for wild type and recombinant adeno-associated virus growth. *Curr Gene Ther* **5**, 265–271.

Huang, Q., Deng, X., Yan, Z., Cheng, F., Luo, Y., Shen, W., Lei-Butters, D. C., Chen, A. Y., Li, Y. & other authors (2012). Establishment of a reverse genetics system for studying human bocavirus in human airway epithelia. *PLoS Pathog* **8**, e1002899.

Jartti, T., Hedman, K., Jartti, L., Ruuskanen, O., Allander, T. & Soderlund-Venermo, M. (2012). Human bocavirus – the first 5 years. *Rev Med Virol* **22**, 46–64.

Kantola, K., Sadeghi, M., Antikainen, J., Kirveskari, J., Delwart, E., Hedman, K. & Soderlund-Venermo, M. (2010). Real-time quantitative PCR detection of four human bocaviruses. *J Clin Microbiol* **48**, 4044–4050.

Kantola, K., Hedman, L., Arthur, J., Alibeto, A., Delwart, E., Jartti, T., Ruuskanen, O., Hedman, K. & Soderlund-Venermo, M. (2011). Seroepidemiology of human bocaviruses 1–4. *J Infect Dis* **204**, 1403–1412.

Kapoor, A., Hornig, M., Asokan, A., Williams, B., Henriquez, J. A. & Lipkin, W. I. (2011). Bocavirus episome in infected human tissue contains non-identical termini. *PLoS One* **6**, e21362.

Kim, J. H., Skountzou, I., Compans, R. & Jacob, J. (2009). Original antigenic sin responses to influenza viruses. *J Immunol* **183**, 3294–3301.

Maeda, E., Akahane, M., Kiryu, S., Kato, N., Yoshikawa, T., Hayashi, N., Aoki, S., Minami, M., Uozaki, H. & other authors (2009). Spectrum of Epstein–Barr virus-related diseases: a pictorial review. *Jpn J Radiol* **27**, 4–19.

Muller, S. (2004). Avoiding deceptive imprinting of the immune response to HIV-1 infection in vaccine development. *Int Rev Immunol* **23**, 423–436.

Seishima, M., Shibuya, Y., Watanabe, K. & Kato, G. (2010). Pericarditis and pleuritis associated with human parvovirus B19 infection in a systemic lupus erythematosus patient. *Mod Rheumatol* **20**, 617–620.

Simon, A., Groneck, P., Kupfer, B., Kaiser, R., Plum, G., Tillmann, R. L., Muller, A. & Schildgen, O. (2007). Detection of bocavirus DNA in nasopharyngeal aspirates of a child with bronchiolitis. *J Infect* **54**, e125–e127.

Thijsen, S. F., Luderer, R., van Gorp, J. M., Oudejans, S. J. & Bossink, A. W. (2005). A possible role for Epstein–Barr virus in the pathogenesis of pleural effusion. *Eur Respir J* **26**, 662–666.

Zompi, S. & Harris, E. (2013). Original antigenic sin in dengue revisited. *Proc Natl Acad Sci U S A* **110**, 8761–8762.