Steps towards Serological Diagnosis of Human Bocavirus Infections

Peter Simmonds

Centre for Infectious Diseases, University of Edinburgh, Summerhall, Edinburgh, United Kingdom

(See the article by Kantola et al. on pages 540–6)

The study by Kantola et al. [1] in the current issue of *Clinical Infectious Diseases* represents an important advance in the development of diagnostic assays for the newly discovered, potentially major respiratory pathogen human bocavirus (HBoV). It also provides a series of new insights into the nature and epidemiology of HBoV infection in children, its systemic spread, and the relationship between HBoV detected in respiratory samples and blood with antibody responses to infection. Although not a "state of the art" serological test, the Western blot assay developed for IgG and IgM antibodies elicited by HBoV infection provides the important first steps towards the establishment of serological diagnosis of HBoV infection.

HBoV is one of several new viruses that have been identified and characterized as a result of virus-discovery programs based on molecular cloning. These techniques are increasingly successful in the identification and genetic characterization of a wide range of novel viruses infecting humans and animals. HBoV is a distinct, new member of the virus family *Parvoviridae* and is distantly related to 2 other animal parvoviruses: bovine parvovirus 1 and minute virus of canines. Over the past 18 months, there has been an explosion of interest in and new data on the potential disease associations of HBoV, concentrating particularly on its involvement in pediatric respiratory disease. Despite the numerous difficulties with referral bias intrinsic to samples referred to routine clinical virology laboratories, the frequent lack of comprehensive screening of samples for other respiratory pathogens, and the unexpected occurrence of frequent coinfections with other viruses in patients with HBoV, there is a growing consensus that HBoV infections in children frequently lead to severe lower respiratory infections [2, 3]. Indeed, in most studies, HBoV is second only to respiratory syncytial virus in frequency and severity of disease in infants and young children. Although most initial studies concentrated on the involvement of HBoV in respiratory disease, it has also become apparent that infections are systemic and may be associated with other pathologies, arising, for example, from infection of the gastrointestinal tract [4, 5].

The study by Kantola et al. [1] builds on a previously published investigation of HBoV DNA detection in respiratory secretions of children hospitalized in Turku, Finland, with acute respiratory disease and the occurrence of viremia contemporaneous to primary infection [6]. The study accessed a valuable archive of respiratory samples (nasopharyngeal aspirates [NPAs]) from this study group that had been exhaustively examined for the presence of other viruses potentially associated with the presenting disease [7]. The inclusion of rhinoviruses, enteroviruses, and newly discovered coronavirus groups in the total of 16 viruses examined by PCR and serological testing makes this archive one of the best characterized sample collections available for etiological studies of new viral pathogens. Allander et al. [5] had established previously that detection of HBoV DNA sequences in respiratory samples coincided with an acute, resolving viremia, indicating the systemic nature of primary infections. Viremia was specifically associated with high viral loads in respiratory samples (>10,000 copies/mL) and was relatively infrequent in patients who had viral loads below this threshold. The frequent codetection of other respiratory viruses in the latter group led the authors to suggest possible long-term persistence in the respiratory tract after clinical recovery, perhaps similar to that associated with the other human parvovirus, B19.

In the current study, a serological test for detection of antibodies to HBoV was developed that used virus protein 1 and

Received 30 October 2007; accepted 31 October 2007; electronically published 16 January 2008.

Reprints or correspondence: Dr. Peter Simmonds, Centre for Infectious Diseases, University of Edinburgh, Summerhall, Edinburgh, EH9 1QH, United Kingdom (Peter.Simmonds@ed.ac.uk).

Clinical Infectious Diseases 2008; 46:547–9

1058-4838/2008/4604-0010$15.00
DOI: 10.1086/526533

virus protein 2 recombinant proteins expressed from cloned structural gene sequences of HBoV as antigens. Although denatured antigens and a Western blot assay format are not likely to be used in the final design of HBoV serological assays, the findings from their use are, nevertheless, of considerable value in detecting serological responses to HBoV and its potential contribution to the diagnosis of HBoV infection. The assay was used to detect anti-HBoV IgM and IgG responses among the 49 HBoV-infected study subjects identified in the previous study, as well as in a selection of 68 control subjects in whom HBoV DNA sequences were not detected in respiratory samples. By combining the serological testing results with PCR detection of HBoV DNA in NPA and plasma samples, a rather broader picture of the nature of primary HBoV infection emerged. With a few exceptions, increases in IgG titer to HBoV and/or IgM detection was found specifically in the group with high viral loads in NPA samples (>10,000 copies/mL), patients who were viremic, and patients for whom HBoV was frequently (67%) the only respiratory pathogen detected. Serological evidence for primary infection with HBoV was specifically absent in the low viral load, nonviremia group, which is consistent with previous hypotheses for low-level persistence after resolution of primary infection. However, not all of the patients in this group were IgG seropositive, as might have been initially expected; this may be an indication of the transient nature of serological responses to linear epitopes in the denatured antigen used in the Western blot assay, as previously described for B19 by the authors [8] and by other groups. Clearly, serological diagnosis may be considerably enhanced in sensitivity if nondenatured antigens containing conformational epitopes (such as whole capsids) are used.

As with any pioneering study, the findings provide more fascinating questions than answers. Particularly intriguing was the large proportion of study subjects with HBoV viremia and serological evidence for primary infection (IgM detection and/or IgG seroconversion) in the control group of patients whose respiratory samples were repeatedly HBoV-negative by PCR. From the data presented here, 12 (18%) of 68 HBoV infections in children with respiratory disease would have been undiagnosed by the standard PCR screening of respiratory samples (as used in all previously published investigations of HBoV prevalence and disease associations). Are these possibly nonrespiratory HBoV infections, as the authors speculate? Could their respiratory disease be accounted for by coinfection with other respiratory viruses? We were not given enough information on these cases [1] to answer such questions, but clearly the occurrence of an almost identical frequency of HBoV infection among children with HBoV-negative NPAs as among the cohort as a whole (19%) makes this a highly significant group to investigate in the future [6].

Similarly unresolved is the nature of HBoV infections associated with low HBoV loads in NPA samples. More than three-quarters of these infections occurred in study subjects without detectable HBoV viremia or serological evidence of acute infection, in marked contrast to those subjects with HBoV loads >10,000 copies/mL. Although these cases may represent infections persisting beyond the period of acute viremia and IgM reactivity and occurring in patients who had become seronegative because of the insensitivity of the Western blot assay used (see above), the authors discuss the alternative possibility that some HBoV infections may be superficial (i.e., nonsystemic) and may perhaps fail to elicit a detectable serological response; such infections might perhaps also not be the cause of respiratory disease, given the almost universal occurrence of coinfection with other viruses in this group. Whatever the final explanation, the findings support previous conclusions that measurement of viral load is essential when interpreting results from PCR-based HBoV testing of respiratory samples [6].

The development of serological assays provides the opportunity, in the future, to perform population-based studies of overall frequencies of HBoV exposure in different age ranges and to determine the true incidence of HBoV infection in childhood. However, rather than showing a cumulative increase in serological reactivity to HBoV with age, seroprevalence was actually higher in the age range 1–2 years (52%), and it subsequently decreased (to 28% among children >3 years of age). This may be further evidence that the antibody response detectable in the Western blot assay was not durable (because of the use of denatured proteins as antigens). However, a further contributory factor might be differences between HBoV and other parvoviruses, such as B19, in their ability to persist systemically. In the latter case, the maintenance of high levels of IgG reactivity and strong cytotoxic T cell reactivity [9] likely result from the lifelong systemic presence of virus, with low-level persistent replication in tissues despite the rapid, permanent clearance of viremia after acute infection. In contrast, available evidence indicates that HBoV does not persist in the same way, being universally undetectable in those autopsy tissues (lymph node, bone marrow, and lung) in which B19 DNA sequences can be reliably detected [10].

Where do these findings leave future investigations of the role of HBoV in respiratory disease and the development and introduction of HBoV diagnostic testing? Should viral load measurement become routine? Clearly, HBoV infecting the subset of individuals with high viral loads showed a much more apparent etiological link to respiratory disease; previously published investigations of HBoV detection in respiratory disease might, indeed, be usefully reinterpreted and clarified, should viral load data become available on these other study populations. Should serolog-

ical testing also be used to detect HBoV infection in study subjects, in addition to or instead of quantitative PCR? IgM detection to diagnose acute B19 infection is routine and might similarly become the standard test for HBoV infection. Unfortunately, we do not know enough about the time course of respiratory disease and seroconversion to address this issue at present. The finding that 4 of the 28 individuals with high viral loads in NPAs (all of whom were, with 1 exception, viremic) showed no detectable serological response to HBoV infection suggests that respiratory disease may present clinically before the appearance of antibody responses in a significant proportion of individuals. Serological testing and/or viremia testing seems to be required, however, to detect the extraordinarily large number of individuals with acute HBoV infection but repeatedly HBoV-negative respiratory samples by PCR.

Clearly, there are uncertainties with respect to the interpretation of current study data on HBoV infection, the nature of the acute infection process, and the underlying reasons for the frequent association of HBoV with coinfection due to other respiratory viruses. It is similarly unclear at present what path diagnostic methods for this newly discovered parvovirus will take in the future. However, we should not be overly critical of the currently available data on HBoV. There is little or no data on the occurrence and timing of serological responses and viremia during acute (or long-term) infections for other respiratory viruses that are now routinely diagnosed by PCR testing of NPAs. Diagnostic assays, as currently implemented, have rarely, if ever, been evaluated critically for their ability to reliably detect acute (or recurrent) respiratory virus infections. Particularly in the case of coinfections due to multiple viruses, there is also little or no critical assessment of the relevance of the detection of each detected virus to the respiratory disease in the patient.

The investigative framework adopted in the study by Kantola et al. [1] and the availability of a well-characterized archive of intensively tested respiratory samples and associated clinical information on the study subjects should be applied much further in the future development of diagnostic screening. This approach would not only address gaps in our understanding of currently known respiratory pathogens but would also provide the resources to deal with further newly discovered viruses, such as the WU and KI polyomaviruses [11, 12], that also have proposed etiological roles in respiratory disease.

Acknowledgments

Potential conflicts of interest. P.S.: no conflicts.

References

1. Kantola K, Hedman L, Allander T, et al. Serodiagnosis of human bocavirus infection. Clin Infect Dis **2008**; 46:540–6 (in this issue).
2. MacIntosh K. Human bocavirus: developing evidence for pathogenicity. J Infect Dis **2006**; 194:1197–9.
3. Anderson LJ. Human bocavirus: a new viral pathogen. Clin Infect Dis **2007**; 44:911–2.
4. Vicente D, Cilla G, Montes M, Perez-Yarza EG, Perez-Trallero E. Human bocavirus, a respiratory and enteric virus. Emerg Infect Dis **2007**; 13:636–7.
5. Mackay IM. Human bocavirus: multisystem detection raises questions about infection. J Infect Dis **2007**; 196:968–70.
6. Allander T, Jartti T, Gupta S, et al. Human bocavirus and acute wheezing in children. Clin Infect Dis **2007**; 44:904–10.
7. Jartti T, Lehtinen P, Vuorinen T, et al. Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children. Emerg Infect Dis **2004**; 10:1095–101.
8. Kaikkonen L, Lankinen H, Harjunpaa I, et al. Acute-phase–specific heptapeptide epitope for diagnosis of parvovirus B19 infection. J Clin Microbiol **1999**; 37:3952–6.
9. Isa A, Kasprowicz V, Norbeck O, et al. Prolonged activation of virus-specific $CD8^+$ T cells after acute B19 infection. PLoS Med **2005**; 2: e343.
10. Manning A, Willey SJ, Bell JE, Simmonds P. Comparison of tissue distribution, persistence, and molecular epidemiology of parvovirus B19 and novel human parvoviruses PARV4 and human bocavirus. J Infect Dis **2007**; 195: 1345–552.
11. Gaynor AM, Nissen MD, Whiley DM, et al. Identification of a novel polyomavirus from patients with acute respiratory tract infections. PLoS Pathog **2007**; 3:e64.
12. Allander T, Andreasson K, Gupta S, et al. Identification of a third human polyomavirus. J Virol **2007**; 81:4130–6.