Pediatric Pulmonology 49:517–518 (2014)

Response to Letter

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Establishment of an etiological diagnosis in pediatric patients hospitalized with respiratory symptoms constitutes a clinical and laboratory challenge. Until recently, most lower respiratory tract infections of viral etiology were attributed to the respiratory syncytial virus, parainfluenza viruses, adenoviruses, or influenza viruses, all of which can be identified by immunological techniques in the patient care setting. However, in a substantial percentage of patients (40–60% of cases), there was no identifiable causative agent, due to the limited sensitivity of these techniques. In the last 10–15 years, advancements in molecular biology and genome amplification methods have not only increased the rates of identification of known viral pathogens but also led to the discovery of a series of new viruses implicated in the pathogenesis of respiratory illness (human metapneumovirus, coronavirus, human bocavirus, among others).[1,2]

Within this new scenario, genomic detection of the causative agent of respiratory infections has been adopted by many centers as a diagnostic tool, particularly with the advent of multiplex PCR techniques. In addition to excellent sensitivity and faster turnaround, these techniques enable simultaneous detection of several pathogens (particularly viral, but some atypical bacteria as well).[1,3]

Since then, human bocavirus has been detected worldwide and with increasing frequency in patients with acute respiratory symptoms, at prevalences ranging from 1.5% to 19%. However, these data may still not represent the actual rates of HBoV infection in the general population. One justification for this disconnect would be the possibility of subclinical infection with persistent or recurrent HBoV colonization in the nasopharynx. Detection of the agent in nasopharyngeal aspirates (NPA) may or may not reflect the actual occurrence of pulmonary infection.[4–6]

Prof. Matti Korppi makes some very interesting and current points. They are centered on these challenges, and reinforce the need for other techniques (in addition to PCR) to be used in NPA samples, even if quantitative, in an attempt to improve diagnostic certainty in the detection of HBoV infection. Seroepidemiologic studies of HBoV using a variety of techniques—immunofluorescence, Western blot, or ELISA, with anti-HBoV IgG and IgM antibodies—have been published, in an attempt to establish better clinical, virologic, and serologic correlations, but with no definitive results. We cannot disregard the fact that many challenges remain, and are compounded by the absence of an optimal gold standard. Thus far, we have no well-established cell culture system or animal model for the propagation of HBoV, which could help provide a greater understanding of this infection and of the pathogenicity of the agents involved.[3]

Data from the literature can also be conflicting, which precludes the formation of a single, evidence-based judgment on the matter. Deng et al. analyzed pulmonary secretions collected via bronchoscopy from 186 children hospitalized for lower respiratory tract infections. HBoV was detected by real-time PCR alongside 10 other viruses. Patients who tested positive for HBoV had their viral loads measured quantitatively. Overall, 31 patients (24.6%) were positive for HBoV in respiratory secretions. Of these, roughly half ($n = 15$) had high viral loads (10^4 copies/ml). HBoV was detected in isolation in 12 samples (38.7%), with no differences in clinical presentation (symptom frequency, length of hospital stay, and cytokine production). The duration of wheezing and number of inpatient hospital days were greater in patients with higher viral loads. Therefore, even though the authors did not collect NPAs, the possibility of multiple virus detection was not associated with any differences in viral load pattern or disease course in these patients.[5] Conversely, viral load had an impact on clinical manifestations.[6–8]

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Received 17 October 2013; Accepted 27 October 2013.

DOI 10.1002/ppul.22951
Published online 11 December 2013 in Wiley Online Library (wileyonlinelibrary.com).

As in any other investigation on this topic, the information presented in our study cannot be dissociated from the limitations that hinder a definitive diagnosis. Nevertheless, we believe that, in a clinical practice setting, characterization of the clinical manifestations of patients with HBoV infection as identified by multiplex real-time PCR is important, as this diagnostic tool is accessible to many centers and can identify HBoV genome material with high sensitivity. Furthermore, we would like to stress the clinical similarity between our sample and other published series, conducted in other centers using similar methods.[9]

It also seems clear to us that, despite the use of more accurate techniques, which are not always available in clinical practice, such as quantitative PCR and paired blood sample collection for serological testing, the diagnosis of HBoV infection cannot be established conclusively in all patients. NPA samples can be collected noninvasively. Tests of the increase of IgG antibodies in paired sera are based on the rise in antibody titers from the acute to the convalescent stages of illness, that is, must be obtained 2 weeks apart, and are thus of dubious diagnostic and clinic utility. Therefore, definitive evidence should be obtained when an in vitro culture system and an animal model are incorporated to the research setting to enable definitive elucidation of any unresolved issues.

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