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Review

Human bocaviruses: Possible etiologic role in respiratory infection

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ABSTRACT

Four species of human bocaviruses (HBoV) are currently included in the Bocavirus genus. There is satisfactory evidence demonstrating an association between HBoV1 and respiratory disease in children, and there is evidence that HBoV2 (and possibly the HBoV3 and HBoV4 species) are associated with gastroenteritis. In particular, HBoV1 has been associated with a prolonged period of persistence in the mucosa of the respiratory tract. Virus persistence does play a role in the high frequency of co-infections with proper pathogens of the upper and lower respiratory tracts. The high detection rate of multiple respiratory viruses in up to 83% of respiratory specimens and the presence of asymptomatic HBoV1 infections complicate the elucidation of the pathogenic role of the agent. Overall, a large amount of data are available concerning HBoV1, whereas little information is available about other bocavirus species. High viral loads are often associated with symptoms, and viremia may be associated with systemic manifestations such as encephalopathy. The effects and mechanisms of latency, persistence, reactivation, and reinfection are poorly understood. Thus, particularly in co-infections, the pathogenic contribution of the detected bocavirus species cannot be accurately stated. This review summarizes the current knowledge of HBoV species and provides perspectives for future clinical studies.

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1. Introduction

In 2005, Tobias Allander et al. [1] reported that by using a system of genomic virus screening, an unknown human parvovirus was discovered in the respiratory secretions of children with respiratory infection. The newly identified virus shared sequence similarities and genomic organization with the bovine parvovirus and the minute virus of canines [2], which are two members of the genus bocavirus in the Parvovirinae subfamily of Parvoviridae [1]. Thus, the new agent was named human bocavirus-1 (HBoV1). Three additional species were later characterized and named HBoV2–4 [3–5].

HBoVs have been identified in up to 5% of pooled human plasma [6], and they have been considered to be of possible concern in transfusion medicine and the blood products industry [7,8]. With increasing incidence, HBoV have been detected in febrile patients and intravenous drug users [9]. In August 2015, a PubMed search revealed 633 records for "human bocavirus" and 741 for "bocavirus." Similar agents have been detected in the stool and respiratory samples of children with acute conditions.

From the 5′ to the 3′ terminus, bocavirus genome contains three open reading frames (ORFs): the first and the second ORFs encode NS1 and NP1 (nonstructural proteins), and the third one encodes the capsid proteins VP1 and VP2. NS1 is essential to replicate single-stranded DNA genome and DNA packaging [10]. NP1 plays a role in nuclear localization [11], blocks interferon production by interacting with IRF-3 [12], and induces both cell arrest and apoptosis in HeLa cells [13]. The capsid proteins VP1 and VP2 bind to surface cell receptors, transport the genome into the nucleus, and they are immune response targets. VP1 and VP2 share the C-terminal sequence, with the exception of the N-terminus of VP1, which contains a unique phospholipase-A motif. The VP1 protein may exert a direct pathogenic effect on human airway cells [14]. VP2 represents the major antigenic determinant for serology and immunization [15], while upregulating IFN-beta production [16].

HBoVs undergo genetic variation due to mutation and recombination events [17]. Their variability has also been documented in the clinical setting [18]. HBoVs are cytopathogenic in differentiated epithelial cells of human airways [19].

2. Diagnosis of HBoV infection

Respiratory diseases have accounted for the largest application segment of the clinical microbiology market over the last year because of the growing list of novel agents, technologic advances (e.g., multiplex gene amplification assays) and the need to understand the causes of a variety of respiratory disorders that, thus far, have gone undiagnosed. It is not yet feasible [20,21] to isolate different HBoV types, therefore, diagnosis is based on genome detection and serology (e.g., EIA, WB, immunofluorescence) [22]. Upon the development of the first gene amplification assay for HBoV1 [23], different PCR tests have emerged, and some are type-specific [24]. Most laboratories use in-house end-point and real-time PCR assays targeting the NP-1, NS-1 or VP1/2 gene [25], but other nucleic acid-based detection HBoV diagnosis methods have been described [26–29]. A number of multiplexing assays have been developed, approved, and brought to market. Some multiplex respiratory assays (e.g., the Luminex RVP assay and the RespiFinder assay [30,31]) detect HBoVs.

When HBoV detection is associated with high viral loads, a cause-effect relationship may be inferred, particularly in young children [32]. More recently, the potential of next-generation sequencing is being explored in the field [33]. The antigens used in serology are based on HBoV1: either recombinant VP2 or virus-like particles (VLP) carrying VP2 [34]. EIA methods have been established to detect IgG and IgM antibodies and IgG affinity [35]. These methods cannot rule out cross-reactivity with the capsid proteins of HBoV2–4. Thus, IgG cross-reactivity with HBoV2–4 must be kept in mind when addressing the high prevalence of HBoV1 IgG. Competition assays with HBoV2–4 VLPs have been recently introduced. Based on published results, the prevalence of HBoV infections is likely in the descending order of HBoV1–4.

To diagnose primary infection, at least two of the following markers must be present: positive IgM, a fourfold increase of IgG titer, low IgG avidity, and medium-high viral load by PCR.

To clinically diagnose an HBoV infection, it is necessary to screen respiratory tract or stool samples (depending on whether the primary symptoms are respiratory or gastrointestinal), as well as a serum sample [36–40]. The serum sample is of great relevance because viremia is observed only during active infection [6,36–40], whereas HBoV can be shed by otherwise healthy patients, most likely due to persistent infection without viremia [6,39].

Studies of children with respiratory tract infection have reported a prevalence of bocaviruses ranging from 1.5% to 19% [41–56]. In our own experience, the frequency of HBoV detection in children with respiratory disorders (age 1–14 years) was similar in Italian and German studies [53–56]. Table 1 shows the results of molecular tests obtained in the years 2010–2014. Note that the detection rate (6.9–8.5%) did not change from year to year and that it was slightly higher in immunosuppressed children (10.5%). Multiplex PCR assays were used in these studies,. Assays could detect 15–21 respiratory pathogens. Surprisingly, when mucosal samples from adults with chronic sinusitis were studied, 17.6% carried HBoV as the single viral agent [55].

In clinical association studies, the diagnoses of reported HBoV infections have been overwhelmingly based on PCR. However, PCR is not an optimal diagnostic tool because of prolonged positivity in the respiratory and gastrointestinal tracts, particularly when low viral loads are present. This fact may lead to high detection rates in asymptomatic subjects. Primary infections that are diagnosed serologically or by the presence of HBoV1 DNA in serum have been linked to respiratory symptoms. However, neither the diagnostic sensitivity of HBoV DNA detection in serum nor the precise duration of DNAemia in connection with primary infection not been determined. Serum/plasma PCR may require precise timing of the sample collection.

An additional and relevant question is whether DNA quantitation improves HBoV diagnosis. Studies that have stratified patients according to the viral load in nasopharyngeal secretions show that high viral loads are associated with few co-infections and increased illness severity. High viral loads in respiratory samples ($>2 \times 10^8$ genomes/ml) have been clearly linked to respiratory symptoms. In a study of wheezing children [57], 96% of children had medium-high HBoV1 loads ($>10^4$ genomes/ml) in the nasopharynx (in the absence of co-infections), which indicated that serum IgM against HBoV and/or an increasing IgG titers were present in most patients compared with positive serology in only 38% of the patients with low DNA loads [57]. Because other studies have failed to show an association between symptoms and HBoV copy numbers, further study is required to assess the diagnostic utility of HBoV genome quantification in the respiratory tract.

Most studies have been based on the PCR detection of HBoV1 in respiratory tract, but only a few have confirmed the HBoV1 infection through serology or PCR in serum/plasma. Molecular and serologic methods are now appropriate to investigate both acute and persistent bocavirus infections in a variety of clinical settings, including neonatal and pediatric patients, oncologic patients, transplant recipients, and ICU patients. Diagnosis should not be based on qualitative PCR in respiratory or gastrointestinal samples due to the possibility of low-level HBoVi1 persistence/recurrence. When HBoV serology or serum samples for PCR are not available, the next best option is quantitative PCR, with a cutoff of $>10^4$ HBoV1