What role for human rhinoviruses in the lower respiratory tract?

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SUMMARY

Human rhinoviruses (HRV) usually cause upper airway infections. However, viral replication in the tracheobronchial tree has been disclosed, although its clinical role is poorly known. We evaluated the prevalence of HRV in 159 bronchoalveolar lavages from 88 patients and describe a lung transplant recipient with a high HRV load in association with acute rejection. HRV was detected in 22/88 patients (25.0%): 7/18 lung transplant recipients, 11/41 immunocompetent, and 4/29 immunocompromised (p=n.s.). No lung disease was significantly associated with HRV positivity. It should be recommended to include HRV in the virological diagnostic work-up of lower respiratory specimens to elucidate their role.

KEY WORDS: Human rhinoviruses, Revrese-transcription PCR, Lower respiratory tract infections, Bronchoalveolar lavage, Lung transplantation

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Human Rhinoviruses (HRV) are the most common viral agents in humans, the over 110 serologic types being responsible for about 30% to 50% of all the cases of common colds and associated upper respiratory tract complications such as otitis media and sinusitis in both adults and children. Although HRV are generally temperature restricted in replication with optimal growth at 33-35°C, the temperatures observed in the tracheobronchial tree are often lower than body core temperatures, also in relation to external temperature and frequency of ventilation, and so are permissive for HRV replication (McFadden *et al.*, 1985). Moreover, it has been shown that many HRV serotypes can replicate efficiently at core body temperature with modest differences in terms of infectious titres (<0.5-1.0 $\log_{10}$ $TCID_{50}$)(Papadopoulos *et al.*, 1999). Several studies have linked HRV infection to illnesses in the lower respiratory tract in immunocompetent patients, including exacerbations of pre-existing airways disease in those with asthma, chronic obstructive pulmonary disease (COPD) or cystic fibrosis, and pneumonia and bronchiolitis in children aged <5 years hospitalized for HRV infection (Hayden, 2004). However, a recent study on hospitalized adults with pulmonary disease found a prevalence of HRV in bronchoalveolar lavage (BAL) specimens of 31.2% without evidencing a significant difference of prevalence in relation to the presence of COPD, pneumonia or acute respiratory illness (Minosse *et al.*, 2008). On the other hand, limited information is available regarding HRV as a cause of potential severe lower respiratory tract infections (LRTI) in immunocompromised individuals (Parody *et al.*, 2007, Kaiser *et al.*, 2006). Herein we evaluate the prevalence of HRV infection in 159 consecutive BAL samples and describe a lung transplant recipient in whom a high HRV load was detect-

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ed in the absence of findings suggestive of LRTI. Samples were collected over a period of 11 months (April 2007 - February 2008) from 88 patients: 41 immunocompetent (with fever and/or respiratory signs and symptoms and/or radiological abnormalities in the presence or not of underlying bronchopneumopathy), 47 immunocompromised patients, including 29 bone marrow and solid organ transplant and 18 lung transplant recipients (BAL procedure performed regularly as routine follow-up, also in the absence of clinical, functional or radiological abnormalities). BAL procedure was performed as previously described (Costa *et al.*, 2007) and samples, thawed and liquefied with 1:1 N-acetylcisteine, were evaluated with a panel that detected 17 respiratory viruses by rapid shell vial culture (Costa *et al.*, 2007) and/or molecular methods (real time PCR, real time RT-PCR) following automated extraction of total nucleic acids with the NucliSens easyMAG platform (bioMeriéux, Marcy l'Etoile, France), including cytomegalovirus, herpes simplex viruses, varicella-zoster virus, adenoviruses, influenza viruses A and B, parainfluenzaviruses, respiratory syncytial virus, Epstein-Barr virus, human herpesvirus-6 and -7, enteroviruses, human metapneumovirus, human coronaviruses, human bocavirus, and HRV. In particular, HRV-RNA was reverse transcribed with random primers using SuperScript™ II Reverse Transcriptase (Invitrogen, Milan, Italy), according to manufacturer's instructions, detected by a real time RT-PCR with a retrotranscription. Real time quantitative RT-PCR targeted the conserved 5'-UTR region (forward primer 5'-TGG ACA GGG TGT GAA GAG C-3'; reverse primer 5'-CAA AGT AGT CGG TCC CAT CC-3'; probe 5'FAM-TCCTC-CGGCCCCTGAATG-TAMRA3'; selected with the aid of Primer Express Software, version 2.0.0 [Applied Biosystems, Foster City, CA]) and was executed with the 7300 Real Time PCR System (Applied Biosystems) based on TaqMan platform. The linearity range of the assay was 1-10^7 copies/reaction. Results are summarized in Table 1. The overall prevalence of HRV, as detected by real time RT-PCR, was of 13.8% in BAL specimens (22/159) and 25.0% in patients (22/88), namely 7/18 (38.9%) lung transplant recipients, 11/41(26.8%) immunocompetent patients, and 4/29 (13.8%) immunocompromised patients (p=n.s.). A coinfection with at least another virus was present in all the cases. No clinical context or lung disease (including exacerbations of COPD, interstitial pneumonia, acute or chronic respiratory failure) was significantly associated with HRV positivity. Moreover, the viral load of HRV was <1000 genome equivalents/ml BAL in all the cases, but one in which a load of 49943 was detected. This was a 66-year-old man with a history of severe COPD who had undergone a single lung transplant. A BAL procedure performed in the first month posttransplantation resulted negative for HRV and positive for CMV and human herpesvirus-6, for which he underwent a course of valganciclovir. At three months BAL tested negative for CMV, while HRV was detected in concomitance with HHV-6. At the time of this procedure, the patient presented no significant respiratory signs or symptoms (mod-

TABLE 1 - *Results of human Rhinoviruses (HRV) detection in the study population. BMT, bone marrow transplant; SOT, solid organ transplants other than lung transplant; LT, lung transplant. GEq, genome equivalents. Differences between immunocompetent and immunocompromised patients and between BMT, SOT, and LT were not significant.*

HRV positivity	*Samples N (%)*	*Patients N (%)*	*Viral load (GEq/ml BAL) Median (range)*
Total, N	22/159 (13.8%)	22/88 (25.0%)	353.5 (125-49943)
Immunocompetent	11/59 (18.6%)	11/41 (26.8%)	387.5 (150-994)
Immunocompromised	11/100 (11.0%)	11/47 (23.4%)	321.0 (125-49943)
- BMT	1/5 (20%)	1/4 (25%)	
- SOT	3/37 (8.1%)	3/25 (12.0%)	
- LT	7/58 (12.1%)	7/18 (38.9%)	

ified Borg dyspnea index, 0); blood test results, C-reactive protein, arterial blood gas analysis, and spirometry evidenced no significant abnormalities. Chest radiography and computed tomography scan disclosed mild consolidation. Transbronchial lung biopsy (hematoxylin & eosin, periodic acid-Schiff, Masson's trichrome stainings) revealed minimal acute vascular rejection (grade A1) and low-grade small airway inflammation (grade B1), without viral inclusions. Immunohistochemistry for CMV was negative. In a recent study on HRV infection in BAL in a cohort of 68 lung transplant recipients (Kaiser *et al.*, 2006), Kaiser *et al.* described three patients with lower respiratory symptoms and graft dysfunction, two of whom had acute rejection (A0 to A4, Bx to B3; A0 to A3, Bx to B2, respectively) and persistent infection over a period of 12 months. In our study a mild acute rejection was found in the patient with high HRV load, although in the absence of graft dysfunction and respiratory symptoms. However, considering lung transplant recipients, no significant difference in terms of HRV positivity was found in relation to the presence of acute or chronic rejection. In contrast to immunocompetent subjects, HRV clearance in immunocompromised patients may be delayed with prolonged shedding, although we did not find it in patients repeatedly tested such as lung transplant recipients. Evidence demonstrating that HRV disease is not exclusively limited to the upper airways and may cause lower respiratory complications, together with the frequency of HRV infections and the increasing number of immunocompromised patients, emphasize the need to include HRV in the virological diagnostic work-up of lower respiratory specimens. Compared to other organ transplant recipients, lung transplant patients are potentially at higher risk of complications following exposure to HRV, and in our study HRV infection tended to be more frequent in lung transplant patients. It may be difficult to evaluate the clinical role of these findings in determining lower respiratory symptoms, graft dysfunction and acute rejection, also taking into account the viral load and the high frequency of coinfections.

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