ORIGINAL ARTICLE 10.1111/1469-0691.12242

The significance of rhinovirus detection in hospitalized children: clinical, epidemiological and virological features

J. C. Rahamat-Langendoen[1], A. Riezebos-Brilman[1], E. Hak[2], E. H. Schölvinck[3] and H. G. M. Niesters[1]

1) *Division of Clinical Virology, Department of Medical Microbiology, University of Groningen, University Medical Center Groningen,* 2) *PharmacoEpidemiology and PharmacoEconomics, University of Groningen, University Center for Pharmacy and* 3) *Beatrix Children's Hospital, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands*

Abstract

Recent developments in molecular diagnostic tools have led to the easy and rapid detection of a large number of rhinovirus (HRV) strains. However, the lack of clinical and epidemiological data hampers the interpretation of these diagnostic findings. From October 2009 to January 2011, we conducted a prospective study in hospitalized children from whom samples were taken for the detection of respiratory viruses. Clinical, epidemiological and microbiological data from 644 patients with 904 disease episodes were collected. When HRV tested positive, strains were further characterized by sequencing the VP4/VP2 region of the HRV genome. HRV was the single respiratory virus detected in 254 disease episodes (28%). Overall, 99 different serotypes were detected (47% HRV-A, 12% HRV-B, 39% HRV-C). Patients with HRV had more underlying pulmonary illness compared with patients with no virus (p 0.01), or patients with another respiratory virus besides HRV (p 0.007). Furthermore, cough, shortness of breath and a need for oxygen were significantly more present in patients with HRV infection. Particularly, patients with HRV-B required extra oxygen. No respiratory symptom, except for oxygen need, was predictive of the presence of HRV. In 22% of HRV-positive disease episodes, HRV infection was hospital acquired. Phylogenetic analysis revealed several clusters of HRV; in more than 25% of these clusters epidemiological information was suggestive of transmission within specific wards. In conclusion, the detection of HRV may help in explaining respiratory illness, particular in patients with pulmonary co-morbidities. Identifying HRV provides opportunities for timely implementation of infection control measures to prevent intra-hospital transmission.

Keywords: Infection control, nosocomial transmission, respiratory infection, rhinovirus, sequence analysis
Original Submission: 28 December 2012; **Revised Submission:** 13 March 2013; **Accepted:** 4 April 2013
Editor: L. Kaiser

Clin Microbiol Infect

Corresponding author: J. C. Rahamat-Langendoen, Department of Medical Microbiology, University Medical Center Groningen, PO Box 30 001, Mailcode EB80, 9700 RB Groningen, the Netherlands
E-mail: j.c.rahamat@umcg.nl

Introduction

In recent years, human rhinoviruses (HRVs) have been increasingly recognized as a potential cause of acute otitis media, bronchiolitis, asthma and pneumonia in children [1–3]. The development of sensitive and rapid molecular techniques markedly improved the detection rate of HRV and revealed the high genetic diversity. Over 150 serotypes of HRV have been described, classified into three main species: HRV-A, HRV-B and HRV-C [4]. HRV-C is the most recently discovered species and is thought to contribute more to recurrent wheezing and exacerbations of asthma compared with HRV-A and HRV-B [1,5]. Also, recently published data suggest that HRV-A and HRV-C cause more severe illness than HRV-B, with the greatest virulence during the winter [6]. Currently, molecular diagnostics are increasingly integrated into routine practice, allowing detection and quantification of HRV, and also raising questions about their value in direct patient care and infection control. We performed a hospital-based prospective study to determine the clinical, epidemiological and viral characteristics associated with HRV infection in children.

Materials and Methods

Patients and sample collection

In October 2009 a prospective study of respiratory infections in hospitalized children was initiated at the University Medical Center Groningen (UMCG), the Netherlands. The UMCG is a tertiary referral hospital with more than 1300 beds in the northern region of the country. Demographic, clinical and microbiological data were systematically collected from all children under 18 years of age, from whom respiratory samples were taken for the detection of 15 respiratory viruses (influenza A/B, respiratory syncytial virus A/B, coronavirus 229E/NL63/OC43, para-influenzavirus type 1–4, metapneumovirus, adenovirus, bocavirus and rhinovirus). Samples positive for HRV were further characterized by sequence analysis of the VP4/VP2 region. Information on the presence of bacterial respiratory pathogens was included when bacteriological culture was performed on the same day, 1 day before or 1 day after the virological sample was taken.

Clinical data were collected using a standardized case record form, with items regarding the presence of an underlying chronic illness (pulmonary, cardiovascular, gastrointestinal or neurological) and/or immune suppression (transplantation, malignancy or immune suppressive therapy), clinical symptoms (fever, cough, shortness of breath, otitis media, wheezing, vomiting, diarrhoea, need for oxygen or mechanical ventilation), treatment (antibiotics, antivirals or inhalation therapy), clinical diagnosis (upper respiratory tract infection (pharyngitis, coryza or otitis media) or lower respiratory tract infection (pneumonia, bronchiolitis, exacerbation of asthma or croup)) and outcome. Underlying pulmonary illness included asthma, congenital pulmonary illness or anatomic malformations, cystic fibrosis and bronchopulmonary disease. Cardiovascular disease was divided into inborn or acquired heart disease. Patients with partial resection of the bowel or failure to thrive, and those who were waiting for a liver transplantation, were categorized as having gastrointestinal disease as an underlying illness. Neurological disease was not further specified.

Epidemiological data were gathered to determine whether the respiratory infection was community or hospital acquired, including measures that were taken in the hospital to prevent further transmission of respiratory viruses. Hospital-acquired HRV infection was defined as a first day of illness 2 or more days after admission to the hospital. Infection control measures consisted of a combination of droplet and contact precautions (gown, gloves and mask for healthcare workers during patient care, and patient in a single room) and were installed when HRV was detected.

The study was approved by the local Medical Ethical Committee of the UMCG. Informed consent was obtained from a parent or guardian.

Real-time PCR and sequencing

The majority of samples (91%) arrived at the laboratory within 1 day after collection. Samples were divided into aliquots and stored at 4°C if PCR testing was performed on the same or the next day. Longer storage was carried out at −80°C. In general, PCR testing was performed on a daily basis, providing results within 48 h after arrival of the sample at the laboratory.

All respiratory samples, nasopharyngeal swabs, aspirates or sputum, were tested by a laboratory-developed (LDT) real-time PCR, as has been described elsewhere [7]. For rhinovirus detection, a real-time LDT-PCR was introduced using the SuperScript® III Platinum® One-Step qRT-PCR Kit, (Life technologies, Carlsbad, CA, USA). All reactions were performed with Phocine Distemper Virus as an internal control in a total volume of 25 μL containing 12.5 μL of 2× reactionmix, 0.5 μL SuperScript® III RT/Platinum® Taq mix, 0.5 μL of 1:10 Rox reference dye, 300 nM of each forward primer, 600 nM reverse primer and 100 nM of each probe, and 5 μL of genomic RNA template. Primers and probes used are listed in Table 1. The addition of this large set of primers and probes is performed in a specific reaction to avoid bias and favouring against other respiratory viruses. Our rhinovirus PCR has been optimized during recent years based on the available genetic information, in particular regarding species C. By re-optimizing the assay with new primers and probes, together with the use of Invitrogen SuperScript enzymes, we detected retrospectively more HRV-positive samples at a lower cycle threshold (Ct) value. To ensure that these HRVs were not enteroviruses, all samples were sequenced. We only detected HRV, eventually 99 serotypes, as well as enterovirus 68, which is genetically identical to HRV.

The Ct value (the number of amplification cycles needed for a PCR to become positive) was used as relative estimate for the amount of HRV present in the samples.

TABLE 1. Primers and probes for HRV detection used in this study

Primers/probes	Sequence (5′→ 3′)	Position[a]	Tm
Rhino-fwdB-mod-TM	GGTGTGAAGACTCGCATGTGC	408–427	60.1
Rhino-fwdA-mod-TM	GGTGTGAAGAGCCCCGTGTG	408–426	62.4
Rhino-fwd-C-TM	GGTGTGAAGAGCCNANTGYGCTC	408–429	58.9
Rhino-fwd-D-TM	GGTGYGAAGANCCNANTGTGC	408–427	58.9
Rhino-fwd-E-TM	GGTGTGAAGACYTGCATGTGC	408–427	57.9
Rhino-fwd-F-TM	GGTGTGAAGAGYCNCGTGTGCT	408–428	58.1
Rhino-rev3	CCAAAGTAGTYGGTYCCRTCCC	523–544	58.4
Rhino-Probe-TM	TCCTCCGGCCCCTGAATGCG	438–457	70.2
Rhino-Probe3	TCCTCCGGCCCCTGAATGTGG	438–458	69.1

Tm, melting temperature.
[a]Primer positions are given according to the orientation of the primer; numbers are given according to an HRV-A16 reference strain (GenBank no. L24917).