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Short Communication

Human Rhinovirus Infection in Children with Cystic Fibrosis

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SUMMARY: Nasopharyngeal swabs obtained from 78 pediatric patients with cystic fibrosis (CF), including 47 with acute pulmonary exacerbation and 31 in a stable clinical condition, were evaluated for 17 respiratory viruses. Human rhinovirus (HRV) was the most frequently detected virus in patients with pulmonary exacerbation and in those who were clinically stable (21.3% vs. 12.9%; $P = 0.52$). HRV-A was the main RV detected in patients with pulmonary exacerbations. However, no prevalence of particular HRV-A subtypes was found. This study highlights that RV is frequently found in the respiratory secretions of patients with CF and the impact of HRV-A appears higher than that of the other HRV types in patients with pulmonary exacerbations.

Viral respiratory infections seem to play an important role in conditioning the short- and long-term pulmonary morbidity of patients with cystic fibrosis (CF) because they increase the rate of hospitalization and antibiotic use (1). They are also the main trigger of recurrent pulmonary exacerbations, which cause progressive lung damage that is characteristic of the disease (2,3).

Respiratory syncytial virus (RSV), influenza virus, and human rhinovirus (HRV) are the most frequent causes of viral respiratory infections (1–3). However, the importance of RV as a causative agent of pulmonary exacerbations remains unclear, mainly because it has been found in the respiratory secretions of patients with CF with and without exacerbations and is frequently associated with other pathogens (4,5). Moreover, it is not known whether viral load is important in favoring new respiratory manifestations or whether there are differences in the pathogenic impact of various types of HRV.

The present study aimed to identify the role of HRV in acute pulmonary exacerbations of patients with CF.

The study was conducted between May 1, 2012 and April 30, 2013 and involved patients aged <25 years who were regularly followed up at the Cystic Fibrosis Centre of the University of Milan (Italy).

Clinically stable patients with CF examined at a standard follow-up visit and those admitted because of an acute pulmonary exacerbation were eligible for enrolment. An acute pulmonary exacerbation was defined as an increase in respiratory symptoms severe enough to require admission for intravenous antibiotics, as determined by an independent pediatrician (6). At the time of enrolment, clinical, biochemical, and lung function data were recorded, sputum bacterial culture with standard procedure was performed, and nasopharyngeal swabs for viral investigations were collected using a pernasal flocked swab that was stored in a tube containing 1 mL of universal transport medium (Kit Cat. No. 360c; Copan Italia, Brescia, Italy).

The study was approved by the Institutional Review Board of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy. A written informed consent of a parent or legal guardian was required, and patients aged >8 years were asked to provide their assent.

The viral nucleic acids were extracted from the swabs using the Nuclisens EasyMAG automated extraction system (Biomeriéux, Craponne, France), and the extract was tested for respiratory viruses using the Respiratory Virus Panel (RVP) Fast assay in accordance with the manufacturer's instructions (Luminex Molecular Diagnostics Inc., Toronto, Canada). The RVP Fast assay simultaneously detects influenza A virus (subtypes H1 or H3); influenza B virus; RSV-A and -B; parainflunza virus-1, -2, -3, and -4; adenovirus; human metapneumovirus; coronaviruses 229E, NL63, OC43, and HKU1; enterovirus/RV; and human bocavirus (7).

The enterovirus/RV-positive nasopharyngeal samples were retested to identify HRV and determine the viral load. The real-time reverse transcription-polymerase chain reaction (RT-PCR) assay was performed using the iAg-Path-ID One-Step RT-PCR Kit (Applied Biosystems, Foster City, CA, USA), and the primers and probe sequences employed have been previously described (7,8). The PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Milan, Italy), and the purified products were sequenced in both the directions using the same forward and reverse primers as those used in PCR. To identify the HRV species (A, B, and C), the newly determined sequences were checked and aligned using the BioEdit program, and the resultant consensus sequences were compared with those from GenBank using the

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Table 1. Demographic and clinical characteristics of the study population

	CF patients with acute pulmonary exacerbation (n = 47)	CF patients in stable clinical condition (n = 31)	P
Gender, no. (%)	11 (23.4)	11 (35.5)	0.36
Median age, years (range)	16.7 (7–25)	17.3 (7–24)	0.69
Genetic mutations, no. (%)			
ΔF508/ΔF508	10 (21.3)	10 (32.3)	0.41
ΔF508/others	31 (65.9)	13 (41.9)	0.06
Others	6 (12.8)	8 (25.8)	0.24
Median FEV_1, % (range)	82 (32–102)	87 (44–112)	0.70
Associated clinical conditions, no. (%)			
Diabetes	2 (4.2)	1 (3.2)	1.00
Liver disease	5 (10.6)	4 (12.9)	1.00
Lung transplantation	5 (10.6)	6 (19.4)	0.32
Bacterial colonization, no. (%)			
Pseudomonas aeruginosa	23 (48.9)	9 (29.0)	0.13
Staphylococcus aureus + *Pseudomonas aeruginosa*	12 (25.5)	14 (45.2)	0.12
Staphylococcus aureus + Gram-negative bacteria other than *Pseudomonas* spp.	9 (19.1)	5 (16.1)	0.96
Staphylococcus aureus + *Aspergillus fumigatus*	3 (6.4)	3 (9.7)	0.67
Median CRP, mg/dL (range)	2.13 (0.03–13.24)	0.37 (0.03–1.79)	0.036

CRP, C-reactive protein; FEV_1, forced expiratory volume in 1 s.

nucleotide-nucleotide BLAST algorithm (http://www.ncbi.nlm.nih.gov). A plasmid containing the corresponding target viral sequence was then used to quantify the positive samples, as described previously (8). The quantitative results were expressed as $\log_{10}$ RNA copy numbers/mL of nasopharyngeal swab after data multiplication by an appropriate dilution factor.

The between-group comparisons of the categorical data were conducted using contingency table analysis and χ^2 or Fisher's exact test, as appropriate. The continuous variables were analyzed using Wilcoxon's rank-sum test because the data were not normally distributed on the basis of the Shapiro-Wilk statistic. All analyses were two-tailed and conducted using SAS version 9.2 (Cary, NC, USA), and P values of ≤ 0.05 were considered statistically significant.

Of the 78 children enrolled (22 boys, 28.2%; mean age ± standard deviation [SD], 16.6 ± 7.9 years), 47 (60.3%) had been admitted for an acute pulmonary exacerbation and 31 (39.7%) were treated as outpatients and considered to be clinically stable because they had no acute respiratory exacerbation and no serious problem during the previous 3 months. Table 1 shows the demographic, clinical, and biochemical characteristics of the study population. Apart from C-reactive protein (CRP), which was significantly higher among patients with acute pulmonary exacerbation than among controls (P = 0.036), the other characteristics were similar in the two groups.

The distribution of infecting viruses is summarized in Table 2. HRV was the most frequent in both the groups, and the other respiratory viruses were identified in a minority of cases. In all but one case, RV was the only virus. Sequencing analysis revealed that HRV-A was more common among patients with pulmonary exacerbations than among clinically stable patients. Subtyping of HRV included in the different RV groups was possible in all but three cases, which revealed that different HRV subtypes were concomitantly circulating and infecting patients with CF, without differences among the clinical groups. HRV-A 31, 38, 40, 45, and 46 and HRV-C 32 and 51 were identified in patients with exacerbations, whereas HRV-A 90, HRV-B 101, and 2 HRV-C 19 were found in clinically stable patients. The HRV load was similar in patients with and without acute pulmonary exacerbations (5.85 ± 1.36 vs. 4.90 ± 1.76 $\log_{10}$ cp/mL, P = 0.46). No correlation was found between the associated clinical condition and HRV load as well as between bacterial colonization, colonizing bacteria, and viral infection in patients with exacerbations and clinically stable patients.

Table 2. Viral findings

	CF patients with acute pulmonary exacerbation (n = 47)	CF patients in stable clinical condition (n = 31)	P^*
Single infection, no. (%)	14 (29.8)	9 (29.0)	0.85
HRV, no. (%)	10 (21.3)	4 (12.9)	0.52
A	8 (17.0)	1 (3.2)	0.07
B	0 (0.0)	1 (3.2)	0.39
C	2 (4.3)	2 (6.4)	1.00
Influenza virus, no. (%)	2 (4.2)	2 (6.4)	1.00
A/H1N1	1 (2.1)	1 (3.2)	1.00
A/H3N2	1 (2.1)	1 (3.2)	1.00
Bocavirus, no. (%)	1 (2.1)	1 (3.2)	1.00
Metapneumovirus, no. (%)	1 (2.1)	0 (0.0)	1.00
Respiratory syncytial virus, no. (%)	0 (0.0)	1 (3.2)	0.39
Enterovirus, no. (%)	0 (0.0)	1 (3.2)	0.39
Dual infection, no. (%)	1 (2.1)	0 (0.0)	1.00
HRV + metapneumovirus	1 (2.1)	0 (0.0)	1.00

HRV, human rhinovirus.

The findings of the present study revealed that HRV is frequently found as a single infectious agent in the respiratory secretions of patients with CF. However, as

this observation was noted in patients with and without acute pulmonary exacerbations, it does not support the hypothesis that HRV can be considered as the trigger of pulmonary exacerbations in all the cases. Our data are in line with those reported by de Almeida et al. (4) and Stelzer-Braid et al. (5), who were unable to demonstrate any significant association between RV infection and the development of pulmonary exacerbation in patients with CF. Furthermore, it is well known that the presence of a virus in the nasopharynx of a patient with pulmonary disease does not necessarily signify that it is the cause of the disease because it may simply indicate a coincidental upper airways infection, a carrier state, or prolonged shedding of a pathogen that caused a previous infection (9). This may be particularly important in the case of HRV because a number of epidemiological studies have shown that it can be found in the respiratory secretions of 12–22% of asymptomatic subjects (10,11).

In a recent study, Kieninger et al. observed that the HRV load in the bronchoalveolar lavage of children with CF was extremely high, particularly during pulmonary exacerbation, and higher HRV load in patients with CF was found to be associated with lower levels of antiviral and antinflammatory mediators as well as increased levels of proinflammatory mediators (12). In the present study, determination of the HRV load did not seem to provide any further information regarding the potential role of RV in the development of pulmonary exacerbations in patients with CF because the HRV load was similar in all our patients. However, analysis of the type of RV suggested that the role of HRV-A differs from that of the other types because it was identified more frequently in patients with pulmonary exacerbations, whereas HRV-C was identified more frequently in clinically stable patients. The clinical relevance of the various RV types is debated. Although a number of studies have found that each RV type could be associated with any type of respiratory disease, it has been reported that HRV-A causes pneumonia more frequently (9,13,14) and HRV-C is more frequently associated with the development of acute wheezing or asthma exacerbations (15). In the case of patients with CF, our data differ from those reported by de Almeida et al. (4), who found that both HRV-A and HRV-C were more frequent in patients with acute pulmonary exacerbations. However, the number of patients in most of these studies (including the present study and that of de Almeida et al. [4]) is very small to draw any definite conclusions. This inference seems even more important when considering the data regarding the HRV subtypes found in the present study. It was noted that numerous HRV subtypes are involved in the determination of respiratory infections, suggesting that only studies comprising hundreds of patients with CF could help determine the relevance of different HRV groups and subtypes in pulmonary exacerbations in patients with this disease.

In conclusion, the present study highlighted that HRV is frequently found as a single infectious agent in the respiratory secretions of patients with CF, regardless of pulmonary involvement. Determination of the HRV load did not seem to provide any further information regarding the potential role of HRV in the development of pulmonary exacerbations, whereas the impact of HRV-A appeared to be higher than that of the other HRV types in patients with pulmonary exacerbations. Further studies are required to evaluate the actual role of HRV groups and subtypes in conditioning the prognosis of CF, verify whether HRV is only a simple bystander, and determine whether the association between HRV and lung exacerbations is related to a specific type, which could be important for the development of specific preventive measures.

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Conflict of interest None to declare.

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