Role of respiratory viruses in pulmonary exacerbations in children with cystic fibrosis

Sandra Asner, a,d Valerie Waters, a,d Melinda Solomon, b,d Yvonne Yau, c,e Susan E. Richardson, c,e Hartmut Grasemann, b,d Farhad Gharabaghi, c,e Dat Tran a,d,*

a Division of Infectious Diseases, Department of Paediatrics, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G1X8
b Division of Respiratory Medicine, Department of Paediatrics, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G1X8
c Division of Microbiology, Department of Paediatric Laboratory Medicine, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8
d Department of Paediatrics, University of Toronto, Toronto, Ontario, Canada
e Department of Pathobiology and Laboratory Medicine, University of Toronto, Toronto, Ontario, Canada

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Abstract

Background: The role of respiratory viruses in cystic fibrosis (CF) exacerbations is incompletely understood.
Methods: Cross-sectional study of CF children with a pulmonary exacerbation. Mid-turbinate swabs were tested by a direct immunofluorescent antibody assay and a multiplex PCR panel (ResPlex II v2.0, Qiagen). Resplex II was also applied to sputum or throat swab samples. Pulmonary function tests and quality of life and severity scores were recorded. Sputum cell counts, bacterial density and cytokines were measured.
Results: 26/43 (60.5%) subjects tested positive for at least one respiratory virus by any diagnostic method applied to any sample type. Virus-positive patients were younger (p=0.047), more likely to be male (p=0.029), and had higher CF clinical severity (p=0.041) and lower quality of life (physical) scores (p=0.023) but similar IL-8, neutrophil percentage and elastase levels.
Conclusions: Compared to non-viral exacerbations, viral-related exacerbations were associated with worse severity and quality of life scores but similar pulmonary inflammation.

Keywords: Cystic fibrosis; Pulmonary exacerbations; Respiratory viruses; Molecular diagnosis

1. Introduction

Cystic fibrosis (CF) affects over 60,000 patients worldwide. Bacterial infections are the main cause of recurrent pulmonary exacerbations leading to progressive lung damage with less known about the role of viral respiratory tract infections [1–3]. The true impact of respiratory viruses in CF may have been previously underestimated as the majority of previous studies [4,5] used viral culture and/or immunofluorescence techniques which are relatively insensitive and restricted in breadth for viral detection [1,2]. To date, published reports of respiratory viral infections in children with CF have primarily focused on the proportion of pulmonary exacerbations associated with viruses [1,4–8]. The relationship between severity of these exacerbations and infection status with respiratory viruses has not been assessed. The extent to which these complications are mediated by direct viral effects or via potentiation of bacterial colonization and infection is unclear [5]. The pulmonary manifestations of CF involve both neutrophil-dominated airway inflammation and chronic bacterial infection. Although the temporal relationship between infection and inflammation remains unclear in early disease, later in disease there is a self-perpetuating cycle of airway obstruction, chronic bacterial infection and vigorous inflammation resulting in structural damage to the airway [9–11]. Less is currently known about the contribution from viral infections. A better understanding of the host inflammatory response following respiratory viral infections may minimize the use of prolonged antibiotic therapy.
The primary objective of this study was to investigate the proportion of CF pulmonary exacerbations associated with a respiratory viral infection, as detected by conventional diagnostics and a commercially available multiplex molecular diagnostic assay. Our secondary objectives were to determine the clinical severity and pulmonary inflammation due to exacerbations associated with viral infections.

2. Materials and methods

2.1. Participants

From November 1, 2009 to March 31, 2010, we conducted an observational, cross-sectional study of children with CF seen in the setting of a pulmonary exacerbation. Patients were recruited from the respiratory medicine clinic at The Hospital for Sick Children, a large pediatric referral center that cares for approximately 280 CF patients. We enrolled all CF patients who met the following inclusion criteria: age < 18 years; and clinical presentation consistent with a pulmonary exacerbation, defined as any of the following clinical symptoms: change in sputum production, increased cough or dyspnea, alone or in combination with temperature above 38 °C, and/or change in respiratory status (tachypnea, indrawing, wheezing or crackles) reported by the attending team. Informed consent (and assent from child as appropriate) was obtained from all participants and ethics approval was obtained from the Research Ethics Board at The Hospital for Sick Children. Exclusion criteria included lack of consent and insufficient command of English or absence of translator.

2.2. Collection of clinical information and specimens

Information on patient demographics and CF-relevant baseline characteristics such as CF-related comorbidities and bacterial colonization were abstracted from health records. The Cystic Fibrosis Questionnaire-Revised (CFQ-R) [12] and the Cystic Fibrosis Clinical Score (CFCS) [13] were prospectively administered to participants through standardized interviews and physical assessments. The CFCS is an acute clinical score, based on signs and symptoms. It was chosen as a severity scale because of its reliability and its ease of use [13]. Body mass index (BMI) percentile was calculated for children ≥ 2 years of age and weight-for-recumbent length percentile for those under the age of 2 [14]. Mid-turbinate flocked swabs (Copan Diagnostics, Murrieta, CA), collected from all enrolled patients, were placed into 3 mL of Universal Transport Medium (UTM-RT COPAN Diagnostics, Murrieta, CA). In addition, children old enough to produce sputum provided sputum samples while FLOQ COPAN throat swabs (inoculated in UTM) were obtained from those unable to produce sputum (refer to Fig. 1 for sample collection and testing).

2.3. Virology studies

Mid-turbinate specimens were examined by 1) direct fluorescent antigen assay (DFA) for 8 respiratory viruses (respiratory syncytial virus (RSV), influenza virus [A,B] (INFA/INFB), parainfluenza [types 1–3] (PIV), adenovirus (ADV) (SimulFluor®, Millipore, Temecula, CA) and human metapneumovirus (hMPV) (Diagnostic HYBRIDS, Athens, OH); 2) a nucleic acid amplification-based assay (ResPlex II v2.0, Qiagen, Mississauga, ON, Canada) for detection of 18 respiratory viruses (RSV [A, B], coronaviruses [OC43, 229E, NL63, HKU1], rhinovirus (HRV), cocksackie/echovirus, PIV [types 1–4], INFA, INFB, bocavirus, ADV [B, E] and hMPV); and 3) reverse transcription polymerase chain reaction (RT-PCR) for specific identification of the pandemic 2009 influenza A H1N1 (pH1N1) virus (Astra influenza Screen and Type (Astra Diagnostics, Hamburg, Germany or RT-PCR Kit 1.0, Astra Diagnostics, Hamburg, Germany)). We also applied the ResPlex II v2.0 panel to FLOQ COPAN throat swabs and Sputolysin (10.14 mg/ml, CALBIOCHEM)-treated sputum sample after ensuring that Sputolysin would not affect the performance of the assay (Fig. 1). To evaluate the potential interference of Sputolysin with the efficiency of nucleic acid extraction and performance of the assay, we randomly chose four known positive mid-turbinate samples (RSV B, PIV 1, HRV/INFA and RSVA) from another study and divided them each into two equal aliquots. The potential interference of Sputolysin was tested on mid-turbinate swabs instead of sputum samples as non Sputolysin-treated sputum samples would not enable appropriate nucleic acid extraction because of the sputum viscosity. We mixed one aliquot with an equal volume of 10% Sputolysin in PBS (phosphate buffered saline) and the other with an equal volume of PBS alone. After incubation for 10 min at room temperature, we extracted total nucleic acids from both aliquots with the NucliSENS easyMAG (bioMerieux, St Laurent, QC, Canada) followed by amplification with the Resplex II v2.0 assay [15]. A patient was classified as being virus-positive if a respiratory virus was detected in the mid-turbinate or throat or sputum sample by any one of the three assays used (DFA, Resplex II v2.0 or RT-PCR).

2.4. Bacteriologic confirmation and load assessment

Sputum samples underwent bacterial density analysis for Pseudomonas aeruginosa, Staphylococcus aureus and Haemophilus influenzae based on the predominant organism isolated on the routine bacterial culture using previously described methods [16].

2.5. Inflammatory markers and sputum neutrophil percentage

Levels of IL-8 and 26 additional inflammatory cytokines (PDGF, II-1 B, II-1 RA, II-2, II-4, II-5, II-6, II-7, II-9, II-10, II-12, II-13, II-15, II-17, Eotaxin, FGF, G_CSF, GM_CSF, IFN_G, IP-10, MCP_1, MIP_1A, MIP_1B, RANTES, TNF_alpha, VEGF) were measured on Sputolysin-treated sputum samples using a commercial multiplex bead-based assay (Bio-Plex Cytokine Assay, Bio-Rad) [17,18]. The detection range for all cytokines was 0–32,000 pg/ml as per the manufacture guidelines [26]. Neutrophil elastase (NE) activity was measured using a modified enzymatic assay based on NE cleavage of nitroanilide from N-methoxysuccinyl-al-a-la-pro-val-p-nitroanilide and results were reported in units/ml (1 unit=nmol/min). Lower limit of detection was 0.006 units/ml [10]. Sputum neutrophil percentage is the...
percentage of neutrophils based on a count of 400 white blood cells.

2.6. Lung function testing

Children old enough to perform spirometry had standard spirometry done at enrollment and routinely at every visit. We reported forced expiratory volume in 1 s (FEV₁) as a percentage of predicted value for sex and height using the equation of Corey et al. [19]. We defined baseline FEV₁ as the best FEV₁ in the 6 months prior to the current pulmonary exacerbation, and exacerbation FEV₁ as the FEV₁ at the time of presentation with a pulmonary exacerbation.

2.7. Statistical analyses

Standard descriptive and comparative statistics were used on data categorized by viral infection status (virus-positive and virus-negative patients). For normally-distributed continuous variables (total bacterial densities and bacterial densities for
S. aureus, P. aeruginosa and H. influenzae), we calculated means and analyzed differences between the comparator groups using Student’s t-test. For skewed data (age, obesity measures, FEV1% predicted, CFSC and CFQ-R scores, IL-8 levels, sputum neutrophil percentage and count, sputum neutrophil elastase), we derived medians and used the Mann–Whitney method for comparisons. The χ² test or Fisher’s exact test was used to compare categorical variables between groups as appropriate. Using standard formulae, we estimated the sensitivities and specificities (with respective binomial 95% confidence intervals (CI)) of mid-turbinate swabs, sputum samples and throat swabs for virus detection relative to the combination of these sample types as the reference standard. Differences in test performance were calculated using McNemar’s test. A p value < 0.05 was considered statistically significant. Data were analyzed by SPSS statistical software (version 16.0, SPSS Inc, Chicago, IL, USA).

3. Results

3.1. Viral detection studies

A total of 112 CF patients were screened for inclusion in the study between November 2009 and March 2010. Of these, 47 (42%) met eligibility criteria, 4 of whom (8.5%) declined participation in the study. Among the 43 enrolled patients, 26 (60.5%) were classified as virus-positive: 25/26 (96.2%) from mid-turbinate swabs and 6/26 (23.0%) from either sputum samples or throat swabs by any one of the three assays used (8 by DFA, 2 by RT-PCR and 26 by multiplex PCR). Of those 26 virus-positive subjects, 17 (65.4%) tested positive for one virus and the remaining 9 (34.6%) for two or more viruses (all by ResPlex II v2.0). From the 17 participants who tested positive for only one respiratory virus, coxsackie/echovirus was the most commonly identified pathogen (29.4%) (Table 1). RSV was reported from all participants who tested positive for two viruses (6/6) (Table 1).

3.2. Patient characteristics and clinical status at the time of pulmonary exacerbation

Virus-positive patients were significantly younger (p=0.047) and more likely to be male (p=0.029) than virus-negative patients. Also, they were significantly more likely to present with fever (p=0.019) but not with upper respiratory tract infection symptoms (p=0.941) (Table 2). There were no other significant differences between the two groups. At the time of exacerbation, virus-positive patients had significantly higher CF clinical score (CFCS) (27 vs 24; p=0.041) and lower quality of life (physical) CFQ-R score (53.4 vs 75.3; p=0.030) (Table 3). When the patient’s viral infection status was determined without incorporating the positive rhinovirus and coxsackie/echovirus results, the physical CFQ-R score of virus-positive patients remained significantly lower (58.5 vs 194.5; p=0.022). However, CFCS was no longer significantly different between virus-positive and negative patients. Antibiotic use, hospitalization requirements and mean percent predicted exacerbation FEV1 were not significantly different between the two groups (Table 3).

3.3. Pulmonary inflammation

Bacterial densities were examined in sputum samples from 8/26 (30.8%) virus-positive and 7/17 (41.2%) virus-negative patients. The mean total bacterial density observed in virus-positive patients was two logs lower (albeit not statistically significant) than that found in virus-negative patients (3.9×10⁷ vs 1.8×10⁹ CFU/mL; p=0.299). Virus-positive and negative patients had similar IL-8, neutrophil percentage and neutrophil elastase levels (Table 3). There was also no difference in the 26 additional cytokines levels between both groups (Supplementary Table 1).

3.4. Comparison of viral detection in mid-turbinate swabs and sputum or throat swab samples

A total of 21/43 (48.8%) patients had concomitant sputum samples and 11/43 (25.6%) had concomitant throat swabs tested for respiratory viruses by ResPlex II v2.0. Overall, the detection rate of respiratory viruses using mid-turbinate swabs was similar to that of throat swabs (7/11 vs 6/11) but higher than that of sputum samples (11/21 vs 6/21) (Supplementary Table 2). For classification of infection status based on detection of at least one respiratory virus, mid-turbinate swabs had a 76% (16/21) concordance rate with sputum samples (concordant pairs: 6 positive/positive, 10 negative/negative; discordant pairs: 5 positive/negative, 0 negative/positive) and 72.7% (8/11) concordance rate with throat swabs (concordant pairs: 5 positive/positive, 3 negative/negative; discordant pairs: 2 positive/negative, 1 negative/positive). The sensitivities of mid-turbinate swabs and sputum samples relative to the combination of mid-turbinate and/or sputum samples were 100.0% (95% CI, 67.9–100.0) and 54.5% (95% CI, 24.6–81.9) respectively, while the specificities were both 100.0% (95% CI, 0.0–100.0). We also compared the number of additional positive virus results, using McNemar’s test. The test was not statistically significant for the comparison of mid-turbinate and sputum samples (p=0.041) but was significant for mid-turbinate swabs and throat swabs (p=0.001). The sensitivities (95% CI) of mid-turbinate swabs, sputum samples and throat swabs for virus detection relative to the combination of these sample types as the reference standard were 96.2% (95% CI, 81.9–100.0) and 72.7% (95% CI, 24.6–81.9) respectively. The specificities were both 100.0% (95% CI, 0.0–100.0) and 54.5% (95% CI, 24.6–81.9) respectively.

Table 1

Identified viruses among virus-positive subjects identified by any diagnostic method applied to any sample (mid-turbinate swabs, throat swabs or sputum samples).

| Virus          | Single viral infection (n=17) | Viral co-infection (n=9) |
|----------------|-------------------------------|-------------------------|
| Coxsackie/echovirus | 5 (29.4)                      | 4 (44.4)                |
| Rhinovirus      | 3 (17.6)                      | 3 (33.3)                |
| RSV A          | 2 (11.8)                      | 4 (44.4)                |
| RSV B          | 1 (5.9)                       | 2 (22.2)                |
| Parainfluenza 2 | 2 (11.8)                      | 1 (11.1)                |
| Parainfluenza 3 | 1 (5.9)                       | 0                       |
| Adenovirus B   | 2 (11.8)                      | 1 (11.1)                |
| pH1N1 b        | 1 (5.9)                       | 1 (11.1)                |
| hMPV c         | 0                             | 2 (22.2)                |
| Coronavirus     | 0                             | 4 (44.4)                |

a Respiratory syncytial virus.
b Pandemic 2009 influenza A H1N1.
c Human metapneumovirus.
Table 2
Baseline characteristics of virus-positive and virus-negative patients.

| Month of exacerbation | Virus-positive (n=26) | Virus-negative (n=17) | p-value |
|-----------------------|-----------------------|-----------------------|---------|
| – November 2009       | 9 (34.6)              | 7 (41.2)              | 0.775   |
| – December 2009       | 6 (23.1)              | 2 (11.8)              |         |
| – January 2010        | 8 (30.8)              | 5 (29.4)              |         |
| – February 2010       | 3 (11.5)              | 3 (17.6)              |         |

Symptom duration at date of swab

| – Mean (range)        | 7.5 (2–21)            | 12.3 (2–21)           | 0.066   |
| – Median (IQR)        | 6 (3–10)              | 14 (2.5–21)           | 0.290   |

Patient characteristics at enrollment

| Age, y, median (IQR)  | 6.9 (3.8–13.5)        | 13 (6.5–15.4)         | 0.047   |
| Male                  | 14 (53.8)             | 4 (23.5)              | 0.029   |

| BMI percentile        | 50 (30–82.5)          | –                     | –       |
| – Median (IQR)        | 24 (5–50)             | 25 (10–50)            | 0.474   |
| Homozygous DF508      | 6 (23.1)              | 9 (52.9)              | 0.588   |
| Baseline predicted FEV1 | 17 (65.4)            | 15 (88.2)             | 0.268   |
| – Median (IQR)        | 68 (57.5–87)          | 85 (63–94)            | 0.243   |
| Upper respiratory tract infection | 11 (42.3) | 7 (41.2) | 0.941 |
| Fever                 | 12 (46.2)             | 2 (11.8)              | 0.019   |

Comorbidities

| Pancreatic insufficiency | 17 (65.4) | 13 (76.5) | 1.0   |
| CF-related diabetes     | 2 (7.7)   | 1 (5.9)   | 1.0   |
| ABPA b                  | 4 (15.4)  | 5 (29.4)  | 0.462 |

Bacterial colonization at enrollment

| Throat swabs (n=16) | 12 | 4 |         |
| – Staphylococcus aureus | 4 | 1 | 0.755 |
| – Pseudomonas aeruginosa | – | – | –      |

| Sputum samples (n=27) | 14 | 13 |         |
| – Staphylococcus aureus | 8 | 9 | 0.340 |
| – Pseudomonas aeruginosa | 2 | 1 | 1      |

Influenza vaccination status

| Received seasonal influenza vaccine | 6 (23.1) | 6 (35.3) | 0.667 |
| Received pH1N1 vaccine             | 9 (34.6) | 11 (64.7) | 0.291 |

Table 3
Clinical status of patients with and without viruses at pulmonary exacerbation.

| Virus-positive (n=10) | Virus-negative (n=17) | p-value |
|-----------------------|-----------------------|---------|
| Predicted FEV1 a      | 17 (65.4)             | 15 (88.2) | 0.447 |
| – Median (IQR)        | 27.2 (19–39)          | 25 (16–49) | 0.667 |
| Received antibiotics  | 18 (69.2)             | 11 (64.7) | 0.355 |
| Hospitalized          | 9 (34.6)              | 5 (29.4)  | 0.504 |
| CFCS c                | 25 (96.1)             | 16 (94.1) | 0.234 |
| – Median (IQR)        | 17 (9)                | 16 (9.4)  | 0.066 |
| CFQ-R d (physical)    | 19 (69.2)             | 15 (64.7) | 0.405 |
| – Mean (range)        | 58.3 (33.3–78.2)      | 79.2 (61.1–91.7) | 0.028 |
| – Median (IQR)        | 53.4 (0–100)          | 75.3 (11.1–100) | 0.030 |
| Sputum IL-8           | 11 (42.3)             | 6 (35.3)  | 0.386 |
| – Median (IQR), pg/ml | 1785.8                | 1403      | 0.615 |

Note. Data are n (%) unless otherwise indicated. * No observations.

a Interquartile range (25th–75th percentile).
b Allergic bronchopulmonary aspergillosis.

65.5–100.0). Mid-turbinate swabs trended toward being more sensitive than sputum samples (p=0.0625). We did not calculate sensitivities and specificities for the mid-turbinate/throat swabs paired samples due to limited numbers.

4. Discussion

The proportion of virus-associated exacerbations documented in our study is similar to those demonstrated in recent studies using molecular-based techniques (60.0% and 49.8%) [20] and significantly higher than those found in prior studies using conventional diagnostic methods (<15%) [4,5]. These discrepancies can be attributed to the higher sensitivity and broader range of virus detection afforded by molecular-based methods leading to a better estimation of the true prevalence of respiratory viruses in CF exacerbations.

Rhinoviruses have been reported as major pathogens in CF exacerbations [1,22] with detection rates reaching 87% from samples collected both from exacerbations and routine visits [20]. Ours and another study [1] reported similar rates of rhinovirus infection (17.6% vs 15.9%) from samples collected during exacerbation. Rhinovirus and coxsackie/echovirus comprised half of our identified viruses. Given the ResPlex II v2.0 assay's suboptimal performance in distinguishing rhinovirus from coxsackie/echovirus, our true rate of rhinovirus detection might have been even higher [21].

Our study documented a low proportion of pH1N1 (5.3%) even though it was conducted during the larger second wave of the 2009 influenza with a modest pH1N1 vaccine uptake rate (46.5%). Another study [1] conducted during a typical influenza season reported higher influenza A and B detection rates (25%) despite a high influenza vaccination uptake of 70%.
These discrepancies may be related to the perfect matching of the pH1N1 vaccine to the circulating influenza viruses during our study period.

Compared to another study [1], our rate of viral coinfection was significantly higher (34.6% vs 4.3%). These discrepancies can be attributed to the broader range of virus detection afforded by the ResPlex II v2.0 assay compared to other molecular-based methods used in this study (detection of 18 viruses vs 9 viruses) [1]. Multiplex assays permit the improved identification of cases of infection with multiple agents although the impact of multiple viral infections on the clinical course of disease is at present unknown and worthy of further study [21].

To our knowledge, this is the first study to assess the severity of viral-associated pulmonary exacerbations and their impact on quality of life. Virus-positive subjects had significantly higher severity and lower quality of life scores. Our ability to detect a difference in severity scores between the two groups but not other metrics of pulmonary exacerbation severity may reflect the increased statistical power afforded by the quantitative nature of the severity and quality of life scores. Another study [22] suggested that children in whom a virus other than rhinovirus or coxsackie/echovirus was identified had a significantly greater decline in FEV1 from baseline compared to children with a rhinovirus infection. However, our repeat analyses after excluding the rhinovirus and coxsackie/echovirus-positive patients did not change the above findings other than the difference in the CFCS which became insignificant. This might reflect a decreased discriminative ability of CFCS compared to the CFQ-R, the underpowered sample size after excluding rhinovirus and coxsackie/echovirus-positive patients, and/or a biased estimate resulting from missing CFQ-R data.

Several studies have correlated repeated bacterial infections in CF patients with increased airway inflammation, whereas the pathophysiology of virus-induced CF exacerbation remains unclear [9–11]. In our study, levels of sputum IL-8, neutrophil percentage and neutrophil elastase were within ranges of published data [9,17] whereas sputum neutrophil cell counts were lower than what have been described in the literature [9,17]. Our study included outpatients with milder exacerbations and consisted of a younger patient-population which may have resulted in a milder degree of airway inflammation and therefore in lower sputum neutrophil counts. We also found levels of inflammatory cytokines in both groups to be similar but reported a two-log lower mean total bacterial density in virus-positive subjects compared to the virus-negative ones (albeit not significant), likely due to the younger age of virus-positive subjects. A recent study [23] reported a trend toward lower cytokine production in CF airway epithelial cells following viral infection. Whether respiratory viruses themselves or by interaction with bacteria induce the observed inflammation remains controversial and requires further investigation.

The data available for the optimal method for respiratory viral detection is limited and controversial [1]. Our study reported a higher rate of viral detection with mid-turbinate swabs than with sputum samples in contrast with another recent study which reported higher concordance rates (87% for rhinoviruses detection and 92% for other virus types) [24]. The difference in timing of sampling between this study (23% of samples collected during exacerbation) and ours (100% during exacerbation) might have contributed to these observed differences. A larger study comparing test performance characteristics of paired upper airway and sputum samples collected during exacerbations would provide insights into the above findings.

Our study has several limitations. The observational design limits our ability to exclude the effect of measured or unmeasured covariates in our analysis. Despite the central importance of pulmonary exacerbations as an outcome measure in CF clinical trials, no standardized definition of pulmonary exacerbation that includes children under the age of 6 has been validated. In using a broader definition of pulmonary exacerbation which includes children managed as outpatients, our sample may have included more patients with mild exacerbations than other studies. However, the proportion of hospitalized patients with viral infection in our study (64%) was similar to that of outpatients (58%). Likewise, there are no generally accepted severity scores for pulmonary exacerbations that apply to younger children, including the CFQ-R, a validated and widely used patient-reported outcome. Despite its validation against PFTs as the gold standard, the CFCS has been infrequently used in clinical trials. This highlights the need for further evaluation of its applicability in clinical studies. Our small sample size did not permit subgroup analyses according to individual viruses. Finally, we did not have data on viral infection status in CF children not experiencing an exacerbation as a comparator to strengthen the association between the exacerbation and viral infections. Compared to other viruses such as RSV, rhinoviruses can shed for a prolonged period (>3 weeks vs <1 week) [25] and may have been acquired before the exacerbation. However, virus-positive subjects were more likely to present with fever upon exacerbation.

In conclusion, respiratory viruses, especially coxsackie/echovirus and rhinovirus are frequently associated with pulmonary exacerbations in pediatric CF patients. Systematic detection of respiratory viruses by molecular techniques using mid-turbinate swabs should occur in the setting of a pulmonary exacerbation. The relative contribution of repeated respiratory viral infections in young children to pulmonary inflammation and continuing decline in lung function seen from an early age is not known. A longitudinal study which documents the decline in FEV1 over time and comprehensively evaluates patients for both viral and bacterial infections during exacerbations might help to clarify the contribution of respiratory viruses to progressive lung damage.

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Conflicts of interest

The authors had no financial or other forms of conflicts of interest.

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