Journal of Cystic Fibrosis 13 (2014) 661 – 666

Journal of Cystic Fibrosis

www.elsevier.com/locate/jcf

Original Article

Feasibility of parental collected nasal swabs for virus detection in young children with cystic fibrosis

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Received 3 December 2013; received in revised form 4 February 2014; accepted 24 February 2014
Available online 15 March 2014

Abstract

Background: The detrimental role of viruses has been well described in CF, although the pattern of virus infections has not been investigated in a longitudinal study. The primary aim was to determine the feasibility of fortnightly parent collected swabs in young children with CF.
Methods: Children under three years with CF were recruited. Nasal swabs were collected by parents every fortnight and during periods of symptoms over 12 months. Nasal swabs were posted and virus detected using real-time PCR.
Results: Only 27% of the patients completed the study to 10 months, although 98% of the swabs returned were adequate for analysis. Mould was observed growing on 23% of the returned swabs. There was no evidence to demonstrate relationships with symptoms and viruses, prolonged symptoms, prolonged shedding or patterns of virus infections.
Conclusions: This study highlights the need to further investigate the role of viruses in children with CF using a robust method of frequent collection in children for a longitudinal study, with appropriate storage and shipping techniques to avoid mould growth or other potential contaminants.

Keywords: Virus; Cystic fibrosis; Paediatric

1. Introduction

Cystic fibrosis (CF) is a common hereditary condition amongst Caucasians affecting multiple body systems, in particular the respiratory system. Onslaughts of infection and inflammation lead to progressive lung damage which begins early in life [1]. The role of viruses contributing to worsening symptoms and associations with exacerbations and hospitalisations in children with CF has been well described [2–5].

Children, defined as under the age of 18 years, with CF have increased number of viruses [6], increased viral load [7], longer periods of upper and lower respiratory infections (URI and LRI) [5], and increased rates of hospitalisation [2] compared to controls. Isolation of virus, either by serology or nasal swabs, was associated with worse clinical outcomes including FEV_1, Shwachman scores and days of intravenous antibiotics [3,4].

This evidence suggests that infection with virus plays a significant role in the pathogenesis of CF. However, the epidemiology of virus infections in children with CF, particularly during asymptomatic periods and over a period of time, is not known.

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http://dx.doi.org/10.1016/j.jcf.2014.02.009
1569-1993/

Previous studies in community cohorts have used nasal swabs from infants, collected by parents in their home, to detect virus with a high rate of parent compliance at 74% [8]. Frequent collection of nasal swabs for the detection of virus in a cohort of children with CF would provide valuable information on prevalence of viruses during asymptomatic periods, as well as during periods of symptoms or exacerbations. However, the feasibility of collection in a group such as this, in comparison to a healthy cohort, has not been investigated.

The main aim of this pilot study was to determine the feasibility of fortnightly parent collected swabs in young children with CF. The secondary aims of this study were to identify changes in virus detected between periods of symptoms and no symptoms, and vice versa; and to observe differences in the type of virus detected, and virus shedding, over a 12 month period in a sub-set of children.

2. Methods

This study was conducted between May 2010 and November 2011 at the Princess Margaret Hospital in Perth, Royal Children's Hospital in Melbourne and Royal Children's Hospital in Brisbane, Australia. Children under the age of three years with a diagnosis of CF were recruited for the 12 month study.

2.1. Ethics

Ethics was obtained at each site from the Princess Margaret Hospital for Children Ethics Committee EC00268 (Approval number 1762/EPP), Royal Children's Hospital Human Research Ethics Committee EC00238 (Approval number 30086), and Children's Health Services Human Research Ethics Committee EC00175 (Approval number HREC/10/QRCH/24). Consent was obtained from the parents of all participants recruited into the study.

2.2. Study protocol

Baseline demographics including age, CF genotype and pancreatic sufficiency were obtained. Parents were asked to complete a daily diary for presence of solicited symptoms including: fever, wheeze, shortness of breath, moist cough, pneumonia, ear infection, runny nose, sore throat, cough, muscle aches, chills, sore head, irritability, lethargy or vomiting.

Parents were taught by research staff on how to collect an anterior nasal swab from their child using a flocked cotton swab (147CV viral transport tube, COPAN). The swab transport tube contained a foam pad soaked in viral transport medium. Nasal swabs were collected every fortnight (routine swabs) and within three days of the beginning of respiratory symptoms (symptomatic swabs). Respiratory symptoms were wheeze, shortness of breath, moist cough, pneumonia and cough.

Parents returned the daily symptom diary and the routine and symptomatic nasal swabs by post every fortnight. Parents were contacted by the study coordinator every fortnight and reminded to take routine swabs from their child.

2.3. Symptom classification

Respiratory symptoms were classified as upper respiratory infections or lower respiratory infections based upon criteria from a previous study [9]. Upper respiratory infections were classified upon presentation of symptoms: runny nose or cough with no other respiratory symptoms. Lower respiratory infections were classified upon presentation of symptoms: wheeze, moist cough or shortness of breath.

2.4. Virus analysis

Upon receiving samples at the research laboratory, swabs were frozen at −80 °C until analysis. Quality of collection of the nasal swabs and assessment of extraction efficiency were carried out using previously described methods [10]. Briefly, samples are spiked with Equine Herpes Virus to assess extraction efficiency, and nasal specimens are assessed by determining the presence of a marker of human genetic DNA [10,11].

Real-time PCR assays were performed on samples for detection of the following viruses: picornaviruses (rhinoviruses and enteroviruses), influenzae A & B, human metapneumovirus (HMPV), parainfluenzae virus types I, II and III, respiratory syncytial virus (HRSV-A and HRSV-B), adenovirus, bocavirus, polyomavirus (hPy-V-WU, hPyV-KI), and coronavirus (OC43, 229E, NL63 and HKU1) [10]. Appropriate positive and negative controls were used.

2.5. Statistics

Data are presented as mean and standard deviation (SD) unless otherwise specified. During periods of symptoms (URI, LRI and/or fever) which continued for three or more days, the mean duration of symptoms in children where no virus was detected was compared to periods where virus was detected using a Mann–Whitney test. Data were excluded from this analysis if there were no swabs taken during periods of symptoms, and if symptom information was missing either side of the symptom event.

3. Results

3.1. Feasibility

A total of 74 parents of children with CF were approached with intent to stay in the study for the 12 month study duration. Consent was formally withdrawn from nine participant's families for reasons including; time commitment, child refused swabs, additional time with physiotherapy and other treatments, and parent unwell and unable to devote additional time to research. The samples these participants returned were included in the analysis. Only 20 children completed the study to at least 10 months.

A total of 930 swabs were returned. Two swabs were excluded as they were not labelled with a patient ID. Of the remaining swabs 738 were routine, 168 were symptomatic swabs, and 7 swabs were returned unlabelled as either routine