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Original Article

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

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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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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

or symptomatic. Fifteen swabs (1.6%) were excluded as the sample that was provided was not adequate for analysis. The mean (±standard deviation) time between sample collection and freezing in the laboratory was 6.1 ± 8.3 days.

Mould was observed growing on 23% of the swabs, ranging from low growth (14%) to high growth (9%). Mould growth was highest in Perth with 34% of the swabs affected, followed by Melbourne with 23% of the swabs affected, then Brisbane with 17% of the swabs affected. Swabs were still analysed regardless of mould contamination. The time from swab collection to freezing the swabs at −80 °C in the laboratory, and season had an effect on mould growth (Table 1).

3.2. Cross-sectional analysis

409 swabs were collected from 54 children over a period of 2.87 years, with the mean number of swabs returned per patient as 8 ± 5 swabs. The mean time in the study for this group was 5.3 ± 3.9 months with the mean age at the start of the study as 1.79 ± 0.86 years. Table 2 displays the demographics of this group.

Of the 96 symptom swabs that were returned, 37 (38.5%) were positive for at least one virus. Of the 313 routine swabs that were collected, 66 (21.1%) were positive for at least one virus. Fig. 1 demonstrates viruses detected from routine and symptomatic swabs by season. Of the viruses tested, rhinovirus was the most common virus detected. More symptomatic swabs were returned during winter, which was also the period when the highest variety of viruses was detected.

There were 12 cases where more than one virus was detected. The majority of cases were rhinovirus detected in conjunction with coronavirus (n = 1), polyomavirus (n = 4), adenovirus (n = 3), bocavirus (n = 2) or parainfluenzae (n = 1).

Table 1
Mould growth by site, season and time to freezing.

		Low growth	High growth	Total with mould
Site (% of swabs infected/swabs collected by site)				
Brisbane		11	6	17
Melbourne		13	10	23
Perth		20	13	34
Season (% of swabs infected/swabs collected by in that season)				
Autumn		12	11	24
Winter		15	10	25
Spring		14	8	22
Summer		14	8	22
Time to freezing (% of swabs infected/swabs collected by site)				
Brisbane	0–4 days	7	0	7
	5–9 days	23	21	45
	≥10 days	20	35	55
Melbourne	0–4 days	8	3	11
	5–9 days	14	16	30
	≥10 days	22	33	55
Perth	0–4 days	7	3	11
	5–9 days	25	6	31
	≥10 days	28	26	55

Table 2
Demographics of participants.

	Cross-sectional population	Longitudinal population
n	54	20
Age (years) at first swab (mean ± SD)	1.79 ± 0.86	1.47 ± 0.81
Number of swabs collected per patient	8 ± 5	25 ± 3
Time (months) in study	5.3 ± 3.9	11.9 ± 1.1
Pancreatic sufficient (n(%))	10 (18.5)	1 (0.5)
CF genotype (n(%))		
Homozygous Phe508del	31 (57.4)	13 (65)
Heterozygous Phe508del	20 (37.0)	7 (35)
Other	3 (5.6)	0 (0)

One case was identified where bocavirus and polyomavirus were detected together.

In participants who returned more than one swab, a sub-set analysis was undertaken to determine differences in virus detected from a swab collected at the time of URI and/or LRI ("symptomatic") followed by an asymptomatic swab. Analysis was conducted on 23 children from 37 visit pairs with a mean time difference between symptomatic and asymptomatic swabs of 18 ± 8 days. As Table 3 demonstrates there was no clear pattern of virus detected between periods of symptoms followed by clearance of symptoms.

A second analysis was conducted to look at the differences in virus detected from an asymptomatic swab followed by a symptomatic swab. Analysis was conducted on 18 children from 35 visit pairs with a mean time difference between asymptomatic and symptomatic swabs of 16 ± 12 days. As Table 3 demonstrates there was no clear pattern of virus detected between periods of no symptoms followed by onset of respiratory infection.

3.3. Longitudinal analysis

An average of 25 ± 3 swabs over a period of 11.9 ± 1.1 month was collected from 20 children. These children were not part of the cross-sectional population. Table 2 displays the demographics for this population. On average, children had 2.9 ± 3.1 LRI and 5.9 ± 3.4 URI over the study period. This equated to, adjusting for the amount of time each patient was enrolled in the study, an average of 0.2 ± 0.2 LRI/child/month and 0.5 ± 0.3 URI/child/month.

Data were observed for patterns of viral infections and symptoms. 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 13.4 ± 8.7 days compared to periods where virus was detected which was 14.8 ± 12.3 days (p = 0.93). There were no clear patterns between onset of symptoms and isolation of virus, duration of symptoms and isolation of type of virus, or isolation of virus and duration or intensity of symptoms.

There were only eight cases where a new virus was detected following clearance of another virus. There were not enough data to analyse to determine if infection with one type of virus increases susceptibility for infection with another virus. Over

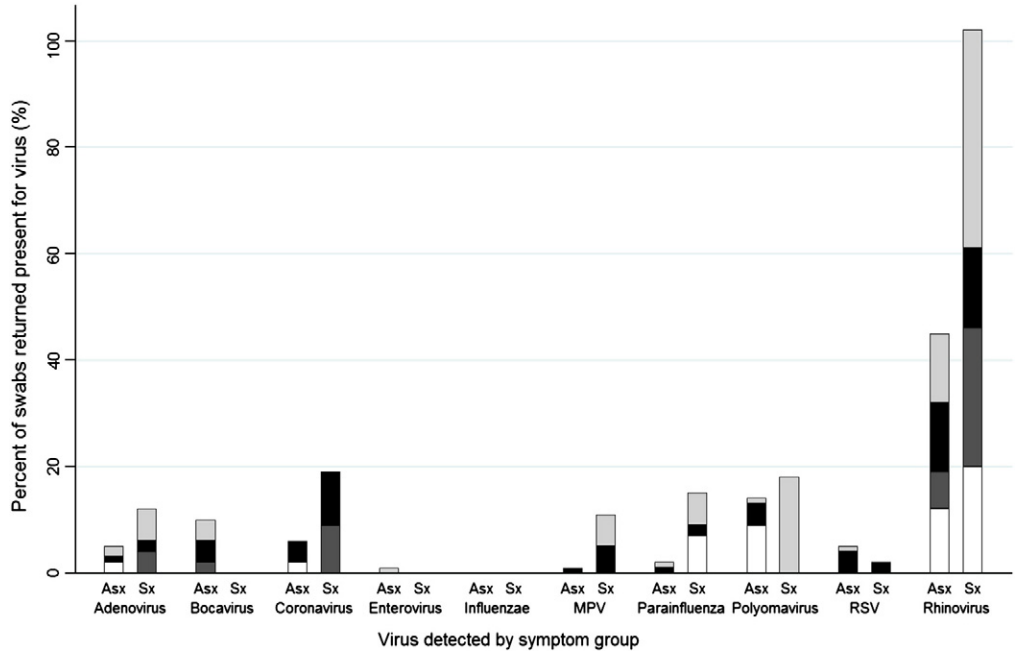

Fig 1. Percent of nasal swabs returned which were positive for virus. Data are presented by virus, month collected, and the presence or absence of respiratory symptoms. Asx = asymptomatic; Sx = symptomatic; White shading = summer, Dark grey shading = autumn, Black shading = winter; Light grey shading=spring.

the course of the study five children were hospitalised with one child admitted twice.

3.4. Virus shedding

Periods of virus shedding were analysed in the longitudinal population of 20 participants. Overall, swabs were still positive for virus for 13.0 ± 9.3 days, with swabs negative after 15.2 ± 7.7 days. Table 4 shows the shedding time by virus, although data for each virus is limited due to small numbers of infections.

4. Discussion

The primary aim of this pilot study was to determine if fortnightly parental collected nasal swabs were feasible in infants and young children with cystic fibrosis. While the sample collection was of good quality, the number of participants who completed the study was small. This indicates that parental collected fortnightly swabs in a population of young children with cystic fibrosis may not be feasible without increased support to participating families.

Detection of mould on swabs was unexpected, and infection rates of 23% were high. The presence of mould was consistent across seasons and was associated with location and time from collection to freezing. We are confident that the presence of mould did not compromise the detection of virus based on our results, and results from other studies [12,13]. While there was an increase in the variety of viruses detected on swabs during winter in the present study, this is likely to be clinically related, rather than a methodological issue due to storage temperatures. However, we would recommend that parents store swabs in the home freezer and return either by courier on ice, or deliver the swabs to the site using cold-blocks and insulated bag to limit mould contamination.

Table 3
Virus isolated between pairs of symptomatic and asymptomatic swabs.

	n	Time between swabs (days)	Symptoms (n)		
			URI	LRI	Both
Symptomatic swab followed by an asymptomatic swab					
No virus detected at either visit	21	18 ± 8	10	3	8
Virus detected at both visits	5	15 ± 8	2	2	1
Virus detected at symptomatic (first) visit only	8	19 ± 9	5	2	1
Virus detected at asymptomatic (second) visit only	3	15 ± 2	1	2	0
Asymptomatic swab followed by a symptomatic swab					
No virus detected at either visit	17	19 ± 14	5	5	7
Virus detected at both visits	1	15	0	1	0
Virus detected at asymptomatic (first) visit only	6	14 ± 9	3	2	1
Virus detected at symptomatic (second) visit only	11	14 ± 11	6	0	5

Table 4
Duration of shedding by virus.

Virus	Time to next negative swab (days)	
	n	Mean ± SD or [actual values]
Adenovirus	2	9.0 ± 7.1
Bocavirus	12	20.4 ± 10.1
Coronavirus	9	12.8 ± 3.7
Enterovirus	1	[5]
Influenza	1	[27]
MPV	3	13.7 ± 5.5
Parainfluenzae	3	16.3 ± 4.0
Polyomavirus	12	17.3 ± 10.0
Rhinovirus	58	14.5 ± 7.0
RSV	4	12.8 ± 2.8
Total		**15.2 ± 7.7**

The collection of swabs from parents was of good quality, with only 15 of 932 (1.6%) swabs inadequate. However, of the 75 children enrolled in the study, only 20 children (26.6%) completed 10 or more months of fortnightly collection. This is in comparison to another study in a community cohort of healthy preschool children where the return rates of parental collected nasal swabs during periods of acute respiratory infections over a period of 12 months were 74% [8].

This indicates that in this population collection of fortnightly swabs may be a large burden on these families. Children with CF need to undergo physiotherapy twice daily, plus follow a strict medication regime. These treatments, plus participating in a research study requiring a daily diary and fortnightly swabs may be too much for some parents. A previous Australian study has reported poor adherence to physiotherapy, with 50.4% of patients aged 6 months to 6 years not compliant [14]. This study also reported sleep problems (53.5%) and eating problems (40.2%) in this age group of children with CF, as well as poor parent mental health with depression (33.3%), anxiety (16.4%) and stress (34.2%) all within the clinical range [14].

During the study, depending on the site, parents were contacted by phone every two weeks. Diary cards were paper based, and parents needed to post the swabs every two weeks using reply paid envelopes which could be posted using normal postage methods. To improve the compliance in a study such as this, a number of methods could be employed. Firstly, it is important that a single researcher establishes a good relationship with the parent and the family. This helps the family to feel that they have a support person they can contact during the study. Web-based diaries that could be filled out by parents, or depending on the budget of the project personal electronic diaries, have been shown to improve diary card completion [15,16]. While reply-paid envelopes that could be posted in standard Australia Post letterboxes were considered the most efficient and cost-effective way of posting swabs, perhaps if parents froze the nasal swabs and then a courier service collected them it may help to improve compliance. However, courier services, particularly with frozen samples, are expensive.

While the burden of a daily diary and fortnightly swabs in this age group may be too great for participants and their parents, fortnightly swabs were not frequent enough to provide us with enough information about the pattern of viral infection in this group. There was no evidence from the present study to demonstrate relationships with symptoms and viruses, prolonged symptoms with virus isolation, prolonged virus shedding or patterns of virus infections in children with CF.

Rate of infections with URI in this study at 0.5 ± 0.3 URI/child/month (this equates to 5.8 ± 3.4 URI/child/year) was at the upper end of the number of infections reported in previous studies in healthy preschool children with rates of 5.8 ARI/child-month, 2.8 illness/child/year and 4.1 ARI/child/year [8,17,18]. However, the data from the referenced studies were collected in children attending daycare, or in the first year of life and may be an over-representation of the number of infections expected in healthy children.

Rhinovirus was the most commonly detected virus in this study, at a mean prevalence of 14% in the cross-sectional cohort, varying between 7 and 41% due to season. Similar rates have been observed in previous studies which range from a mean prevalence of 8% in infants [19] to between 7 and 27% in children [6,7] and up to 84% in adults [3] with cystic fibrosis. Prevalence of other viruses reported in the present study varied compared to other studies in cystic fibrosis. Prevalence of coronavirus was similar, although slightly higher in the present study at 3% compared to others between 0 and 1.5% [6,20]. Prevalence of parainfluenzae was less in the present study at 1% compared to up to 17% in other studies [2,3,19,20]. As expected rates of RSV were lower in this study at 1% compared to infant studies which reported a prevalence of 23% [2,3,6,19,20]. No influenza was detected in the cross-sectional group in the present study, although studies have reported varied rates between 1.5 and 30% [2,3,6,19]. The prevalence of adenovirus was similar to another study in adults, although less than others in children [2,3,20]. Differences in the prevalence of virus between studies can be explained by a number of factors: age of children (e.g. RSV is more common in infants), time of year when samples were taken (e.g. more influenza in winter), if samples were taken at time of an exacerbation or when the participants were well or symptom free, and if nasal/throat swabs or bronchoalveolar lavage or nasopharyngeal aspirate was used.

Data from the present study must be interpreted with caution as only a small sample size was used, and no data were collected to compare viral infections with bacterial infection, or structural and/or functional changes in this group. What this study does show is that even during periods of no symptoms, virus may be present in young children with CF; and infections do not seem to differ compared to healthy children, although the consequences of infections may be greater in older children [2–4].

In conclusion, researchers need to be aware of the equilibrium between the burden of research on families and the scientific value of results. This study highlights the need to further investigate the role of viruses in children with CF with a robust method of frequent collection in children for a longitudinal study. Researchers need to give consideration to good techniques for parent support and select families who would be most compliant without introducing bias.

Conflict of interest statement

All authors declare no conflict of interest.

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References

[1] Sly PD, Brennan S, Gangell C, de Klerk N, Murray C, Mott L, et al. Lung disease at diagnosis in infants with cystic fibrosis detected by newborn screening. Am J Respir Crit Care Med 2009;180:146–52.
[2] Hiatt PW, Grace SC, Kozinetz CA, Helioui Raboudi S, Treece DG, Taber LH, et al. Effects of viral lower respiratory tract infection on lung function in infants with cystic fibrosis. Pediatrics 1999;103:619–26.
[3] Olesen HV, Nielsen LP, Schiotz PO. Viral and atypical bacterial infection in the outpatient pediatric cystic fibrosis clinic. Pediatr Pulmonol 2006;41:1197–204.
[4] Smyth AR, Smyth RL, Tong CYW, Hart CA, Heaf DP. Effect of respiratory virus infections including rhinovirus on clinical status in cystic fibrosis. Arch Dis Child 1995;73:117–20.
[5] van Ewijk BE, van der Zalm MM, Wolfs TFW, Fleer A, Kimpen JLL, Wilbrink B, et al. Prevalence and impact of respiratory viral infections in young children with cystic fibrosis: prospective cohort study. Pediatrics 2008;122:1171–6.
[6] Wat D, Gelder C, Hibbitts S, Cafferty F, Bowler I, Pierrepoint M, et al. The role of respiratory viruses in cystic fibrosis. J Cyst Fibros 2008;7:320–8.
[7] Kieninger E, Singer F, Tapparel C, Alves MP, Latzin P, Tan H-L, et al. High rhinovirus burden in lower airways of children with cystic fibrosis. Chest 2013;143:782–90.
[8] Lambert SB, Allen KM, Druce JD, Birch CJ, Mackay IM, Carlin JB, et al. Community epidemiology of human metapneumovirus, human coronavirus NL63, and other respiratory viruses in healthy preschool-aged children using parent-collected specimens. Pediatrics 2007;120:e929–37.
[9] Kusel MMH, Kebadze T, Johnston SL, Holt PG, Sly PD. Febrile respiratory illness in infancy and atopy are risk factors for persistent asthma and wheeze. Eur Respir J 2012;39:876–82.
[10] Lambert SB, Ware RS, Cook AL, Maguire FA, Whiley DM, Bialasiewicz S, et al. Observational Research in Childhood Infectious Diseases (ORChID): a dynamic birth cohort study. BMJ Open 2012;2:e002134.
[11] Lu X, Holloway B, Dare RK, Kuypers J, Yagi S, Williams JV, et al. Real-time reverse transcription-PCR assay for comprehensive detection of human rhinoviruses. J Clin Microbiol 2008;46:533–9.
[12] Johansen CA, Hall RA, van den Hurk AF, Ritchie SA, MacKenzie JS. Detection and stability of Japanese encephalitis virus RNA and virus stability in dead infected mosquitoes under different storage conditions. Am J Trop Med Hyg 2002;67:656–61.
[13] Pyankov OV, Agranovski IE, Pyankova O, Mokhonova E, Mokhonov V, Safatov AS, et al. Using a bioaerosol personal sampler in combination with real-time PCR analysis for rapid detection of airborne viruses. Environ Microbiol 2007;9:992–1000.
[14] Ward C, Massie J, Glazner J, Sheehan J, Canterford L, Armstrong D, et al. Problem behaviours and parenting in preschool children with cystic fibrosis. Arch Dis Child 2009;94:341–7.
[15] Palermo TM, Valenzuela D, Stork PP. A randomized trial of electronic versus paper pain diaries in children: impact on compliance, accuracy, and acceptability. Pain 2004;107:213–9.
[16] Stone AA, Shiffman S, Schwartz JE, Broderick JE, Hufford MR. Patient compliance with paper and electronic diaries. Control Clin Trials 2003;24:182–99.
[17] Kusel MMH, De Klerk N, Holt PG, Kebadze T, Johnston SL, Sly PD. Role of respiratory viruses in acute upper and lower respiratory tract illness in the first year of life. Pediatr Infect Dis J 2006;25:680–6.
[18] Martin ET, Fairchok MP, Kuypers J, Magaret A, Zerr DM, Wald A, et al. Frequent and prolonged shedding of bocavirus in young children attending daycare. J Infect Dis 2010;201:1625–32.
[19] Armstrong D, Grimwood K, Carlin JB, Carzino R, Hull J, Olinsky A, et al. Severe viral respiratory infections in infants with cystic fibrosis. Pediatr Pulmonol 1998;26:371–9.
[20] Asner S, Waters V, Solomon M, Yau Y, Richardson SE, Grasemann H, et al. Role of respiratory viruses in pulmonary exacerbations in children with cystic fibrosis. J Cyst Fibros 2012;11:433–9.