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Respiratory viral infections in Western Australians with cystic fibrosis

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ABSTRACT

Background: Viral respiratory infections (VRI) in people living with Cystic fibrosis (CF) is less well understood than respiratory bacterial infections, particularly adults with CF and few studies have compared children with adults. This study evaluated the frequency of respiratory viruses in patients with cystic fibrosis (CF) in Western Australia (WA). We determined the VRI in CF and compared them with non-CF patients. Further, we compared CF patients that were hospitalised with those that were not.

Patients/methods: Nucleic acid from sputum of 157 CF and 348 non-CF patients was analysed for influenzavirus A (Flu A) and B, (Flu B), respiratory syncytial virus (RSV), human metapneumovirus (hMPV), human rhinovirus (RV), and parainfluenza viruses (PIV 1-3) by RT-PCR, during the 2016 winter respiratory season.

Results: No significant difference in the frequency of respiratory virus detection between CF and non-CF patients was found. RV was the most frequently detected virus in CF patients, and in hospitalised CF. RSV and hMPV were found less frequently in CF patients and RSV was not found in any hospitalised CF patient. A trend for fewer influenzavirus detections in adult CF patients was observed, however the trend was opposite for paediatric patients. RV and Flu A were the most common viruses detected in hospitalised CF patients.

Conclusion: There was no significant difference in VRI between CF and non-CF patients. RV and influenza A were most commonly found in hospitalised CF patients, suggesting that infection with these viruses may contribute to hospitalisation for CF respiratory exacerbations.

1. Background

Currently, CF mortality is predominantly attributed to its respiratory manifestations [1]. This includes recurrent bacterial infections with chronic inflammation, mucoid impaction of the bronchi and bronchiectasis [1,2]. Together these contribute to the respiratory exacerbations observed in CF, which cause irreversible damage to the airways, disease progression and ultimately premature death [1,3].

It has been observed that the range of bacteria infecting the respiratory tract differs between otherwise healthy individuals and those with CF [4]. The most notable bacterial pathogen in CF is *Pseudomonas aeruginosa* [5,6] with other bacterial pathogens including *Staphylococcus aureus*, *Burkholderia spp* and *Haemophilus influenzae* [4,7,8].

Although bacteria are strongly associated with pulmonary exacerbations in people with CF [3,7], the role of viral respiratory infections (VRI) is less well understood. This is despite the fact that pulmonary exacerbations due to VRI have demonstrated a poorer response to treatment, greater deterioration in lung function, and reduced time to next exacerbation [9,10]. Furthermore, several studies have demonstrated that, although infants with CF acquire viral infections at the same frequency as their non-CF counterparts, they are more likely to develop symptoms and become hospitalised [11,12].

Historically CF studies have concentrated on children, however the upward trend in survival highlights the need for comparable studies to be conducted in adults living with CF. The studies of respiratory exacerbations in adult CF patients to date have mostly investigated bacterial infections [6,7] with few studies focusing on viral respiratory infection (VRI), and those that have either lacked control populations or had low statistical power. Even fewer studies have been conducted over the span of a defined respiratory virus season. Nevertheless, recent studies have identified that human rhinovirus (RV) is frequently associated with pulmonary exacerbations in people with CF [13,14], and viruses such as

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influenza virus A (Flu A) and B, (Flu B), respiratory syncytial virus (RSV), human metapneumovirus (hMPV), and parainfluenza viruses 1, 2 and 3 (PIV 1-3) can be regularly detected [9,10,15,16].

This study using molecular methods to detect respiratory viruses was conducted to explore the extent of VRI in adults and children with CF compared to their non-CF counterparts throughout the 2016 Western Australian (WA) respiratory virus season. In addition, comparisons were made between CF respiratory exacerbations that did or did not result in hospitalisation, as an indicator for respiratory exacerbation severity.

2. Materials and methods

2.1. Specimens and patients

This prospective study was conducted using sputum samples received at the PathWest Laboratory Medicine WA Queen Elizabeth II Medical Centre laboratory which performs the microbiology investigations for the WA adult (>18yrs) and paediatric (<18yrs) tertiary CF medical centres. The sputa were collected during the 2016 WA winter respiratory virus season (March to November) from adult and paediatric CF and non CF patients (Table 1).

The non-CF control group of specimens were matched for date of collection, age range and gender corresponding to the CF samples received each day during the study (Table 1).

Sputum samples were used since this type of respiratory sample is routinely collected from both CF and non-CF patients during acute exacerbations of lower respiratory tract disease. Standard sputum collection procedures were followed and no induced sputa was used. Age, sex and hospitalisation data was obtained from the PathWest laboratory information system.

2.2. Sample preparation and nucleic acid extraction

Sputum was sampled for bacteriology, mycology and mycobacteriology culture using standard laboratory methods, if requested, and then processed for respiratory virus detection. The sputum was suspended in virus transport medium prior to vortexing. Each sample was treated with 20 μL of 0.25 μM dithiothreitol (DTT) per 500 μL and digested for 1 h at 37 °C.

Viral RNA (vRNA) was extracted from all samples using the MagNA Pure 96 automated extraction system (Roche Diagnostics Australia Pty Ltd, http://www.roche-australia.com) with a treated sputum input volume of 200 μL and elution volume of 100 μL using the Viral NA Small Volume extraction protocol. The lysis buffer was spiked with a known concentration of MS2 coliphage RNA during the extraction protocol to serve as an internal RNA extraction and PCR control.

Table 1
Patient characteristics and number of sputum collections.

Characteristic	CF	non-CF	*p*
Number	**157**	**348**	
Adult	95	310	
Child	62	38	
Mean Age	**25**	**39**	
Adult	26	43	*0.1455 (child/adult)*
Children	13	10	
Gender (male%)	**84 (54)**	**153 (44)**	*0.0540 (male/female)*
Adult	53 (56)	136 (44)	
Child	31 (50)	17 (45)	
Patients with > 1 Sputa	**47**	**19**	*0.0001*
Adult	29	16	*0.0001*
Child	18	3	*0.0122*
Total Sputa Samples	**247**	**378**	
Adult	140	334	
Child	107	44	

2.3. Amplification by duplex RT-PCR

All samples were tested for respiratory viruses by real-time reverse-transcriptase PCR (RT-PCR). The viruses targeted were RV, Flu A, Flu B, RSV, hMPV and PIV types 1, 2 and 3. An 8 μL aliquot of vRNA template or control material was added to 12 μL of master mix in each reaction to give a final volume of 20 μL. Each reaction contained 1X qScript XLT 1-step RT-qPCR ToughMix (QuantaBio) with the addition of primers and probes (Supplementary Table 1).

A total of 7 real-time RT-PCRs were performed using the ViiA7 real-time thermocycling instrument (Applied Biosystems). The targets combined in each mix are described in Supplementary Table 1. Following an initial reverse transcription and total denaturation step (10 min at 50 °C and 3 min at 95 °C), each reaction was cycled for 15 s at 95 °C and 1 min at 55 °C, 45 times.

2.4. Data analysis

A two-tailed Fisher's exact test, using a 2 × 2 contingency table, was used for all statistical analyses. Statistical significance was defined as $p < 0.05$.

2.5. Ethics approval

All samples were residual specimens from routine diagnostic testing. Patient identifying data was removed before viral testing. The study was done as an audit of negligible risk to ascertain the benefit of viral testing in addition to bacteriology. Ethics approval was granted by the Office of Research and Development, Curtin University, Perth WA (Ethics Approval Number: RDHS-107-16).

3. Results

A total of 247 sputa collected from 157 people living with CF were included in the study. Of these, 95 (60%) samples were from adults and 62 (40%) samples were from children. A total of 378 sputa were collected from 348 non-CF patients. Of these, 334 (88%) samples were from adults and 44 (12%) samples were from children.

The patient characteristics of the CF and non CF patients are shown in Table 1. There were more multiple sputum samples received from adults ($p = 0.0001$) and children ($p = 0.0.0122$) with CF than from their non-CF counterparts during the study period. In the CF group 28 of 109 (26%) samples from children and 101 of 140 (72%) samples from adults cultured *Pseudomonas aeruginosa,* and 19 (17%) paediatric samples and 54 (39%) of adult samples cultured *Staphylococcus aureus*. *Burkholderia cepacia* complex was cultured from only 10 adult sputum samples. Almost half (43%) of the control group sputa did not undergo bacteriology, mycology or mycobacteriology assessment either because this testing was not requested or the specimen was deemed to be contaminated by oral flora on microscopy. Of the control group that underwent sputum culture 65% of sputa were reported as either growing normal oral flora or no growth. *Streptococcus pneumoniae* was cultured from 4 samples and *Mycoplasma pneumoniae* was detected by PCR in 9 samples. *Pseudomonas aeruginosa* was cultured from only 4 samples.

Viral respiratory infection detection rates from CF sputa was not increased compared to the non-CF sputa (40% and 45% of sputa with RVI, respectively) (Table 2). There were no differences between rates of VRI between CF and non-CF sputa from adults and children, nor between CF and non-CF males and females (Table 2). There were no differences in VRI rates between hospitalised and non-hospitalised CF patients and this held true for adults, children, males and females (Table 3). The culture rate of *P. aeruginosa* and *S. aureus* was similar between CF patient sputa with or without respiratory virus detection (56% vs 49% and 21% vs

Table 2
Respiratory virus detections in CF and non-CF patients.

Characteristic	CF patient sputa (%)	non-CF patient sputa (%)	*p*
Total	99/247 (40)	171/378 (45)	*0.4548*
Adult	49/140 (35)	149/334 (45)	*0.2221*
Child	47/107 (44)	22/44 (50)	*0.7515*
Male	50/131 (38)	70/160 (44)	*0.5852*
Female	46/116 (40)	100/218 (46)	*0.5298*

Table 3
Respiratory virus detections in hospitalised vs non-hospitalised CF patients.

Characteristic	Hospitalised CF patient sputa (%)	Non-hospitalised CF patient sputa (%)	*p*
Total	32/64 (50)	65/182 (36)	*0.2294*
Adult	15/34 (44)	33/106 (31)	*0.3469*
Child	16/29 (55)	33/78 (42)	*0.5683*
Male	16/31 (52)	37/100 (37)	*0.3577*
Female	18/33 (55)	30/82 (36)	*0.2732*

34%, respectively).

RV was the most frequently detected respiratory virus in CF patient sputa and non-CF patient sputa (Table 4). There were no statistical difference between CF and non-CF rates of RV detection overall, and this held true for both adult and paediatric samples (Table 4). Flu A was detected almost twice as frequently in the non-CF patients (10.6% compared to 5.7%) but this did not reach significance. RSV and hMPV were common detections in CF and non-CF paediatric sputa. RSV was detected similarly in CF and non-CF patients, however RSV was detected significantly more frequently in children with CF than adults with CF (7.5% and 0.7%, respectively, $p = 0.0122$). The detection of hMPV was also similar between the CF and non-CF patients (2.4% and 3.4%, respectively), and hMPV was detected in significantly more children than adults with CF ($p = 0.0165$) (Table 4). The number of detections of Flu B and PIV-3 was low (3.3% and 1.6%, respectively) in both the CF and non-CF patients.

Patient hospitalisation was used as an indicator of acute lower respiratory disease severity (Table 5). RV detection was associated with hospitalisation in CF patients (39%) compared with non-hospitalised CF patients (26.4%) although the difference did not reach statistical significance, this held true in both adults and children. A similar trend was seen with Flu A detection in hospitalised CF patients (10.9%) compared to non-hospitalised CF patients (3.8%), and this was true for adults and children. Interestingly, despite RSV detection being relatively common in CF patients, no hospitalised CF patient was found to have an RSV infection.

4. Discussion

Adults and children living with CF have an increased susceptibility to acute respiratory infections resulting in respiratory exacerbations [2,3,9,12,16]. These exacerbations cause irreversible damage to the airways, a decrease in pulmonary function and, ultimately, disease progression leading to premature death [3]. As such, respiratory infections are the precursor to the main cause of mortality in CF patients [11,17–19]. The relationship between exacerbations and bacterial infections, especially in paediatric populations, has been well documented [7]. However, despite being known to cause respiratory exacerbations with increased severity [3,9,10,20], there is limited information on the aetiology and frequency of VRI, especially in the adult CF population [9,10]. Furthermore, nosocomial *P. aeruginosa* acquisition is common and disease progression due to *P. aeruginosa* acquisition presents an important challenge to people with CF [5,21]. Therefore exploring the inter-relationship between CF and VRI could yield much needed data to assist in respiratory exacerbation prevention and management strategies for CF patients.

This study conducted during the WA 2016 respiratory virus season suggests that people with CF submitting sputum develop VRI at the same frequency as patients with other lower respiratory tract diseases who submit sputum (Table 2), which is consistent with previous studies [9,11]. This data suggests that people living with CF do not have more viral airway infections than people without CF. Likewise, we found no significant difference between the detection of VRI in males and females with CF and we did not find an association between VRI and either *P. aeruginosa* or *S. aureus* culture positivity. As the acute respiratory diseases in the non-CF patients were not characterised in this study it is not possible to investigate this further.

Overall, there were no significant differences in respiratory virus detections between hospitalised and non-hospitalised CF patients. The most frequently detected respiratory virus in adults and children with CF was RV, however, RV was not detected more often in CF patients than in non-CF patients. Previous studies have also reported RV to be the most common cause of VRI in CF patients [9–14,22], possibly due to the upregulation of the cellular receptors for RV, ICAM-1 and LDL, in CF epithelial cells [23]. Moreover, RV infection can increase adherence of *Staphylococcus* spp. and *Streptococcus* spp. to epithelial cells as a possible mechanism to facilitate secondary bacterial infections [24]. Therefore our findings support the conclusions of previous studies that people with CF have high rates of RV infection, further implicating this virus as a potential major cause of respiratory illness in CF patients [3,9,13,14,25]. Preventing hospitalisation is a major goal for the management of chronic respiratory diseases, such as chronic obstructive pulmonary disease (COPD), asthma [26] and CF. Since RV was detected in almost 40% of CF patients in this study, preventing RV infections could reduce the respiratory disease burden in CF thereby preventing or delaying respiratory disease progression.

There were fewer Flu A and Flu B detections in CF patients than non-

Table 4
Respiratory virus detections during the 2016 respiratory season in Western Australians with and without cystic fibrosis (%).

	CF	Non-CF Control	Adult CF	Adult Control	Paediatric CF	Paediatric Control
RV	28.7	21.4	29.3	20.0	29.0	31.8
Flu A	5.7	10.6	5.7	11.7	5.6	2.3
Flu B	1.6	4.2	1.4	5.1	3.7	2.3
RSV	3.6	4.5	0.7[#]	3.8	7.5[#]	9.1
hMPV	2.4	3.4	0.0*	3.0	4.7*	6.8
PIV 1	0.0	0.7	0.0	0.9	0.0	0.0
PIV 2	0.4	0.0	0.7	0.0	0.0	0.0
PIV 3	0.8	1.8	0.0	1.8	1.8	0.0

[#] Significant difference between RSV in adult and paediatric CF ($p = 0.0122$).
* Significant difference between hHMP in adult and paediatric CF ($p = 0.0165$).

Table 5
Respiratory virus detections in hospitalised and non-hospitalised Western Australians with cystic fibrosis (%).

	CF-Hosp	CF Control	Adult CF-Hosp	Adult CF Control	Paediatric CF-Hosp	Paediatric CF Control
RV	39.0	26.4	38.2	25.5	37.9	26.9
Flu A	10.9	3.8	11.8	3.8	10.3	3.8
Flu B	3.1	2.2	0.0	1.9	6.9	2.6
RSV	0.0	4.4	0.0	0.9*	0.0[#]	9.0*[#]
hMPV	1.5	1.6	0.0	0.0	3.4	7.7
PIV 1	0.0	0.0	0.0	0.0	0.0	0.0
PIV 2	0.0	0.5	0.0	0.9	0.0	0.0
PIV 3	1.5	0.5	0.0	0.0	3.4	1.3

* Significant difference between RSV in adult non-hospitalised CF control and paediatric non-hospitalised CF control groups ($p = 0.0228$).
[#] Although there were no paediatric hospitalised CF samples positive for RSV and 10.6% of non-hospitalised paediatric CF samples had RSV the difference did not reach statistical significance ($p = 0.1883$).

CF patients. Although the influenza vaccination status of the patients was not known, the WA CF treatment centres pursue an active influenza vaccination programme which may account for this. Flu A was more common in the hospitalised CF patients compared to non-hospitalised CF patients, consistent with the association of Flu A with hospitalisation [27].

RSV and hMPV were more commonly detected in children with and without CF than in adults with and without CF. This is unsurprising as these viruses have previously been identified as common causes for childhood VRI [28–30]. Interestingly, RSV was found in less than 1% of CF adults, while hMPV was not detected in any CF adult. Moreover, in addition to being less commonly detected in children with CF than non-CF children RSV was not detected in hospitalised children with CF. The reason for this requires further study.

There were several limitations in this study. Repeat samples collected from the same patients were included as separate events. It is acknowledged that a second collection may be due to a single event, however the usual practice of the WA CF Units is to collect a single sputum for each respiratory exacerbation, such that only 9 (4%) of the 247 CF sputa and 20 (5%) of the 378 non-CF patient sputa were collected from the same patient within one week. Use of sputum from non-CF patients may have introduced heterogeneity into the control group as non-CF patients were likely to be afflicted with a variety of acute and chronic lower respiratory tract diseases. However, restricting the control group to specific chronic lower respiratory diseases would have reduced the number of sputa available for the study period and introduced other biases due to poorer demographic matching. It is possible that more invasive lower respiratory tract sampling may have provided a different spectrum of respiratory virus detection but bronchoscopic washings and bronchioalveolar lavage (BAL) are not routinely performed in CF and non-CF patients during lower respiratory exacerbations. Moreover, the use of sputum provided the opportunity to assess for both upper and lower respiratory tract VRI potentially increasing the sensitivity of the study. We used hospitalisation as an indicator of respiratory disease exacerbation severity. Using hospitalisation as a marker of severity is imperfect, as reasons other than respiratory disease may result in hospitalisation. Also it is known that people with CF are often colonised by bacteria in the respiratory tract, particularly with *P. aeruginosa*, and that it is difficult to determine if symptoms are due to the viral or bacterial infection. Using a controlled study in part addressed these confounding factors in that a comparison of viruses infecting the respiratory tract could be made between CF and non-CF patients. This study was conducted over a single respiratory virus season in one geographic region of Australia. Further studies conducted over several years and from different locales would be needed to confirm these results. As expected, the *P. aeruginosa* culture positivity rate was higher in CF patient sputa than in non-CF sputa, especially in adult CF patients, but we did not find an association between VRI and *P. aeruginosa* culture. Due to the high rate of *P. aeruginosa* culture in CF patient sputa a much larger cohort would be required to demonstrate an association with VRI.

5. Conclusions

In this study we compared the respiratory virus detections in sputum collected from CF and non-CF patients for clinical investigation of acute respiratory disease during the 2016 WA respiratory virus season from March to November. There were no significant differences in VRI between CF and non-CF patients. RV infections were the most commonly detected VRI in CF patients. There was an observed increased trend for RV and Flu A to be detected in hospitalised CF patients compared to non-hospitalised CF patients. It is remarkable that although RSV was commonly detected in children with CF, infection with this virus was not associated with hospitalisation. Further studies to investigate the impact of viral infections of CF patients is warranted.

Authors contribution statement

Brian Brestovac: Contributions include – conceptualization, formal analysis, supervision and major role in writing.Charleigh Lawrence: data curation, investigation, writing (original draft). David Speers: conceptualization, formal analysis, review & editing. Leanne Sammels: data curation, methodology, project administration, supervision, review & editing. Siobhain Mulrennan: Resources, formal analysis, review & editing.

Declaration of competing interest

There are no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

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Appendix A. Supplementary data

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