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Ten years of viral and non-bacterial serology in adults with cystic fibrosis

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SUMMARY

Viral infections are associated with pulmonary exacerbations in children with cystic fibrosis (CF), but few studies have addressed the frequency in adults. This paper investigates the frequency and impact of viral infections in adults with CF receiving intravenous antibiotics. Pre- and post-treatment spirometry, inflammatory markers and antibody titres against influenza A, influenza B, adenovirus, respiratory syncytial virus, *Mycoplasma pneumoniae*, *Chlamydia psittaci*, and *Coxiella burnetti* were analysed over a 10-year period. Non-bacterial infections were identified in 5·1% of 3156 courses of treatment. The annual incidence of admissions per patient associated with viral infection was 4·9%. The presence of viral infection in association with a pulmonary exacerbation did not adversely affect lung function or inflammatory markers in the short term. Adults with CF have a lower incidence of respiratory viral infections associated with pulmonary exacerbations requiring intravenous antibiotics compared to children and infants with CF.

INTRODUCTION

Cystic fibrosis (CF) is characterized by chronic airway infection, pancreatic insufficiency, elevated sweat chloride concentration, impaired fertility and hepatobiliary disease. Mortality in patients with CF is primarily due to respiratory failure secondary to chronic bacterial infection. Lower respiratory tract infection occurs with a number of pathogens predominantly *Staphylococcus aureus*, *Haemophilus influenzae*, *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex (Bcc). Chronic infection with *P. aeruginosa* is associated with an increase in mortality and morbidity in patients with CF [1]. Respiratory viral infections may be associated with significant clinical deterioration [2] and predispose to infection with *P. aeruginosa* [3].

The reported prevalence of respiratory viral infection ranges from 13% to 52% [4–12], and is higher in younger patients [9]. In infants and young children with CF respiratory viral agents, mainly respiratory syncytial virus (RSV), have been detected in over half of respiratory exacerbations [5, 6, 13]. In older children, up to a third of infective exacerbations may be due to viral agents [10, 11, 14]. In mixed populations of children and adults, viral agents, mainly RSV and parainfluenza have been reported in 20% of exacerbations [9]. Data on the impact and prevalence of respiratory viruses in adults with CF is limited to one published study which showed that 11 out of 36 patients with symptoms of an exacerbation had serological evidence of infection with influenza virus A, B,

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cytomegalovirus, adenovirus or human rhinovirus A or B [8].

The aim of this study was to determine the prevalence and clinical impact of serologically defined infection to respiratory viruses, *Mycoplasma pneumoniae*, *Chlamydia psittaci*, and *Coxiella burnetii* in a large cohort of adults with CF receiving intravenous (i.v.) antibiotic treatment.

METHODS

Patients

A retrospective analysis was performed of serological studies to respiratory viruses and atypical organisms collected between 1 January 1994 and 31 December 2003 from patients attending the Leeds Regional Adult CF Unit. Data as to whether or not patients were receiving regular elective three-monthly or non-elective on-demand i.v. antibiotic therapy were recorded. The indications for non-elective i.v. antibiotics were increased respiratory symptoms, $>10\%$ reduction in spirometry measurements, reduced exercise capacity and increased sputum purulence. Blood was sampled for viral and atypical organism serology at the beginning and end of each course of i.v. antibiotic treatment. Spirometry measurements, peripheral blood white cell count (WCC), plasma viscosity (PV) and C-reactive protein (CRP) levels were obtained from the Unit's database. Data for annual influenza vaccination were obtained from both the Unit's database and general practitioner records.

Sputum microbiology

Data regarding *P. aeruginosa* and Bcc status in the year prior to admission were collected from the microbiology database. For each admission the patient's sputum microbiology status from the preceding 12 months was reviewed. The patients were categorized as Bcc-positive or Bcc-negative, chronic *P. aeruginosa*, intermittent *P. aeruginosa* or non-*P. aeruginosa* (by combining free and never categories) using the 'Leeds' criteria [15]. Patients were classified as 'chronic *P. aeruginosa*' if *P. aeruginosa* was isolated from sputum samples in $>50\%$ of those months where samples were collected and 'intermittent *P. aeruginosa*' if the percentage of months in which *P. aeruginosa* was isolated from sputum samples was $<50\%$. If *P. aeruginosa* was not isolated from the subject's sputum samples in the preceding 12 months, the subject was classified as 'non-*Pseudomonas*'.

Serological studies

Blood samples were assayed for antibody against influenza A, influenza B, adenovirus, RSV, *M. pneumoniae*, *C. psittaci*, and *Cox. burnetii* using complement fixation tests. Serum controls, complement, antigen, and known reactive controls were included with each assay. Titres were expressed as the reciprocal of the highest dilution of serum that completely fixed the complement in the presence of a specific antigen. Positive serological evidence of viral or atypical organism infection was defined as a $\geqslant$four-fold increase in titre between paired samples collected at least 7 days apart. Any rise in influenza A and B titres which were temporally associated with combined influenza A and B vaccinations were excluded.

Statistical analysis

Non-normally distributed data were expressed as median and range, and the Mann–Whitney, χ^2 and Kruskal–Wallis tests were used (SPSS 12.0, SPSS Inc., Chicago, IL, USA). The Fisher's exact probability test was used when frequencies were too low to justify use of the χ^2 test. Normally distributed data were expressed as mean and standard deviation, and the one-way ANOVA was used (SPSS 12.0). Kaplan–Meier survival analysis was used to determine the median time free from *P. aeruginosa* infection and comparison between survival curves was made using the log rank test (SPSS 12.0). A P value of $<0{\cdot}05$ was taken to be significant.

Ethics

Ethical approval for this study was obtained from the Leeds (East) Research Ethics Committee.

RESULTS

Between January 1994 and December 2003 a total of 3453 courses of home and hospital i.v. antibiotics were administered to 305 adult with CF. Of these, 297 treatment courses were excluded from the study as the serological data were incomplete, leaving 3156 courses available for study. The total number of patients with a positive rise in serology for influenza

Table 1. *Serology results*

	Number of courses of i.v. antibiotics
Negative serology	2996 (94·9%)
Influenza A	65 (2·0%)
Influenza B	25 (0·9%)
Adenovirus	46 (1·5%)
Respiratory syncytial virus	15 (0·5%)
Combined*	1 (0·03%)
M. pneumoniae	5 (0·2%)
C. psittacosis	3 (0·1%)
Cox. burnetii	0 (0·0%)

Data are presented as *n* (%).
* Raised titres to both influenza A and RSV.

A, influenza B, adenovirus, RSV, *M. pneumoniae*, *C. psittaci*, and *Cox. burnetii* are shown in Table 1. Serological evidence of a viral infection occurred in 152 (4·8%) of treatment episodes. Only five (0·2%) and three (0·1%) courses of i.v. antibiotic courses were associated with evidence of *M. pneumoniae* or *C. psittaci* infection respectively. There were no cases of *Cox. burnetii* infection over the 10-year period. The mean annual incidence of admissions per patient associated with positive viral serology was 4·9% (see Table 2).

The presence of positive serology was not related to age, gender, length of treatment, time to next course of i.v. antibiotics, levels of inflammatory markers or spirometry measurements at the beginning and end of treatment (see Table 3) (Mann–Whitney *P*, n.s.).

There was a higher prevalence of positive viral serology during winter months (October–March, 6·0%) compared to summer months (April–September, 3·5%) (χ^2 $P<0·05$) (see Fig.). The frequency of positive serology was significantly higher in the winter months for influenza A (χ^2 $P<0·05$) and influenza B (χ^2 $P<0·05$) (see Fig.). There was no significant difference between the frequency of admissions associated with infection by adenovirus, *M. pneumoniae*, *C. psittaci* or RSV in the winter or summer months (χ^2 *P*, n.s.).

Elective and non-elective antibiotic therapy

A total of 2398 courses of treatment were administered to patients receiving non-elective i.v. antibiotic therapy for acute exacerbations. In this group 115 treatment episodes (4·8%) were associated with positive viral serology.

A total of 757 courses of i.v. antibiotics were administered to patients receiving three-monthly elective i.v. antibiotic therapy. A total of 37 (4·9%) treatment episodes were associated with a positive viral serology. There was no significant difference between the prevalence of positive serology in patients receiving elective or non-elective i.v. antibiotic treatment (χ^2 *P*, n.s.). All courses of elective courses of i.v. antibiotics administered were to patients chronically infected with *P. aeruginosa*, apart from one patient chronically infected with *Stenotrophomonas maltiphilia* and one patient chronically infected with *S. aureus*.

Microbiology status and positive serology

Table 2 shows the annual frequency of admissions and the incidence of admissions associated with positive viral serology during the study period. Patients with sputum microbiology positive for chronic *P. aeruginosa* or Bcc had a higher frequency of admissions for treatment with i.v. antibiotics than patients classified as intermittent *Pseudomonas* or non-*Pseudomonas* (one-way ANOVA $P<0·05$). There was no significant difference in the annual incidence of admissions associated with positive viral serology between the four sputum microbiology groups (one-way ANOVA *P*, n.s.).

Fifty-three courses of i.v. antibiotics were administered to patients not colonized with either *P. aeruginosa* or Bcc during the 10-year study period The rate of a positive sputum sample for *P. aeruginosa* in the 3 months following admission was higher in the group with evidence of a viral infection (positive serology 28·6%, negative serology 10·9%), however, this was not statistically significant (Fisher's exact test *P*, n.s.). Using Kaplan–Meier survival analysis the median time for patients classified as non-*Pseudomonas* to have a positive sputum sample for *P. aeruginosa* was longer in patients with evidence of a viral infection (positive serology 399 days, negative serology 776 days), however, this was not statistically significant (log rank test *P*, n.s.).

Subgroup analysis according to microbiology status showed no significant effect of positive serology on the length of treatment, time to next course of i.v. antibiotics, levels of inflammatory markers or pre- and post-treatment spirometry. The median age for patients classified as non-*P. aeruginosa*, intermittent *P. aeruginosa*, chronic *P. aeruginosa* and Bcc were 20·7, 20·7, 23·4 and 23·1 years respectively. Patients

Table 2. *Annual frequency of treatment with i.v. antibiotics and incidence of positive serology during study period*

Sputum microbiology classification	Annual frequency of i.v. antibiotic courses per patient	Annual frequency of i.v. antibiotic courses per patient with positive serology	Annual incidence of i.v. antibiotic treatment with positive serology
All patients	2·20 (0·19)	0·11 (0·19)	4·9 % (4·03)
Non-*Pseudomonas*	1·11 (0·46)	0·08 (0·16)	5·8 % (11·7)
Intermittent *Pseudomonas*	1·33 (0·17)	0·04 (0·06)	3·0 % (4·81)
Chronic *Pseudomonas*	2·29 (0·20)	0·11 (0·07)	3·8 % (4·14)
Burkholderia cepacia complex	2·27 (0·44)	0·08 (0·09)	3·6 % (3·93)

Data presented as mean (standard deviation).

Table 3. *Demographics, inflammatory markers and spirometry at beginning and end of i.v. antibiotic treatment*

	Positive viral serology	Negative viral serology
Sex		
Male (*n*)	69 (4·9 %)	1346 (95·1 %)
Female (*n*)	83 (4·8 %)	1650 (95·2 %)
Age	23·2 (16·2–39·2)	23·3 (15·3–49·0)
Number of days of i.v. antibiotic treatment	14 (6–76)	14 (3–253)
Number of days to next admission	91 (0–1043)	86 (0–2322)
Start of treatment		
WCC	10·1 (2·1–26·6)	10·5 (1·7–33·0)
PV	1·86 (1·49–2·57)	1·84 (1·41–2·98)
CRP	25·0 (5·0–222·3)	18·4 (5·0–339·0)
FEV1	1·46 (0·47–4·55)	1·39 (0·26–4·69)
FVC	2·41 (0·78–5·44)	2·32 (0·28–7·53)
End of treatment		
WCC	8·55 (1·9–19·6)	8·1 (1·6–31·6)
PV	1·81 (1·42–2·30)	1·78 (1·39–2·46)
CRP	8·4 (5·0–93·0)	10·0 (4·0–224·0)
FEV1	1·70 (0·49–4·54)	1·62 (0·13–4·99)
FVC	2·81 (1·06-5·62)	2·74 (0·61-7·27)

Data are presented as median (range) unless otherwise indicated.
WCC White cell count ($\times 10^9\,l^{-1}$) normal range 4·00–11·00; PV, plasma viscosity (mPa s^{-1}) normal range 1·50–1·72; CRP, C-reactive protein (mg l^{-1}) normal range $<5{\cdot}0$; FEV1, forced expiratory volume in 1 s (l); FVC, forced vital capacity (l).

chronically infected with *P. aeruginosa* or Bcc were significantly older than the patients either intermittently infected or free from infection with *P. aeruginosa* (Kruskal–Wallis $P<0{\cdot}05$).

CONCLUSIONS

Most acute respiratory viral infections in people with CF are probably self-limiting, but may result in increased hospitalization, greater antibiotic use, and worse symptoms [5, 16]. Influenza A was the commonest viral infection identified in this study. There are no randomized controlled trial data to support routine influenza vaccination of adults with CF [17]. However, we advise all patients to receive annual influenza vaccination and if at all possible to avoid close contact with people with acute viral-like illnesses in an effort to reduce the frequency of viral infections.

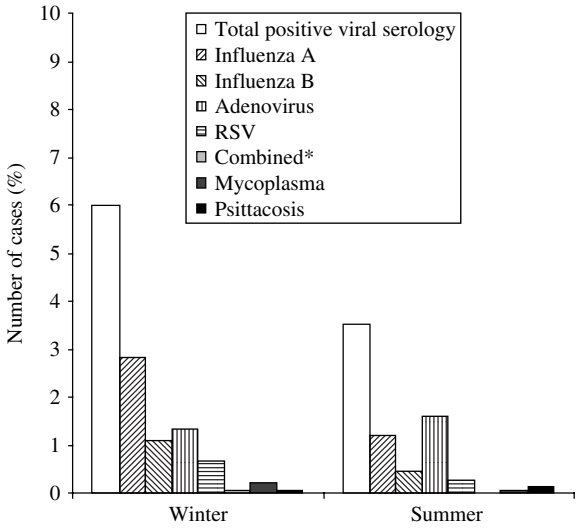

Fig. Frequency of positive viral serology. * Combined = raised titres to both influenza A and RSV.

Viral and bacterial infections may be synergistic in their capacity to cause airway inflammation and lung damage [9]. Coincidental RSV infections in patients with intermittent and chronic *P. aeruginosa* infection are associated with a significant rise in anti-pseudomonal antibody levels [9]. There are several possible mechanisms by which respiratory viruses may induce pulmonary damage in patients with CF. Viral infection may promote inflammatory cell recruitment and activation through intercellular adhesion molecules [18] and induce the expression of stress response genes such as haem-oxygenase-1 and genes encoding for antioxidant enzymes such as glutathione peroxidase [19]. The latter can further affect already reduced epithelial lining fluid levels of glutathione in inflammatory conditions like CF [20]. In the present study we found no significant difference in baseline lung function, inflammatory markers, clinical response to antibiotic therapy, and time to next treatment between patients with or without evidence of a viral infection. This would suggest in the short term that viral infection has no greater clinical impact than exacerbations due to bacterial causes.

It has been reported that a greater proportion of first *P. aeruginosa* infections occur during the winter months [3]. During the study of Petersen *et al.*, RSV infections were more common in patients who developed chronic *P. aeruginosa* infection than in patients with intermittent or without *P. aeruginosa* infection [9]. They postulated that viral infection may result in an increased risk of acquisition of chronic *P. aeruginosa* infection and prevention of viral infection may reduce the risk of subsequent infection with *P. aeruginosa*. This is important because chronic *P. aeruginosa* infection is associated with a worse prognosis [1] and an accelerated decline in lung function [21]. The data from the study of Petersen *et al.* does not demonstrate a causal link between viral infection and subsequent *P. aeruginosa* infection, an alternative explanation could be that factors related to the risk of acquisition of chronic *P. aeruginosa* infection are also associated with an increased risk of viral infection. Ong *et al.* reported that there was a higher rate of viral infections occurring in patients with sputum positive for *P. aeruginosa*, however, this was non-significant [8]. Armstrong *et al.* reported 31 infants with CF hospitalized with symptoms of acute respiratory infection [5]. Sixteen of these infants had either a bronchoalveolar lavage or nasopharyngeal aspirate positive for a respiratory virus and subsequently 25% of them were infected with *P. aeruginosa*. In addition, 26% of the 15 infants where no respiratory viruses were detected, subsequently had *P. aeruginosa* isolated [5]. Collinson *et al.* found that of six patients with a new acquisition of *P. aeruginosa* during the study period, five occurred during symptoms suggestive of an upper respiratory tract infection (URTI) [22]. Of the five first growths of *P. aeruginosa* associated with an URTI, three were associated with a Picornavirus infection. The data presented from the current study do not support the hypothesis that viral infections may predispose patients with CF to infection with *P. aeruginosa*. We found that following an admission for i.v. antibiotics associated with a viral infection there was no increase in the frequency or decrease in the time to a sputum sample positive for *P. aeruginosa*.

There is a wide variation in the incidence of viral infections in groups of patients without CF. Falsey *et al.* prospectively followed 540 adults aged >21 years with a diagnosis of congestive cardiac failure or chronic pulmonary disease over a 4-year period using culture, polymerase chain reaction (PCR) and serology [23]. Within this population of patients, RSV and influenza A infection developed annually in 4–10% and 0–5% respectively [23]. Tan *et al.* reported on 60 patients hospitalized with either life-threatening asthma, acute asthma or an exacerbation of chronic obstructive pulmonary disease (COPD) [24]. Using PCR they found within the respiratory secretions the presence of viral nucleic acid in 52% of patients [24]. They found that picornaviruses and adenoviruses were predominately found in patients

with near-fatal asthma, while influenza virus infection was predominantly found in patients with exacerbations of COPD. Unlike patients with asthma or COPD, adults with CF can have chronic lower respiratory tract infection with bacteria such as *S. aureus*, *P. aeruginosa* and Bcc. The incidence of viral infections in adults with CF could be expected to be lower as there may be factors associated with the infecting bacteria. Interactions between the host and the bacteria may be more important than viral infections as a cause of exacerbations in patients with CF. It has been postulated that the inflammatory response and clinical symptoms associated with exacerbations may be a result of 'blooms' of planktonic bacteria from the anaerobic lung biofilms [25]. This hypothesis is supported by data demonstrating the different patterns of gene expression in airway epithelium cells in response to exposure to mucoid or motile *P. aeruginosa*. Exposure to motile *P. aeruginosa* promotes expression of pro-inflammatory genes related to host defence, whereas the response to mucoid *P. aeruginosa* is not pro-inflammatory [26].

The frequency of viral infections in adults with CF is higher during the winter months. The data presented suggests that the increased frequency is related to influenza A and influenza B infection which would be in keeping with the known epidemiology of these infections. The frequency of RSV was not significantly higher during the winter months which is contrary to the Health Protection Agency data which demonstrates a large peak of RSV reports during the winter period [27]. However, this may be explained by the majority of these cases occurring in children.

There are several limitations to this study. The prevalence of virally induced respiratory exacerbation in patients with CF may have been underestimated due to a number of factors. Between 10% and 30% of patients with confirmed viral respiratory tract infection have negative serology [28]. Modern molecular-based technologies such as immunofluorescence or PCR are more sensitive and quicker than serology or tissue culture. A recent study using multiplex reverse transcriptase PCR (RT–PCR) to detect influenza A, influenza B, parainfluenza viruses 1, 2, and 4, RSV and adenovirus examined 52 samples of sputum from 38 patients with CF during two short periods over 2 years [29]. They reported 12·5% and 32% of the samples were positive in the first and second periods respectively, giving an overall prevalence of 23%. The only other study examining the frequency of viral infections in adults used serology as a basis for diagnosis and reported a higher prevalence of 30·6% but this was only based on 36 patients over a 1-year period [8]. It is our Unit's protocol to advise patients to take appropriate oral antibiotics at the first signs of respiratory symptoms. This may have prevented a secondary bacterial-associated clinical deterioration and the requirement for i.v. antibiotic treatment. Patients with a mild and self-limiting respiratory exacerbation may not have presented to the Unit at the time of exacerbation. Our screening programme did not include detection of serological response to rhinovirus infection which is commonly associated with acute respiratory illness [30]. Diagnosis of rhinovirus infection by serological methods is difficult due to the large number of serotypes. The incidence of rhinovirus infection declines with age but has been reported in adults with CF [8]. Further data, particularly for evidence of rhinovirus infection, could have been obtained through the use of PCR and nasal pharyngeal aspirate specimens [12, 22]. Over the study period in our hospital viral serology was the standard tool for screening patients who were suspected of having viral infections.

Our Unit has stopped routine serology measurements due to the low prevalence of positive serology reported in this study, and is currently using nasopharyngeal swabs combined with immunofluorescence as a method of diagnosis for viral respiratory infections. We intend to introduce PCR-based detection in the near future. Serology is still performed where there is concern regarding the possibility of atypical organisms causing infection or where patients are not responding to conventional i.v. antibiotic therapy.

In conclusion, adults with CF have a lower incidence of respiratory viral infections associated with pulmonary exacerbations requiring i.v. antibiotic treatment compared to children and infants. The serological data presented suggest that most of the exacerbations requiring i.v. antibiotics were not caused by a viral infection. Patients with serological evidence of viral infection did not have a worse short-term clinical outcome. Contrary to previous reports the incidence of viral infections was not higher in patients chronically infected with *P. aeruginosa*. Further prospective studies using modern diagnostic techniques should be undertaken to provide additional data regarding the epidemiology and clinical impact of respiratory viral infections in patients with CF.

DECLARATION OF INTEREST

None.

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