

Haemophilus infection in cystic fibrosis

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Abstract

Twenty seven patients with cystic fibrosis under the age of 12 years and 27 matched patients with asthma were followed up in a prospective study for one year. The isolation rate of non-capsulated strains of *Haemophilus influenzae* from cough swabs and sputum specimens taken at routine clinic visits every two months was significantly greater in cystic fibrosis than in asthma. *Haemophilus parainfluenzae* was equally common in both groups. During exacerbations the isolation rate of *H influenzae* in cystic fibrosis was significantly greater than at other times, whereas in asthma there was no significant difference.

The distribution of biotypes of *H influenzae* and *H parainfluenzae* was similar in the two groups. In cystic fibrosis, biotype I was associated with exacerbations. Biotype V was more common than in previous studies, but was not associated with exacerbations.

In recent years, non-capsulated *Haemophilus influenzae* has become well established as an important human pathogen in both adults and children.¹⁻³ Non-capsulated strains of *H influenzae* and *Haemophilus parainfluenzae* colonise the lower respiratory tracts of patients with cystic fibrosis but may also be found in the upper respiratory tracts of normal children.⁴ Some cystic fibrosis centres adopt a policy of treating all patients from whom *Haemophilus* spp have been isolated, irrespective of the clinical state of the patient.⁵ *H influenzae* and *H parainfluenzae* have been subdivided by Kilian on the basis of three biochemical characteristics (ornithine decarboxylation, indole production, and urea hydrolysis) into several biotypes.⁶ Biotype I of *H influenzae*, which is known to be associated with acute otitis media in normal children,³ was isolated significantly more often from patients with cystic fibrosis than from healthy controls.⁷ To assess the importance of *H influenzae* and *H parainfluenzae* in cystic fibrosis and the value of biotyping, we conducted a prospective study to determine the rates of isolation and the distribution of biotypes from the respiratory tracts of children with cystic fibrosis. Children with asthma were used as controls because they were likely to have increased respiratory secretions without significant bacterial infection.

Patients and methods

PATIENTS

All patients with cystic fibrosis under the age of

12 years who lived within easy reach of the hospital were invited to take part in the study. The next suitable patient seen in the asthma clinic, matched for age and sex, was invited to act as a control. Consent was obtained from the parents and the study was approved by the hospital ethical committee. Patients were seen routinely every two months and weekly during acute respiratory exacerbations. At each visit weight and height were recorded and where possible lung function tests were performed using a Wright peak flow meter and an electronic spirometer. The best of three readings was recorded in each case and was expressed as a percentage of the predicted value for height. Pulse, respiratory rate, and physical signs were scored by one of us (RJR) and the parents were asked to score symptoms of cough, wheeze, and breathlessness on a scale of 0 (none) to 4 (severe). The two scores were added together for analysis. At the start of the study, peak flow records and symptom charts were kept for two weeks to assess baseline variability during a period when the child was well. The parents contacted the hospital at any time when the child was unwell with increased cough, wheezing, or breathlessness and peak flow records and symptom scores were recorded until symptoms had resolved.

During acute exacerbations, patients with cystic fibrosis received a two week course of amoxycillin. Those aged less than 1 year, between 1 and 8 years, and over 8 years received 125, 250, and 500 mg of amoxycillin respectively every eight hours. If *Staphylococcus aureus* had been isolated recently amoxycillin was combined with flucloxacillin or occasionally erythromycin was prescribed. If the child was allergic to amoxycillin or the *Haemophilus* spp was resistant to ampicillin, co-trimoxazole was prescribed. Patients who were chronically infected with *Pseudomonas aeruginosa* were treated with intravenous antibiotics when necessary. In contrast, asthma patients did not receive antimicrobial treatment but inhaled therapy was adjusted and, if necessary, courses of oral steroids were prescribed for acute exacerbations. Cough swabs were repeated after one and two weeks of treatment in both groups.

Cough swabs were taken by a standard method either by the outpatient sister or by one of us (RJR). The swab was held at the back of the child's throat and he or she was asked to cough. In the case of young children it was sometimes necessary to induce a gag reflex. The swabs were placed in Stuart's transport medium and labelled sequentially with a number so that the microbiologists were unaware of the identity

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of the patient. In addition, whenever possible a sputum specimen was sent for culture in the usual way. A throat swab was placed in viral transport medium and sent for viral culture at the start of each exacerbation.

METHODS

Each cough swab was broken off into a bijoux bottle containing 0.5 ml of 2% N-acetylcysteine. It was shaken briefly in a Whirlmixer (Fisons) to distribute the swab contents. Using a Pasteur pipette a drop was placed on to plates containing heated blood agar, chocolate agar, crystal violet agar, and a selective chocolate agar (containing cefsulodin 8 mg/l, which inhibited *Pseudomonas* spp but allowed *Haemophilus* spp to grow). The drops were spread in the usual way and the plates were incubated at 37°C in 5% carbon dioxide for 18 hours. Isolates were identified as belonging to *Haemophilus* spp by typical colonial morphology, microscopic appearance, and growth requirement for 'X' and 'V' factors. All isolates of *H influenzae* were designated non-capsulated if they failed to agglutinate with type specific *H influenzae* type b (Public Health Laboratory Service) and polyvalent (Difco, types a-f) antisera or if they autoagglutinated. Other organisms were identified by standard methods.⁸ The biotypes of isolates were determined using the RapID/NH system (Mercia Diagnostics). All isolates of *H influenzae* and *H parainfluenzae* were tested for β lactamase production by the technique of Escamilla⁹ and additional disc diffusion sensitivity tests were carried out by Stokes' method¹⁰ with discs containing ampicillin 2 and 10 μ g. Discs containing sulphamethoxazole 50 μ g, trimethoprim 2.5 μ g, chloramphenicol 10 μ g, and cefotaxime 30 μ g were used to test isolates which were resistant to ampicillin.

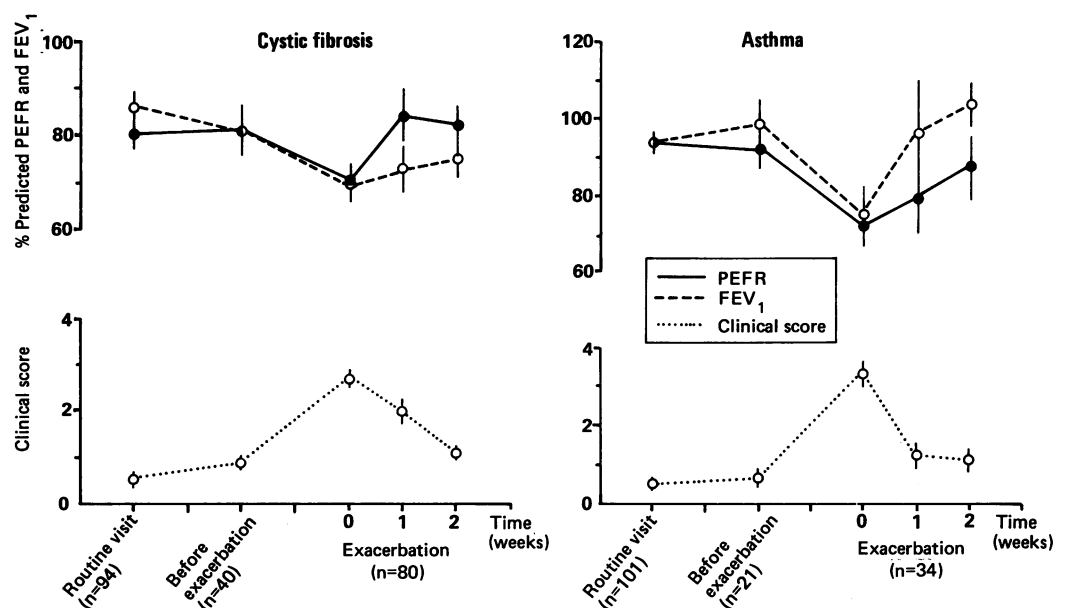
Data were analysed by χ^2 tests, one way analysis of variance, and paired *t* tests using SPSSX programmes.

Results

There were 27 patients in each group with a predominance of boys in both groups (cystic fibrosis, $n=18$ and asthma, $n=17$). The median ages in the cystic fibrosis and asthma groups were 7.5 and 7.1 years respectively. The median Shwachman score¹¹ in the group with cystic fibrosis was 82 (range 43-94), and the median Chrispin-Norman x ray score¹² was 6 (range 2-20). Five patients with cystic fibrosis were chronically infected with *P aeruginosa*. In the asthma group, 19 patients were taking inhaled steroids and six were taking inhaled sodium cromoglycate. Twenty one patients in each group were able to perform reliable respiratory function tests.

There were 80 exacerbations in the group with cystic fibrosis and 34 in the group with asthma. Exacerbations were associated with a fall in mean peak expiratory flow rate (PEFR) and forced expiratory volume in one second (FEV₁) in both groups ($p<0.05$) and a rise in mean clinical score. There was an improvement in mean PEFR and FEV₁ by the second week of antibiotic treatment ($p<0.05$) in the group with cystic fibrosis (figure).

Four hundred and ninety cough swabs or sputum specimens (cystic fibrosis, $n=303$ and asthma, $n=187$) were examined between January and December 1988. *H influenzae* was isolated on at least one occasion from 21 patients with cystic fibrosis and eight with asthma ($p<0.001$), and on three or more occasions from six cystic fibrosis patients but no asthmatics. All strains of *H influenzae* isolated were non-capsulated. *H parainfluenzae* was isolated on at least one occasion from six patients with cystic fibrosis and two with asthma. Table 1 shows the isolation rates of *H influenzae*, *H parainfluenzae*, and other pathogens from specimens obtained at routine clinic visits, in the month preceding an acute exacerbation, during exacerbation, and after starting treatment (after exacerbation). At routine clinic visits, before and during exacer-



Graphs to show the mean (SE) values for peak expiratory flow rate (PEFR), forced expiratory volume in one second (FEV₁), and clinical score in the two groups according to type of visit.

Table 1 Number (%) of isolates of *H influenzae* and *H parainfluenzae* and other pathogens in the two groups according to type of visit

Type of visit	Cystic fibrosis				Asthma			
	No of specimens	<i>H influenzae</i>	<i>H parainfluenzae</i>	Other pathogens only	No of specimens	<i>H influenzae</i>	<i>H parainfluenzae</i>	Other pathogens only
Routine Before	94	24 (25.5)	3 (3.2)	12 (12.7)	101	8 (7.9)	3 (3)	0
Exacerbation	40	15 (37.5)	2 (5)	5 (12.5)	21	2 (9.5)	1 (4.8)	1 (4.8)
Routine After	80	31 (38.8)	2 (2.5)	14 (17.5)	34	1 (2.9)	2 (5.9)	0
Exacerbation	89	7 (7.9)	0	14 (15.7)	31	3 (9.7)	1 (3.1)	1 (3.1)
Total	303	77 (25.4)	7 (2.3)	45 (14.8)	187	14 (7.5)	7 (3.7)	2 (1.0)

bations, *H influenzae* was isolated more frequently from patients with cystic fibrosis than from asthma patients ($p < 0.01$). After antibiotic treatment, the rate of isolation of *H influenzae* from patients with cystic fibrosis was similar to the isolation rate from asthma patients. The isolation rate of *H influenzae* in the cystic fibrosis group increased from 25.6% at routine visits to 38.8% during exacerbations ($p < 0.05$). In contrast, the isolation rate of *H parainfluenzae* remained low at all visits and was equal in the two groups. Of those specimens from which other pathogens only were isolated, *P aeruginosa*, *S aureus*, and group A haemolytic streptococci accounted for 30, 14, and one isolates respectively in cystic fibrosis, whereas in asthma the two isolates were both group A haemolytic streptococci.

Viral cultures performed during exacerbations yielded coxsackievirus, rhinovirus, and Herpes simplex virus in three patients with cystic fibrosis and cytomegalovirus in one patient with asthma.

Seventy nine strains of *H influenzae* and 14 strains of *H parainfluenzae* were biotyped. All but one strain of *H parainfluenzae* were biotype III. The distribution of biotypes of *H influenzae* was not significantly different in the two groups (table 2). Biotype V was more common than biotype I in both groups and occurred with equal frequency at routine clinic visits and during exacerbations. Biotype I was isolated more frequently around the time of an exacerbation (11 isolates) than at other times (four isolates). Patients with cystic fibrosis frequently harboured a particular biotype for several months, but some showed a change in biotype before an exacerbation.

Nine isolates of *H influenzae* (cystic fibrosis, $n = 8$ and asthma, $n = 1$) were ampicillin resistant

and all except one produced β lactamase. Five of eight β lactamase producing strains were biotype V. Two strains of *H parainfluenzae* were ampicillin resistant.

In patients with cystic fibrosis from whom *H influenzae* only was isolated during an exacerbation, a 14 day course of oral amoxicillin was always associated with a clinical response. Eradication of *H influenzae* was achieved on 15 out of 22 (70%) occasions. It is our practice to treat all patients with cystic fibrosis from whom *S aureus* is isolated, irrespective of symptoms.

Discussion

The role of non-capsulated *H influenzae* in adults with lower respiratory tract infections is now widely recognised, but in children its role remains unresolved. This is because it is frequently isolated from the upper respiratory tract in normal children and it is known that invasive infections in children are almost always due to capsulated type b strains of *H influenzae*. Nevertheless, non-capsulated strains are undisputed pathogens of otitis media and sinusitis in children,³ and in a large study of community acquired pneumonia in children, more than half the strains of *H influenzae* producing pneumonia were non-capsulated.¹³

The results of this study suggest that *H influenzae* is a significant pathogen in children with cystic fibrosis. *H influenzae* was more frequently isolated from the lower respiratory tract in cystic fibrosis than in asthma. Furthermore, a rise in the isolation rate of *H influenzae* preceded the development of acute exacerbations in the patients with cystic fibrosis and clinical improvement coincided with a reduction in the isolation rate after antimicrobial treatment (table 1). In this age group *H influenzae* may be a more frequent pathogen than *P aeruginosa*. *S aureus* has been less frequently isolated in recent years because it is usually eradicated by elective antimicrobial treatment. Placebo controlled double blind trials would be needed to establish whether elective treatment of *H influenzae* isolates in young cystic fibrosis patients reduces the frequency of acute exacerbations.

In adults with chronic bronchitis the symptoms produced by infection with *H influenzae* and *H parainfluenzae* are indistinguishable,¹⁴ but no similar study has been carried out in children. This study has not found any difference between the groups in the isolation rate of *H parainfluenzae* or in the distribution of its

Table 2 Distribution of biotypes of *H influenzae* from patients with cystic fibrosis and asthma

Biotype	No (%) of strains	
	Cystic fibrosis	Asthma
I	15 (24.2)	4 (23.5)
II	10 (16.1)	2 (11.8)
III	4 (6.4)	4 (23.5)
IV	2 (3.2)	0
V	21 (34.0)	5 (29.4)
VI	3 (4.8)	0
VII	3 (4.8)	0
Non-typable	4 (6.4)	2 (11.8)
Total	62 (100)	17 (100)

biotypes. As *H parainfluenzae* was much less frequently isolated than *H influenzae*, a larger study would be needed to rule out the possibility that it may be associated with exacerbations in cystic fibrosis.

In this study the biotype distribution of *H influenzae* isolates from children with cystic fibrosis and asthma was similar. Previous studies have suggested that biotype I may be more common and biotype II less common in cystic fibrosis than in normal children.⁷ The value of biotyping strains of *H influenzae* in determining pathogenicity lies mainly in the detection of biotype I.³ As in this study less than one third of isolates during exacerbations were biotype I, biotyping may be of limited value in clinical practice. Other methods of typing non-capsulated *H influenzae*, for example based on outer membrane proteins, may be more useful.¹ Temporal changes in the biotype distribution of *H influenzae* have been described. Therefore, the finding that biotype V was more common than in previous studies may merely reflect the passage of time, geographical differences, or selection of ampicillin resistant strains.¹⁵ Even when strains of *H influenzae* are apparently sensitive to ampicillin, only 70% of courses of amoxicillin eradicate *H influenzae* from the lower respiratory tract, as previously reported by Pressler *et al.*⁵ This may be due to inadequate penetration of the antibiotic into the sputum. The use of very high doses of amoxicillin has been advocated in adults with bronchiectasis¹⁶ and may be effective in children.

The isolation rate of viruses in this study was low, but nasopharyngeal cultures taken on the first day of illness and paired blood samples for viral serology might have yielded more positive results.¹⁷ Secondary bacterial infection may occur in cystic fibrosis as a result of damage to the respiratory epithelium during viral infection. *H influenzae* may colonise the upper respiratory tract initially and then migrate to cause lower respiratory tract infections in the damaged bronchi of cystic fibrosis patients. *H influenzae* is capable of producing histamine *in vitro*,¹⁸ which might contribute to inflammation and airflow obstruction in cystic fibrosis. This study has shown that antibiotic treatment for *H influenzae* was associated with clinical improve-

ment and with the eradication of the organism in most cases. Therefore, we feel that antimicrobial treatment should be considered seriously in patients with cystic fibrosis from whom non-capsulated *H influenzae* is isolated, particularly during exacerbations.

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- 1 Murphy TF, Apicella MA. Nontypable Haemophilus influenzae: a review of clinical aspects, surface antigens, and the human immune response to infection. *Rev Infect Dis* 1987;9:1-15.
- 2 Ispahani P, Youngs ER. Non-capsulate Haemophilus influenzae: a neglected pathogen in adults. *Br Med J* 1985;290:1870-1.
- 3 Long SS, Teter MJ, Gilligan PH. Biotype of Haemophilus influenzae: correlation with virulence and ampicillin resistance. *J Infect Dis* 1983;147(S):800-6.
- 4 Hoiby N, Kilian M. Haemophilus from the lower respiratory tract of patients with cystic fibrosis. *Scandinavian Journal of Respiratory Disease* 1976;57:103-7.
- 5 Pressler T, Szaff M, Hoiby N. Antibiotic treatment of Haemophilus influenzae and Haemophilus parainfluenzae infections in patients with cystic fibrosis. *Acta Paediatr Scand* 1984;73:541-7.
- 6 Kilian M. A taxonomic study of the genus Haemophilus with the proposal of a new species. *J Gen Microbiol* 1976;93:9-62.
- 7 Watson KC, Kerr EJC, Hinks CA. Distribution of biotypes of Haemophilus influenzae and H parainfluenzae in patients with cystic fibrosis. *J Clin Pathol* 1985;38:750-3.
- 8 Cowan ST. *Cowan and Steel's manual for the identification of medical bacteria*. 2nd Ed. London: Cambridge University Press, 1974.
- 9 Escamilla J. Susceptibility of Haemophilus influenzae to ampicillin as determined by use of modified, one-minute beta lactamase test. *Antimicrob Agents Chemother* 1976;9:196-8.
- 10 Stokes EJ, Ridgway GL. *Clinical bacteriology*. 5th Ed. London: Edward Arnold, 1980:215.
- 11 Shwachman H, Kulczycki LL. Longterm study of 105 patients with cystic fibrosis. *Am J Dis Child* 1958;96:6-15.
- 12 Chrispin AR, Norman AP. The systematic evaluation of the chest radiograph in cystic fibrosis. *Pediatr Radiol* 1974;2:101-6.
- 13 Shann F, Gratten M, Germer S, Linnemann V, Hazlett D, Payne R. Aetiology of pneumonia in children in Goroka hospital, Papua New Guinea. *Lancet* 1984;ii:537-41.
- 14 Rhind GB, Gould GA, Ahmand F, Croughan MJ, Calder MA. Haemophilus parainfluenzae and H influenzae respiratory infections: comparison of clinical features. *Br Med J* 1985;291:707-8.
- 15 Watson KC, Kerr EJC, Baillie M. Temporal changes in biotypes of Haemophilus influenzae isolated from patients with cystic fibrosis. *J Med Microbiol* 1988;26:129-32.
- 16 Cole PJ, Roberts DE, Davies SF, Knight RK. A simple oral antimicrobial regimen effective in severe chronic bronchial suppuration associated with culturable Haemophilus influenzae. *J Antimicrob Chemother* 1983;11:109-13.
- 17 Wang EEL, Prober CG, Manson B, Corey M, Levison H. Association of respiratory viral infections with pulmonary deterioration in patients with cystic fibrosis. *N Engl J Med* 1984;311:1653-8.
- 18 Sheinman BD, Devalia JKL, Davies RJ, Crook SJ, Tabaqchali S. Synthesis of histamine by Haemophilus influenzae. *Br Med J* 1986;292:857-8.