Eur Respir J 1999; 14: 1340–1344
Printed in UK – all rights reserved

European Respiratory Journal
ISSN 0903-1936

Determinants of chronic infection with *Staphylococcus aureus* in patients with bronchiectasis

P.L. Shah*, S. Mawdsley*, K. Nash*, P. Cullinan*, P.J. Cole*,**, R. Wilson*,**

Determinants of chronic infection with Staphylococcus aureus *in patients with bronchiectasis. P.L. Shah, S. Mawdsley, K. Nash, P. Cullinan, P.J. Cole, R. Wilson. ©ERS Journals Ltd 1999.*

ABSTRACT: *Staphylococcus aureus* is an uncommon pathogen in bronchiectasis not caused by cystic fibrosis (CF). The object of this study was to identify characteristics that cause patients to be prone to infection with *S. aureus*.

The study population consisted of patients with bronchiectasis attending the authors' unit, excluding those with a diagnosis of overt CF. All patients had a high resolution computer tomographic scan (HRCT) of the thorax which demonstrated bronchiectasis. Cases that were currently chronically infected with *S. aureus* (isolated consecutively on more than two occasions >3 months apart) were identified (n=12) and compared with 74 control patients who had not been chronically infected with *S. aureus*. Patients were carefully evaluated to determine the aetiology of their disease. Odds ratios (OR) and 95% confidence intervals (CI) as measures of the association between disease characteristics and chronic infection with *S. aureus* were calculated.

The results for patients chronically infected by S. aureus demonstrated significant associations with allergic bronchopulmonary aspergillosis (ABPA; OR=8.8, 95% CI 1.8–41.9), atypical variants of CF (OR=12.0, 95% CI 1.8–81.7) or equivocal sweat sodium values (OR=4.0, 95% CI 1.0–15.3). The associations persisted when the analysis was based on cases (n=28) in whom *S. aureus* had ever been isolated from sputum. In the latter analysis there was also a significant association with predominant upper zone disease on HRCT.

These results suggest that patients with bronchiectasis in whom *S. aureus* is isolated from sputum should be carefully evaluated to exclude allergic bronchopulmonary aspergillosis or atypical cystic fibrosis.

Eur Respir J 1999; 14: 1340–1344.

*Royal Brompton Hospital, London, UK.
**Host Defence Unit, Imperial College of Science Technology and Medicine at National Heart & Lung Institute, London, UK.

Correspondence: R. Wilson
Host Defence Unit
National Heart & Lung Institute
Emmanuel Kaye Building
Manresa Road
London, SW3 6LR
UK
Fax: 44 1713518338

Keywords: Allergic bronchopulmonary aspergillosis
bronchiectasis
cystic fibrosis
Staphylococcus aureus

Received: April 13 1999
Accepted after revision August 4 1999

Staphylococcus aureus is a gram positive coccus which is only occasionally cultured from the sputum of patients with bronchiectasis [1], whereas in patients with cystic fibrosis (CF) it is almost always the initial bacterial infection [2–4]. In CF *S. aureus* infection may stimulate an inflammatory response that causes lung damage and facilitates subsequent chronic infection with *Pseudomonas aeruginosa* [2–4]. Once chronic infection with *P. aeruginosa* has occurred *S. aureus* is less frequently cultured and it has been suggested that this is due to anti-staphylococcal factors produced by *P. aeruginosa* [5]. Several reports of sputum microbiology and bronchoscopic sampling suggest that *S. aureus* occurs in ~4–10% of patients with non-CF bronchiectasis [6–8]. The reason for this difference in the type of bacterial infective organisms is unclear. The object of the present study was to identify common characteristics of a group of patients with non-CF bronchiectasis who were chronically infected with *S. aureus*.

Methods

Study definitions

Chronic infection with *S. aureus* was defined as patients who were infected with *S. aureus* during the study period and the bacterium had been isolated from consecutive sputum samples taken on more than two occasions >3 months apart. Intermittent infection with *S. aureus* was defined when the bacterium had been isolated on at least one occasion from sputum samples in the past. Never infected with *S. aureus* was defined when the organism had not been isolated in any of the sputum samples at any time point.

Study population

Twelve patients were identified who had bronchiectasis and chronic infection with *S. aureus*. These patients were seen in the authors' clinic (setting of tertiary referral/research centre) between January and May 1997. Sputum cultures in these patients had consistently identified *S. aureus* over a median (interquartile range) time period of 18 (7.5–22.5) months. The case records of 127 other patients with a diagnosis of bronchiectasis who attended the clinic during the same time period were reviewed. Patients with a diagnosis of overt CF characterized by a consistent history, clinical presentation and confirmatory sweat sodium concentration >80 $mmol \cdot L^{-1}$ were excluded. Patients in whom a recent high resolution computed tomography

(HRCT) of the chest was available to review and had a series of sputum culture were studied further (n=74). None of these 74 patients had been chronically infected with *S. aureus*. Sixteen of the 74 patients were classified as intermittently infected with *S. aureus* and 58 patients as never been infected with *S. aureus*.

Study groups

Some of the patients were infected by more than one organism but the stratification into the three groups was made on the basis of infection with *S. aureus*; group A: chronically infected with *S. aureus* (n=12); group B: intermittently infected with *S. aureus* (n=16); group C: never infected with *S. aureus* (n=58).

The primary case control analysis was between patients who were chronically infected with *S. aureus* (group A) compared to patients who were not chronically infected with *S. aureus* (groups B and C). A secondary analysis using the case definition of ever infected with *S. aureus* (groups A and B) compared to a control group who had never been infected with *S. aureus* (group C) was also performed.

Study protocol

All patients that were evaluated had computed tomographs (CT) of the thorax with 2 mm sections obtained at 10 mm intervals and a high spatial frequency algorithm used for reconstructing the images [9]. They fulfilled both radiological and clinical criteria for bronchiectasis. All patients had a sputum culture and underwent careful assessment to determine the aetiology of the bronchiectasis [1]. The investigations and sputum collections were performed when patients were clinically stable. The routine practice was to co-ordinate all the investigations over a 3 day period with all measurements being made at research standards.

Fresh sputum samples had been collected in sterile containers with the assistance of a chest physiotherapist. The sputum samples were taken directly to the microbiology laboratory and mechanically homogenized with an equal volume of Ringer's solution. Further stepwise 10-fold dilutions were carried out to enable quantitative analysis. Aliquots of 25 µL from each of these dilutions were inoculated onto blood (aerobic and anaerobic) and MacConkey (aerobic) plates which were incubated at 37°C for 24 h, and a chocolate agar plate which was incubated in 10% CO_2 for 48 h. Further stepwise dilutions were performed for semi-quantitative analysis. Bacteria cultured were identified by standard techniques.

In addition to a careful history and examination the protocol of investigations performed in all patients included pulmonary function tests, immunoglobulin (Ig) (A, M, G, E and G subclass) levels, alpha 1-antitrypsin, autoantibody profile, aspergillus precipitins, specific Aspergillus IgE radioallergosorbent test (RAST), sputum microscopy to examine for eosinophils, ciliary function studies, sweat test and measurements of nasal potential differences. Ciliary function was evaluated by taking a biopsy from the inferior turbinate of a nostril with a cytology brush and estimating ciliary beat frequency by a photometric method [10]. Any abnormalities of ciliary beat were further investigated by electron microscopy ultrastructure analysis. Sweat tests were performed by pilocarpine iontophoresis [11] and individuals with high borderline values of sodium (60–80 $mmol \cdot L^{-1}$) had a repeat sweat test after pre-treatment with fludrocortisone 5 mg daily for 2 days [12]. Nasal potential differences were measured by the technique described by ALTON *et al.* [13].

Allergic bronchopulmonary aspergillosis (ABPA) was defined by the presence of four or more of the following abnormalities: peripheral blood eosinophilia of $\geq 0.5 \times 10^9 \cdot L^{-1}$, raised total serum IgE, positive immediate skin-prick sensitivity to *Aspergillus fumigatus* or specific Aspergillus IgE RAST, serum IgG antibody to *A. fumigatus* (Aspergillus precipitins), history or presence of pulmonary infiltrates on radiographs or permanent radiographic abnormalities consistent with ABPA and fungal hyphae of *A. fumigatus* on microscopic examination of sputum [14].

Statistical analysis

Descriptive statistics are quoted as medians (interquartile range) and compared using nonparametric analysis (Mann-Whitney U-test or Chi-squared analysis). Odds ratios (OR) and 95% confidence intervals (CI) in cases and controls were calculated to measure the association between disease characteristics and chronic infection with *S. aureus*. Their significance was estimated using a maximum likelihood method.

Results

Demographic and clinical characteristics

Demographic characteristics and pulmonary function were similar in the three groups (table 1). There was a trend for the patients infected with *S. aureus* to be younger and

Table 1. – Demographic characteristics and pulmonary function

	Chronically infected with *S. aureus*	Intermittently infected with *S. aureus*	Never infected with *S. aureus*
n	12	16	58
Age yrs	36 (27.7–48.4)	36 (27.3–55.0)	48 (38.0–53.2)
Male n[#]	4 (33)	6 (37.5)	24 (41.4)
BMI	20.8 (19.2–24.5)	19.8 (18.5–22.9)	23.8 (19.9–26.0)
FEV_1 $L \cdot s^{-1}$	2.4 (2.2–2.9)	2.26 (1.3–2.9)	2.3 (1.6–3.0)
FEV_1 % pred*	76.0 (44.0–86.7)	75.1 (46.3–92.5)	75.0 (47.2–98.0)
FVC $L \cdot s^{-1}$	3.4 (3.0–4.4)	3.3 (2.6–4.2)	3.4 (2.8–4.0)
FVC % pred*	94.8 (70.9–102.0)	89.5 (77.1–108.5)	96.0 (81.0–109.0)
Smoking status			
Nonsmokers[#]	11 (91.7)	10 (76.9)	41 (73.2)
Exsmokers[#]	1 (8.3)	2 (15.4)	3 (5.4)
Current smokers[#]	0	1 (7.7)	12 (21.4)

Data are presented as the median with the interquartile range in parentheses, unless indicated otherwise. *S. aureus*: *Staphylococcus aureus*; BMI: body mass index; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity. *: percentage predicted values according to Knudson charts; [#]: percentage is given in parenthesis.

more likely to be nonsmokers. The length of follow-up varied in both groups from 6 months to >20 yrs. The clinical course in the three groups appeared to be similar. Use of antibiotics, oral corticosteroids, inhaled corticosteroids and bronchodilators were similar in the groups. The only difference was the use of oral flucloxacillin in patients chronically infected with *S. aureus*. The prevalence of dyspnoea, repeated infections, sinusitis, nasal polyps and gastrointestinal symptoms were similar in the three patient groups (table 2). A family history of bronchiectasis was present in 16.7% of patients chronically infected with *S. aureus* compared to 7.7% in patients who were intermittently infected and 10.5% in patients who had never been infected. The prevalence of infertility was: 0% in patients who were chronically infected with *S. aureus*, 23.3% in patients who were intermittently infected and 15.1% in those who had never been infected with *S. aureus*.

Although patients estimates of daily sputum volumes were similar, patients with chronic infection tended to have more purulent sputum (table 3). Concomitant bacterial infection was similar in the group (table 3). The predominant areas of the lung involved by bronchiectasis as identified by HRCT of the thorax is shown in table 4. Upper zone bronchiectasis was more common in both groups that were infected with *S. aureus.*

Aetiology of bronchiectasis

In the group as a whole eight (9.3%) patients fulfilled criteria for the diagnosis of ABPA. Ciliary abnormalities were detected in 14 (16.3%) patients, panhypogammaglobulinaemia in five (5.8%) patients and 59 (68.6%) patients were classified as idiopathic or bronchiectasis from other causes. However, 23 of this group of patients had a reduced IgG subclass level on at least one occasion. After further investigation five patients were diagnosed as having atypical variants of CF. They all had normal pancreatic function, liver function and body mass indices (24.6, range 18.3–25.3), with a history of normal growth and weight gain in childhood. Sweat sodium concentrations in these patients were; normal (<60 $mmol \cdot L^{-1}$) in two individuals (patients 1 and 2), or high with suppression following pretreatment with fludrocortisone (patients 3, 4 and 5). Nasal potential difference measurements were only possible in two of these individuals and the values obtained were -37 mV (abnormal, patient 3) and -25mV (equivocal, patient 1). The remaining patients had nasal polyps and hence accurate measurements of nasal potential differences were not possible. Genotyping in patient 3 demonstrated only one copy of the ΔF508 mutation and the other mutation was unidentified. The other four patients were diagnosed on the basis of genetic analysis as compound heterozygotes; ΔF508/S549N in two patients (patients 4 and 5), ΔF508/3849 + 10 Kbc→T (patient 1), G542X/3849 + 10 Kbc→T (patient 2). Detailed information on the aetiology of the bronchiectasis in the different patient groups is provided in table 5.

Table 2. – Prevalence of clinical features

	Chronically infected with *S. aureus*	Intermittently infected with *S. aureus*	Never infected with *S. aureus*
n	12	16	58
Dyspnoea	75	71.4	56.1
Repeated infections	100	93	96.6
Sinusitis	75	46.7	61.4
Nasal polyps	16.7	7.1	8.8
Gastrointestinal	33	14.3	46.6

Results are given as the percentage of patients with that symptom in each group. *S. aureus*: *Staphylococcus aureus*.

Table 3. – Sputum purulence and bacterial infection

	Chronically infected with *S. aureus*	Intermittently infected with *S. aureus*	Never infected with *S. aureus*
n	12	16	58
Sputum purulence			
Mucoid	0	0	5.3
Muco-purulent	25	50	49
Purulent	75	50	45.6
Concomitant bacterial infection			
P. aeruginosa	25	31.3	22.4
H. influenzae	25	18.8	17.2
S. pnemoniae	8.3	12.5	6.9

Results are given as the percentage of patients with that feature in each group. *S. aureus*: *Staphylococcus aureus*: *P. aeruginosa*: *Pseudomonas aeruginosa*: *H. influenzae*: *Haemophilus influenzae*: *S. pneumoniae*: *Streptococcus pneumoniae*.

Case-control analysis

The case-control comparison demonstrated significant associations between the 12 patients with chronic infection by *S. aureus* and ABPA, atypical variants of CF and intermediate sweat sodium values (table 6). Analysis of the relationship between the distribution of bronchiectasis on CT scanning and current chronic infection did not demonstrate any significant associations (global disease OR= 1.4 (95% CI 0.4–4.7), upper zone disease OR=3.6 (95% CI 0.9–14.5), mid zone disease (OR not estimated as none of the patients chronically infected with *S. aureus* had mid zone disease), lower zone disease OR=0.6 (95% CI 0.2–2.1)). There were no significant associations with other features such as clinical history, or other investigations including concomitant bacterial pathogens or pulmonary function. Two of the patients had ABPA and were atypical variants of CF (patients 1 and 2).

The associations were maintained when the analysis was based on whether *S. aureus* had ever been isolated (groups A and B) from sputum samples compared to never been isolated (group C). The five patients diagnosed as atypical

Table 4. – Distribution of bronchiectasis as identified by high resolution computed tomography of the thorax

Predominant area involved	Chronically infected with *S. aureus*	Intermittently infected with *S. aureus*	Never infected with *S. aureus*
n	12	16	58
Global	50	37.5	55.2
Upper	25	25	3.4
Mid	0	12.5	5.2
Lower	25	25	36.2

Results are given as the percentage of patients with bronchiectasis predominantly involving that area in each group. *S. aureus*: *Staphylococcus aureus*.

Table 5. – Aetiology of bronchiectasis

	Overall group	Chronically infected with *S. aureus*	Intermittently infected with *S. aureus*	Never infected with *S. aureus*
n	86	12	16	58
Ciliary dyskinesia	14 (16.3)	1 (8.3)	3 (18.8)	10 (18.2)
Panhypogamma-globulinaemia	5 (5.8)	0 (0)	2 (12.5)	3 (5.2)
ABPA	8 (9.3)	4 (33.3)	2 (12.5)	2 (3.4)
Other	59 (68.6)	7 (58.3)	9 (56.3)	43 (74.1)
subclass deficiency	23 (26.7)	0 (0)	6 (37.5)	22 (37.9)
atypical CF	5 (5.8)	3 (25)	2 (12.5)	0 (0)

Data are presented as the median with the percentages in parentheses. *S. aureus*: *Staphylococcus aureus*. CF: *cystic fibrosis.*

variants of CF patients had all grown *S. aureus* in their sputum previously. The association between previous *S. aureus* in sputum and intermediate sweat test results and ABPA were maintained (table 7). There was also a significant association with predominant upper zone disease on HRCT (OR=6.4 (95% CI 1.8–23.2), p=0.003).

Discussion

The study demonstrated that infection with *S. aureus* in patients with bronchiectasis is more frequently associated with ABPA and atypical variants of CF and hence may be a useful marker for these conditions. The primary analysis was based on whether patients were chronically infected with *S. aureus* but the implications are the same whether *S. aureus* is isolated chronically or intermittently. The associations observed were not due to any other characteristics present in the study groups. Concomitant bacterial infection including *P. aeruginosa* was similar in both the case and control groups. Disease severity appeared similar in the three groups but the retrospective nature of the study limited this evaluation.

There was a strong association between infection with *S. aureus* and atypical variants of CF. Three of the five patients with this condition had abnormal sweat tests which were suppressed with fludrocortisone, and normal pancreatic function, normal body mass indices, normal liver function, and growth in childhood. Therefore, prior to genotyping they would not be classified as patients with CF. The 3849 + 10 Kbc→T mutation has been well described as a CF mutation associated with normal sweat concentrations [15, 16]. Abnormal nasal potential differences have been reported in these patients with normal sweat tests who possess atypical CF mutations and may be a useful measurement in borderline cases [15–17]. One of the patients in the current study had been categorized as atypical variants of CF on the basis of consistently abnormal nasal potential differences. However, the value of this technique is limited as accurate measurements are not possible in the presence of nasal polyps, acute rhinosinusitis or previous nasal surgery, which are present in a high proportion of patients with bronchiectasis [1].

There was also an association between ABPA and infection with *S. aureus*. In ABPA the germination of aspergillus spores in the airways and continued exposure to fungal elements in combination with specific IgE and IgG antibodies leads to immune mediated damage of bronchial walls. The long term sequelae of this process is fibrosis and bronchiectasis which is often in the upper lobes [18]. The incidence of ABPA is greater in patients with CF [19, 20–22]. This may provide credence to the possibility of a common mechanism rendering these individuals more susceptible to chronic infection with *S. aureus*. One study demonstrated a higher frequency of CF mutations in patients with ABPA compared to patients with chronic bronchitis suggesting that the CF transmembrane conductance regulator (CFTR) may play a role in the aetiology of some patients with ABPA [23]. It is also of interest that the two patients in the current study found to have both ABPA and atypical CF had one copy of the same CFTR mutation (3849 + 10 Kbc→T). However, recent reports of ABPA occurring in patients with CF following lung transplantation suggest that alternative aetiologies should be considered [24, 25].

Table 6. – Associations between disease characteristics and chronic infection with *S. aureus*

	Chronic infection with *S. aureus*	
	OR and 95% CI	p-value
Allergic bronchopulmonary aspergillosis	8.8 (1.8–41.9)	0.001
Atypical variants of cystic fibrosis	12.0 (1.8–81.7)	0.01
Sweat Na+ >60 mmol·L^{-1}	4.0 (1.0–15.3)	0.04
Ciliary dyskinesia	0.4 (0.1–3.4)	0.35
Pan hypogammaglobulinaemia	*	
Other causes	0.8 (0.2–2.6)	0.65
Predominant upper zone disease on HRCT of thorax	3.6 (0.9–14.5)	0.08

*: none of the patients with pan hypogammaglobulinaemia were currently infected with *Staphylococcus aureus* (*S. aureus*). HRCT: high resolution computed tomography.

Table 7. – Associations between disease characteristics and chronic infection with *S. aureus*

	S. aureus ever isolated	
	OR and 95% CI	p-value
Allergic bronchopulmonary aspergillosis	7.6 (1.4–40.8)	0.01
Atypical variants of cystic fibrosis	#	0.0006
Sweat Na^+ >60 mmol·L^{-1}	4.3 (1.5–12.7)	0.006
Ciliary dyskinesia	0.8 (0.2–2.7)	0.65
Pan hypogammaglobulinaemia	1.4 (0.2–9.0)	0.72
Other causes	0.8 (0.3–1.9)	0.53
Predominant upper zone disease on HRCT of thorax	6.4 (1.8–23.2)	0.003

#: all patients with atypical variants of cystic fibrosis had *Staphylococcus aureus* (*S. aureus*) isolated by sputum culture on at least one occasion in the past. HRCT: high resolution computed tomography.

The association between previous infection with *S. aureus* and upper zone bronchiectasis may be of relevance as both ABPA and CF particularly involve the upper lobes. Accentuation of zonal differences in ventilation and perfusion may lead to susceptibility to *S. aureus* infection and subsequent damage. Alternatively *S. aureus* may have a predilection to occur in lung with upper lobe damage which coincidentally occurs in both conditions.

The results of this study merely indicate disease associations and only partially answer questions regarding mechanisms. Interpretation is also limited by possible ascertainment bias. Although there was a standard protocol for most investigations there was a difference in the proportion of patients who had genetic studies performed (7 (58%) in the case group *versus* 2 (3%) in the control group). It is possible that a higher number of CF mutations were found in the case group because they were more extensively studied than the control group. This potential bias is unlikely to have a clinical significance as standard clinical indications for performing genetic investigations were followed. The other potential pitfall is from sputum culture as *S. aureus* occasionally colonizes the oropharynx. Standard collection methods were used with the aid of physiotherapy to minimize oropharyngeal contamination and the samples were taken directly to the laboratory for processing. The hypotheses generated from this study should therefore be investigated further in other populations of patients with bronchiectasis.

The results of this study suggest that the isolation of *Staphylococcus aureus* isolated from the sputum of patients with bronchiectasis should prompt more detailed investigation to exclude allergic bronchopulmonary aspergillosis and atypical variants of cystic fibrosis. It is important to identify allergic bronchopulmonary aspergillosis as recognition and earlier treatment with systemic corticosteroids may prevent progression of the bronchiectasis and development of upper zone fibrosis. The need to identify patients with cystic fibrosis, even those in their middle age with pancreatic sufficiency, has become important with the advent of new treatments targeted at correcting the ion transport defect, cystic fibrosis transmembrane conductance regulator replacement therapy and gene therapy and the provision of appropriate genetic counselling.

References

1. Cole PJ. Bronchiectasis. *In:* Bewis RAL, Corrin B, Geddes DM, Gibson GJ, eds. Respiratory Medicine. 2nd Edn. London, UK, WB Saunders, 1995; pp. 1286–1317.
2. Pier GB. Pulmonary disease associated with *Pseudomonas aeruginosa* in cystic fibrosis: current status of the host bacterium interaction. *J Infect Dis* 1985; 151: 575–580.
3. Fick RB Jr, Sanada F, Hornick DB. Emergence and persistence of *Pseudomonas aeruginosa* in the cystic fibrosis airway. *Semin Respir Inf* 1992; 7: 168–178.
4. Abman SH, Ogle JW, Harbeck RJ, Butler-Simon N, Hammond KB, Accurso FJ. Early bacteriologic, immunologic and clinical courses of young infants with cystic fibrosis identified by neonatal screening. *J Pediatr* 1991; 119: 211–217.
5. Machan ZA, Pitt TL, White W, *et al*. Interaction between *Pseudomonas aeruginosa* and *Staphylococcus aureus*: description of an anti-staphylococcal substance. *J Med Microbiol* 1991; 34: 213–217.
6. Nicotra MB, Rivera M, Dale AM, Shepherd R, Carter R. Clinical, pathophysiologic and microbiologic characterization of bronchiectasis in an aging cohort. *Chest* 1995; 108: 955–961.
7. Pang JA, Cheng A, Chan HS, Poon D, French G. The bacteriology of bronchiectasis in Hong Kong investigated by protected catheter brush and bronchoalveolar-lavage. *Am Rev Respir Dis* 1989; 139: 14–17.
8. Cabello H, Torres A, Celis R, *et al*. Bacterial chronic infection of distal airway in healthy subjects and chronic lung disease: a bronchoscopic study. *Eur Respir J* 1997; 10: 1137–1144.
9. Cole P, Flower CDR, Lavender JP. Clinical and imaging aspects of bronchiectasis. *In:* Potchen EJ, Grainger RG, Greene R, eds. Pulmonary Radiology. Philadelphia, USA, WB Saunders, 1993; pp. 242–258.
10. Rutland J, Cole PJ. Non-invasive sampling of nasal cilia for measurement of beat frequency and study of ultrastructure. *Lancet* 1980; ii: 564–565.
11. Gibson LE, Cooke RE. A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilising pilocarpine by iontophoresis. *Pediatrics* 1959; 23: 545–549.
12. Hodson ME, Beldon I, Power R, Duncan FR, Barnber M, Batten JC. Sweat tests to diagnose cystic fibrosis in adults. *BMJ* 1983; 286: 1381–1383.
13. Alton EWFW, Currie D, Logan-Sinclair R, Warner JO, Hodson ME, Geddes DM. Nasal potential difference: a clinical diagnostic test for cystic fibrosis. *Eur Respir J* 1990; 3: 922–926.
14. Crompton GK. Fungal disease. *In*: Brewis RAL, Corrin B, Geddes DM, Gibson GJ, eds. *Respiratory Medicine*. 2nd Edn. London, UK, WB Saunders, 1995; pp. 853–868.
15. Highsmith WE, Burch LH, Zhan Z, *et al*. A novel mutation in the cystic fibrosis gene in patients with pulmonary disease but normal sweat chloride concentrations. *N Engl J Med* 1994; 331: 974–980.
16. Stewart B, Zabuer J, Shuber AP, Welsh MJ, McCray PB Jr. Normal sweat chloride values do not exclude the diagnosis of cystic fibrosis. *Am J Respir Crit Care Med* 1995; 151: 899–903.
17. Delmarco A, Pradel U, Cabrini G, Bonizzato A, Mastella G. Nasal potential difference in cystic fibrosis patients presenting borderline sweat test. *Eur Respir J* 1997; 10: 1145–1149.
18. McCarthy DS, Simon G, Hargreave FE. The radiological appearances in allergic bronchopulmonary aspergillosis. *Clin Radiol* 1970; 21: 366–375.
19. Rosenberg M, Patterson R, Mintzer R, Cooper BJ, Roberts M, Harris KE. Clinical and immunologic criteria for the diagnosis of allergic bronchopulmonary aspergillosis. *Ann Int Med* 1977; 86: 405–414.
20. Nelson LA, Callerame ML, Schwartz RH. Aspergillosis and atopy in cystic fibrosis. *Am Rev Respir Dis* 1979; 120: 863–873.
21. Brueton MJ, Omerod LP, Shah KJ, Anderson CM. Allergic bronchopulmonary aspergillosis complicating cystic fibrosis. *Arch Dis Chest* 1980; 55: 348–353.
22. Voss MJ, Bush RK, Mischler EH, Peters ME. Association of allergic bronchopulmonary aspergillosis and cystic fibrosis. *J Allergy Clin Immunol* 1982; 69: 539–546.
23. Muller PW, Hamosh A, Macek M Jr, *et al*. Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations in allergic bronchopulmonary aspergillosis. *Am J Hum Genet* 1996; 59: 45–51.
24. Egan JJ, Yonan N, Carroll KB, Deiraniya AK, Webb AK, Woodcock AA. Allergic bronchopulmonary aspergillosis in lung allograft recipients. *Eur Respir J* 1996; 9: 169–171.
25. Fitzsimmons EJ, Aris R, Patterson R. Recurrence of allergic bronchopulmonary aspergillosis in the post-transplant lungs of a cystic fibrosis patient. *Chest* 1997; 112: 281–282.