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Evaluation of a new definition for chronic *Pseudomonas aeruginosa* infection in cystic fibrosis patients

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Abstract

Background: Patients were defined each successive month as either 'chronic' when more than 50% of the preceding 12 months were PA culture positive, 'intermittent' when ≤50% of the preceding 12 months were PA culture positive, 'free of PA', with no growth of PA for the previous 12 months, having previously been PA culture positive, or 'never infected', when PA had never been cultured. *Methods*: Cross-sectional analysis of 146 children attending the Leeds Regional Cystic Fibrosis Centre was performed to assess relationship between the new definition and clinical scores and investigations. The response variable was regressed on age and sex and the residuals analysed using the Kruskal–Wallis test. *Results*: The 'chronic' group (18% of patients) had significantly worse Shwachman–Kulczycki (SK) and Northern chest X-ray scores, and % predicted FEV_1 values than the 'free' (28%) or 'never' (20%) categories ($P<0.004$). The 'intermittent' group (34%) had a significantly higher SK score than the 'chronic' group ($P<0.0001$), and a significantly lower % predicted FEV_1 value than the 'free' or 'never' groups ($P<0.0003$). 'Chronic' patients were significantly associated with a positive, and 'never' patients with a negative, PA antibody result ($P<0.001$). *Conclusions*: The validity and importance of identifying these four subgroups is demonstrated. Previous definitions may over-estimate the prevalence of chronic infection.
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Keywords: Intermittent; *Pseudomonas aeruginosa* infection; Evaluation study; Classification

1. Introduction

Pseudomonas aeruginosa is the most common pathogen causing chronic infection in people with cystic fibrosis (CF) [1]. Chronic infection with this organism has been shown to be associated with a lower FEV_1 in childhood [2], a faster decline in FEV_1 despite optimal respiratory management [3,4], a worse mortality rate [5], and shorter median survival [1].

Currently there is no universally accepted definition for chronic *P. aeruginosa* infection. In the UK the Cystic Fibrosis Trust use the definition proposed by Brett et al. in 1992 as 'the regular culture of *P. aeruginosa* from the sputum or respiratory secretions, on two or more occasions, extending over 6 months or a shorter period if accompanied by a sustained rise of anti-*Pseudomonas* antibodies' [6]. In Copenhagen chronic *P. aeruginosa* infection is defined as 'persistent presence of *P. aeruginosa* for at least 6 consecutive months, or less when combined with the presence of two or more *P. aeruginosa* precipitins', with intermittent *P. aeruginosa* colonisation defined as 'culture of *P. aeruginosa* at least once and the presence of normal levels of precipitating antibodies against *P. aeruginosa*' [7]. In Germany and North America chronic *P. aeruginosa* colonisation is defined as having more than 50% of cough swab or sputum samples positive in a 12-month period [2,8], or by whether a patient's last sputum sample of each year grows *P. aeruginosa* [1].

Modern CF care, particularly the success of *P. aeruginosa* eradication policies [9], has resulted in these definitions becoming less appropriate for many patients with CF. For example, a patient may grow *P. aeruginosa* on two or more occasions and then, following eradication, not grow the organism again for many years.

A validated, universally accepted, and clinically useful classification of patients infected with *P. aeruginosa*,

Abbreviations: PA: *Pseudomonas aeruginosa*; CF: Cystic fibrosis; FEV1: Forced expiratory volume in one second; SK: Shwachman–Kulczycki

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particularly those chronically infected, would improve patient care for the following reasons:

1. *Treatment*: Determining which patients require chronic *P. aeruginosa* treatment protocols [10,11].
2. *Clinic Management*: Enabling cohort isolation of those patients that are at high risk of infecting other CF patients with *P. aeruginosa* [12–14].
3. *Prognosis*: Allowing better prognostic information for patients and parents.
4. *Audit*: Enabling clinical effectiveness of anti-Pseudomonal strategies within the clinic to be assessed quantitatively over time [7].
5. *Research*: Facilitating the comparison of results from different CF centres, and cumulative analyses such as national CF databases.

As there is presently no established 'gold-standard' definition for *P. aeruginosa* infection it is not possible to directly validate any new definition. However, it is possible to assess face-validity by determining if a new definition classifies patients appropriately in relation to relevant clinical scores and investigations. Face validity demonstrates that the measure reflects the content of the concept in question [15], and as the association of chronic *P. aeruginosa* infection with clinical score [16], chest X-ray score [4], percent predicted FEV_1 [2,3], height and weight [3], and *P. aeruginosa* antibody level [6] is already established, the validity of a new definition can be best assessed in this way.

The accuracy of any definition will depend on the frequency of sampling. The UK Cystic Fibrosis Trust suggests sputum sampling at least every 2 months (six times per year) in children and every 3 months (four times per year) in adults [17]. If patients are sampled less than this then any categorisation is likely to be inaccurate. We would recommend that sampling should be taken three monthly as a minimum as recommended by the European Consensus on Antibiotic Therapy against *P. aeruginosa* [10].

The definition of chronic infection should be based on microbiological results from cough swabs or sputum samples as few centres have access to well-validated, prompt *P. aeruginosa* antibody results. The monthly categorisation should be independent of the multiplicity of samples taken within any given month, to avoid bias caused by patients having more frequent sampling during exacerbations.

The aim of this study was to assess the validity of a new definition for chronic *P. aeruginosa* infection that meets these requirements.

2. Methods

2.1. Monthly culture status

Patients were defined each successive calendar month as either:

1. *Pseudomonas aeruginosa* culture positive (one or more *P. aeruginosa* positive cough swabs or sputum cultures that month).
2. *P. aeruginosa* culture negative (all cough swab or sputum cultures that month negative for *P. aeruginosa*).
3. No cough swab or sputum culture performed that month.

2.2. Infection status based on previous 12 months

Four distinct states of *P. aeruginosa* infection, important in the management of individual patients and the clinic, were defined by a local working party of clinicians, microbiologists and nursing staff. All the patients in the clinic were categorised each month according to their monthly *P. aeruginosa* status over the preceding twelve calendar months on the following basis:

Chronic infection	When more than 50% of months, when samples had been taken, were *P. aeruginosa* culture positive.
Intermittent infection	When 50% or less of months, when samples had been taken, were *P. aeruginosa* culture positive.
Free of infection	No growth of *P. aeruginosa* during the previous twelve months, having previously been *P. aeruginosa* culture positive.
Never	*P. aeruginosa* never cultured from sputum or cough swab.

2.3. Microbiological methods

Standard practice for patients receiving all of their care at our Centre is to have a sputum or cough swab sample taken at every clinic visit, with no more than twelve weeks between visits.

Sputum samples were collected from all children who could expectorate. Parents were encouraged to collect sputum samples at home if their children expectorated only rarely. When it was not possible to obtain sputum samples and the child was well, cough swabs were taken. If new infection was suspected clinically sputum induction was attempted using hypertonic saline [18].

P. aeruginosa was isolated from samples using standard microbiological methods. Briefly, sputum samples were homogenised with an equal volume of Sputasol (Oxoid Ltd, Basingstoke, UK) and incubated at 37 °C for 30 min. 0.1 ml of homogenate was added to 5 ml of peptone water, agitated, and 10 μl of this was used to inoculate bactracin (10 mg/l)—chocolate agar. Cough swabs were applied directly without dilution. All plates were incubated at 37 °C in air for 48 h. Presumptive *P. aeruginosa* isolates were identified by colonial morphology, gram stain, positive cytochrome oxidase test, and biochemical profiling using API 20NE (bioMerieux, Marcy l'Etoile, France).

Table 1
Demographic details for patients in each *P. aeruginosa* infection category in April 1999

	Chronic	Intermittent	Free	Never
Number (%)	26 (18%)	50 (34%)	41 (28%)	29 (20%)
Number male (%)	11 (42%)	24 (48%)	29 (71%)	14 (48%)
Mean age in years (S.D.)	13.0 (2.2)	9.6 (4.5)	10.1 (4.0)	6.1 (4.0)
Mean number of months in previous year when sample taken (range)	10.7 (7–12)	9.8 (4–12)	7.7 (2–12)*	7.4 (1–11)*
Mean% of months in previous year when sample taken and +ve for *P. aeruginosa*	82.2%	23.7%	0%	0%

* Three patients in the 'free' group and three patients in the 'never' group were sampled less than our recommended four times per year.

2.4. Patients

Initially an 11-year retrospective analysis of all 232 patients receiving all their CF care at the Leeds Regional Paediatric Cystic Fibrosis Centre between October 1989 and December 2000 was performed to determine the proportion of patients in each category per month, and the future risk of growing *P. aeruginosa* for patients defined as 'free' or 'never' (Total patient months assessed 17 230).

The relationship between categorisation and clinical data was assessed in April 1999. All patients who had received all their CF care at the Leeds Paediatric CF Centre for at least 12 months were included in the cross-sectional analysis. Clinical and demographic data was collected as near as possible to the end of the 12-month period. Two investigators (KB and SC) assessed Shwachman–Kulczycki (SK) [19] and Northern chest X-ray scores [20], and another investigator (TL) then determined patients' *P. aeruginosa* infection categories for April 1999. The percent-predicted FEV_1 was recorded for all patients old enough to perform this test reproducibly [21] and height and weight measurements, and calculated body mass indices were compared to a standard non-CF population [22]. *P. aeruginosa* antibody levels were also recorded, and were considered positive if >16 [6].

The value of the categorisation in predicting future infection status was assessed by taking all those patients who had been attending the clinic and having cough swabs or sputum samples taken for at least 12 months in April 1994, and who were still present in the clinic in April 1999. Patients were classified by the new definition both in April 1994 and April 1999.

The clinical variables associated with each *P. aeruginosa* infection category were then compared. All variables changed linearly with age. Since age and sex were possible confounding factors the analyses have adjusted for these when appropriate with age included in the parametric models (analysis of covariance) as a linear covariate. For non-parametric analyses the response variable was regressed on age (and sex, if appropriate) and the residuals were analysed using the Kruskal–Wallis test. The χ^2 test was used to assess the relationships between *P. aeruginosa* infection category and a positive *P. aeruginosa* antibody test, and infection category in 1994 to infection category in 1999.

3. Results

Microbiological data allowing classification on the basis of *Pseudomonas aeruginosa* sputum culture status was available for all 232 patients receiving full-care at the centre during the period 1990–2000. During the total 17 230 patient months assessed, patients were defined as 'chronic' for 3793 months (22.0%), 'intermittent' for 5355 months (31.1%), 'free' for 4084 months (23.7%), and 'never' for 3998 (23.2%) months.

During this period the risk of a new growth of *P. aeruginosa* in those patients classified as 'free' or 'never' was 4.1 and 2.2% per month, respectively ($P<0.02$).

Clinical data was available for all 146 patients who had been receiving their full care at the centre for at least 12 months in April 1999 (Table 1), although the 28 youngest children were unable to perform FEV_1 assessment. The largest group was the 'intermittent' category, and there was a high proportion of males in the 'free' category.

The association between infection category and SK score was highly significant ($P<0.0001$), with progressively lower SK scores as severity of *P. aeruginosa* categorisation increased from 'never' through to 'chronic' (Table 2). Age and sex had no significant association with SK score, and were removed from the model. The difference between the 'chronic' group and the other categories was significant. Northern chest X-ray score was significantly associated with age and sex, and when these were adjusted for the effect of *P. aeruginosa* infection category remained highly significant ($P<0.0043$), with the 'chronic' score significantly worse than in the 'free' and 'never' categories. The percent-predicted FEV_1 was significantly associated with sex, and following appropriate adjustment the association with infection category was highly significant ($P<0.0003$). The 'chronic' and 'intermittent' groups' percent-predicted FEV_1s were significantly lower than those in the 'free' and 'never' groups.

Table 2
New classification and relationship to Shwachman–Kulczycki score, Northern chest X-ray score, and % predicted FEV_1

	Chronic	Intermittent	Free	Never
Mean SK score (S.D.)	74.5 (14.9)	86.6 (8.9)	88.3 (10.4)	92.7 (5.4)
Mean Northern score (S.D.)	9.2 (3.9)	6.3 (2.8)	5.7 (2.9)	4.0 (1.7)
[mean adjusted for age and sex]	[8.0]	[6.3]	[5.8]	[5.2]
Mean percent-predicted FEV_1 (S.D.)	64.4 (19.2)	69.8 (17.6)	83.1 (19.8)	83.4 (12.3)
[mean adjusted for sex]	[65.1]	[69.6]	[81.1]	[84.9]

Table 3
New classification and relationship to height (cm), weight (kg), and body mass index (BMI)

	Chronic	Intermittent	Free	Never
Mean height (S.D.)	148.3 (14.3)	130.7 (28.0)	135.1 (23.4)	111.6 (24.4)
[mean adjusted for age]	[128.7]	[131.1]	[132.0]	[132.5]
Mean height SDS (S.D.)	−0.94 (0.98)	−0.75 (1.13)	−0.45 (0.92)	−0.49 (0.99)
[mean adjusted for age]	[−1.11]	[−0.74]	[–0.47]	[−0.30]
Mean weight (S.D)	40.7 (11.2)	31.5 (15.3)	33.9 (15.8)	22.4 (11.4)
[mean adjusted for age]	[30.0]	[31.8]	[32.5]	[33.6]
Mean weight SDS (S.D.)	−0.73 (1.00)	−0.52 (1.06)	−0.16 (0.96)	−0.14 (0.99)
[mean adjusted for age]	[−0.71]	[−0.52]	[−0.16]	[−0.16]
Mean BMI (S.D.)	18.1 (2.3)	17.3 (2.3)	17.5 (2.3)	16.0 (1.8)
[mean adjusted for age]	[17.3]	[17.3]	[17.4]	[17.8]
Mean BMI SDS (S.D.)	−0.25 (0.99)	−0.05 (1.02)	0.18 (0.92)	0.30 (0.87)
[mean adjusted for age]	[−0.04]	[−0.06]	[0.21]	[0.07]

Table 4
New classification and relationship to *P. aeruginosa* antibody level (positive if >16)

	Chronic	Intermittent	Free	Never
Mean *P. aeruginosa* antibody level (S.D.)	156.0 (105.9)	35.0 (56.2)	24.0 (72.9)	2.0 (8.0)
Number *P. aeruginosa* antibody positive (%)	26 (100%)	19 (38%)	12 (29%)	2 (7%)
Number *P. aeruginosa* antibody negative (%)	0	31 (62%)	29 (71%)	27 (93%)

Table 5
Table demonstrates association between patients' categorisation in April 1994 with their categorisation in April 1999

1999	1994			
	Chronic	Intermittent	Free	Never
Chronic	7	5	6	2
Intermittent	1	7	11	11
Free	0	5	13	9
Never	0	0	0	7

It was necessary to adjust for age when analysing the association between infection category and height, and following this infection category had no significant association (Table 3). However, when height standardised scores (SDS) were assessed, it was demonstrated that those in the 'chronic' group were significantly shorter than the 'never' group ($P=0.032$). Adjustment for age was also required when assessing the association between infection category and weight and body mass index, after which infection category was not significantly associated with these parameters. Similarly, after age adjustment, there was no significant association between infection category and weight and body mass index SDS scores.

The association between infection category and *P. aeruginosa* antibody level required adjustment for age (Table 4). Following this the association with infection category was highly significant ($P<0.0001$), with a significantly higher *P. aeruginosa* antibody level in the chronic group when compared to the other three categories. When a *P. aeruginosa* antibody level of >16 was considered positive [6], 'chronic' patients were significantly associated with a positive antibody result ($P<0.001$), and 'never' with a negative antibody result ($P<0.001$).

The predictive value of infection status in determining status five years later was assessed among the 84 patients who had been seen in the centre since 1994 (Table 5). Of those patients defined as 'chronic' in 1994 seven (88%) remained chronic in 1999, significantly more than would be expected if this definition had no

predictive value ($P < 0.001$). In contrast, only 29% of those classed as 'intermittent' in 1994 had progressed to 'chronic' by 1999.

4. Discussion

The purpose of this study was to determine the value of a new definition for chronic *Pseudomonas aeruginosa* infection in cystic fibrosis patients, allowing patients to be categorised into one of four groups according to the results of monthly sputum cultures over the preceding year. The results show that this new definition has good face validity, and demonstrates the importance of identifying these four subgroups of patients. We therefore suggest that this new definition is more useful than previous definitions.

The clinical data demonstrates that chronic *P. aeruginosa* infection is associated with worse SK clinical score, worse Northern chest X-ray score, worse percent-predicted FEV_1, and a reduction in height standard deviation score when compared to uninfected patients, in keeping with previous studies [2,7,23,24]. The latter acknowledge that many patients grow *P. aeruginosa* intermittently, but the clinical characteristics of this subgroup have not previously been separately analysed. Nor are there any studies assessing patients free of *P. aeruginosa* for more than 12 months (these have been previously categorised within the intermittent or first detection group) [7,8]. It is clear from our data that these patients are very different clinically and prognostically from other children with cystic fibrosis.

4.1. Relevance for treatment

These present data suggest that our previous definition 'the regular culture of *P. aeruginosa* from the sputum or respiratory secretions, on two or more occasions, extending over six months' would falsely categorise the many patients with a new incidence of *P. aeruginosa* as chronically infected [6]. When a routine cough swab or sputum culture demonstrates a new growth of *P. aeruginosa* the patient is called back to clinic for commencement of early eradication therapy and further cultures are taken [7,9]. Patients with chest exacerbations requiring treatment also receive more frequent cultures than obtained in routine surveillance. This biases heavily towards a definition of chronic *P. aeruginosa* according to our previous definition [6]. Indeed 46 patients would have been classified as chronic in April 1999 compared to 28 under our new definition. Many such patients become free of *P. aeruginosa* following treatment, and it would be inappropriate for them to receive intensive chronic *P. aeruginosa* treatment protocols.

The Copenhagen definition uses a precipitin technique, which is well validated but not widely available [7]. When precipitins have not been measured, chronic *P. aeruginosa* infection is defined as persistent presence of *P. aeruginosa* for 6 consecutive months. Patients classified as 'chronic' under our new definition have only 82.2% of months when sputum samples are taken in which *P. aeruginosa* is cultured. These patients are clinically very different from those who are classified as 'intermittent', and are significantly predisposed to remaining chronically infected. Only 15 of these 28 patients would be identified by the Copenhagen criteria without the support of precipitin results.

The German definition uses a similar chronic *P. aeruginosa* infection definition as in our study, although they further subdivide this based on the presence of non-mucoid or mucoid strains [8]. In practice this subdivision does not affect treatment decisions, as evidence suggests that the entire group should be on intensive antibiotic treatment and mucolytics such as DNase [10,25]. They do not differentiate between those classed as 'intermittent' and 'free', but our data demonstrates that these are very different groups.

4.2. Relevance for clinic management

We have assessed the relative risk of patients in each diagnostic category growing *P. aeruginosa* in their sputum in any given month. For those patients classed as 'never' or 'free', the chance of growing *P. aeruginosa* is small (2 and 4%, respectively), and they are not a high risk for infecting other patients. 'Chronic' patients have an 82% chance of growing *P. aeruginosa*, strengthening the case for cohorting these patients to reduce the risk of cross-infection. Patients who are classed as 'intermittent' have a 24% risk of growing *P. aeruginosa* each month and ideally these patients should also be kept separate from those classed as 'never' or 'free' [26].

4.3. Relevance for predicting future infection status

An important feature for any definition of chronic *P. aeruginosa* infection should be that it has predictive value. As well as demonstrating an association with changes in respiratory function and clinical scores, it should also be shown to be of value in predicting a patient's *P. aeruginosa* infection status in the future. In our study 88% of patients defined as chronically infected remained in this category five years later. This is also of value over the short term, as of the 26 patients defined as 'chronic' in April 1999, 22 remained 'chronic' in April 2000 (85%). This compares favourably to both the Copenhagen definition (11 of 15 remaining chronic (73%)) [7], and our previous definition (34 of 46 remaining chronic (74%)) [6]. In addition, our data demonstrate that with appropriate treatment patients defined as 'intermittent' are most likely to remain in this category when reassessed 5 years later, with as

many patients (29%) in this category reverting to 'free' as progressing to 'chronic'.

A high proportion (71%) of the patients in the 'free' group were male. Females outnumbered males in the other three groups. The relevance of this finding requires further investigation.

4.4. Relevance for research and audit

P. aeruginosa is an important pathogen in cystic fibrosis, and much research into the effects of this organism as well as improved treatment strategies is being undertaken worldwide. For valid comparisons to be made between different studies it is important that a consensus is reached on the most appropriate definition for chronic *P. aeruginosa* infection. The increasing use of national databases to compare the results between centres also requires a definition that does not bias against those centres who perform sputum surveillance more frequently, or places too much emphasis on differing precipitin or antibody tests.

5. Conclusions

This study demonstrates the value of an improved clinical definition for chronic *P. aeruginosa* infection. In particular it demonstrates the importance of identifying patients growing *P. aeruginosa* intermittently to facilitate appropriate treatment. Adoption of a suitable definition is necessary to ensure both improved treatment for people with cystic fibrosis and an increased understanding of the effects of *P. aeruginosa* infection.

References

[1] FitzSimmons S. The cystic fibrosis foundation patient registry report. Pediatr Pulmonol 1996;21:267–75.
[2] Kerem E, Corey M, Gold R, Levison H. Pulmonary function and clinical course in patients with cystic fibrosis after pulmonary colonisation with *Pseudomonas aeruginosa*. J Pediatr 1990;116:714–9.
[3] Pamukcu A, Bush A, Buchdahl R. Effects of *Pseudomonas aeruginosa* colonisation on lung function and anthropomorphic variables in children with cystic fibrosis. Pediatr Pulmonol 1995;19:10–5.
[4] Kosorok MR, Zeng L, West SH, et al. Acceleration of lung disease in children with cystic fibrosis after *Pseudomonas aeruginosa* acquisition. Pediatr Pulmonol 2001;32:277–87.
[5] Henry RL, Mellis CM, Petrovic L. Mucoid *Pseudomonas aeruginosa* is a marker of poor survival in cystic fibrosis. Pediatr Pulmonol 1992;12:158–61.
[6] Brett MM, Simmonds EJ, Ghonheim ATM, Littlewood JM. The value of serum IgG titres against *Pseudomonas aeruginosa* in the management of early infection in cystic fibrosis. Arch Dis Child 1992;67:1086–8.
[7] Frederiksen B, Koch C, Høiby N. Changing epidemiology of *Pseudomonas aeruginosa* infection in Danish cystic fibrosis patients (1974–1995). Pediatr Pulmonol 1999;28:159–66.
[8] Ballmann M, Rabsch P, von der Hardt H. Long term follow-up of changes in FEV1 and treatment intensity during *Pseudomonas aeruginosa* colonisation in patients with cystic fibrosis. Thorax 1998;53(9):732–7.
[9] Valerius NH, Koch C, Høiby N. Prevention of chronic *Pseudomonas aeruginosa* colonisation in cystic fibrosis by early treatment. Lancet 1991;338:725–6.
[10] Döring G, Conway SP, Heijerman HG, Hodson ME, Høiby N, Smyth A, Touw DJ. Antibiotic treatment against *Pseudomonas aeruginosa* in cystic fibrosis: A European consensus. Eur Respir J 2000;16(4):749–67.
[11] Denton M, Wilcox MH. Antimicrobial treatment of pulmonary colonisation and infection by *Pseudomonas aeruginosa* in cystic fibrosis patients. J Antimicrob Chemother 1997;40(4):468–74.
[12] Cheng K, Smyth RL, Govan JR, et al. Spread of a beta-lactam-resistant *Pseudomonas aeruginosa* in a cystic fibrosis clinic. Lancet 1996;348:639–42.
[13] Pedersen SS, Koch C, Høiby N, Rosenthal K. An epidemic spread of multiresistant *Pseudomonas aeruginosa* in a cystic fibrosis centre. J Antimicrob Chemother 1986;17:505–16.
[14] Høiby N. Microbiology of Cystic Fibrosis. In: Hodson ME, Geddes DM, editors. Cystic Fibrosis. London: Arnold, 1999. p. 75–98.
[15] Bryman A, Cramer D. Quantitative data analysis for social scientists. London: Routledge, 1990.
[16] Nixon GM, Armstrong DS, Carzino R, et al. Clinical outcome after early *Pseudomonas aeruginosa* infection in cystic fibrosis. J. Pediatr 2001;138(5):699–704.
[17] Standards of Care: Standards for the clinical care of children and adults with cystic fibrosis in the UK. The Cystic Fibrosis Trust's Clinical Standards and Accreditation Group. Cystic Fibrosis Trust, London, UK, 2001.
[18] De Boeck K, Alifier M, Vandeputte S. Sputum induction in young cystic fibrosis patients. Eur Respir J 2000;16(1):91–4.
[19] Shwachman H, Kulczycki LL. Long-term study of one hundred and five patients with cystic fibrosis. Am J Dis Child 1958;96:6–15.
[20] Conway SP, Pond MN, Bowler I, et al. The chest radiograph in cystic fibrosis: A new scoring system compared with the Chrispin–Norman and Brasfield Scores. Thorax 1994;49:860–2.
[21] Polgar G, Promadhat V. Pulmonary function testing in children. Philadelphia: W.B. Saunders, 1971.
[22] Cole TJ, Freeman JV, Preece MA. Body mass index reference curves for the UK, 1990. Arch. Dis. Child 1995;73:25–9.
[23] Burns JL, Gibson RL, McNamara S, et al. Longitudinal assessment of *Pseudomonas aeruginosa* in young children with cystic fibrosis. J Infect Dis 2001;183(3):444–52.
[24] Høiby N. Prospects for the prevention and control of *Pseudomonas aeruginosa* infection in children with cystic fibrosis. Paediatric Drugs 2000;2(6):451–63.
[25] Hodson ME. Clinical studies of rhDNase in moderately and severely affected patients with cystic fibrosis—an overview. Respiration 1995;62(1):29–30.
[26] *Pseudomonas aeruginosa* infection: prevention and infection control. Report of the United Kingdom Cystic Fibrosis Trust's Infection Control Group. Cystic Fibrosis Trust, London, UK, 2001.