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Evaluating the ''Leeds criteria'' for *Pseudomonas aeruginosa* infection in a cystic fibrosis centre

M. Proesmans*, W. Balinska-Miskiewicz#, L. Dupont¶, X. Bossuyt+, J. Verhaegen§, N. Høiby[f] and K. de Boeck*

ABSTRACT: Four separate categories of chronic *Pseudomonas aeruginosa* (*Pa*) infection in children with cystic fibrosis (CF) have been previously defined, based on airway cultures taken over the previous year.

The aim of the present study was to evaluate this definition in the current authors' paediatric and adult CF clinic using clinical, immunological and lung function parameters.

During follow-up, out of 193 patients, 55 (34%) CF patients had never been infected with *Pa*, 27 (17%) were free of *Pa*, 29 (18%) were intermittently infected and 51 (31%) were chronically infected. Disease severity markers, such as lung function, were significantly worse in the chronic group, especially in the paediatric population. Differences in adult patients were smaller and no longer significant. *Pa* antibodies differed strongly between the groups, and were very high (mean±SD 55.4±5.5) and highly statistically significant from all other groups in the chronic group. They were low and different from all other groups in the never group (1.8±0.6). *Pa* antibodies did not differ between the free of *Pa* and the intermittent group.

In conclusion, the current authors confirmed an agreement between *Pseudomonas aeruginosa* status according to the new definition and clinical status, as well as with the level of *Pseudomonas aeruginosa* antibodies.

KEYWORDS: Anti-pseudomonal antibodies, chronic lung infection, cystic fibrosis, *Pseudomonas aeruginosa*

In cystic fibrosis (CF), *Pseudomonas aeruginosa* (*Pa*) is the most important lung pathogen causing progressive lung infection and shortened survival [1]. The first definition for chronic *Pa* infection in CF was introduced at the Danish CF centre in 1974. This was based on monthly microbiological examination of sputum, defining chronic infection as the continuous presence of *Pa* in sputum for 6 months, whereas presence for shorter periods of time was defined as intermittent infection [2]. This definition was subsequently modified by including the antibody response to *Pa* so that chronic infection required presence of *Pa* in the lower airway for ⩽6 months if the antibody response to *Pa* was significantly increased [3]. Since most CF centres do not see the patients monthly and do not have access to regular *Pa* antibody measurement, the European consensus definition for chronic *Pa* infection is more commonly used, *i.e.* at least three positive cultures over ⩾6 months with at least a 1-month interval between the samples [4].

Chronic *Pa* infection in CF is usually preceded by a stage of intermittent infection [5], and the early detection of *Pa* followed by intensive treatment can delay chronic infection [6]. In agreement with these clinical data, LEE *et al.* [7], from the CF centre in Leeds (UK), introduced a new definition classifying patients into four groups according to airway culture results obtained over the last 12 months. Chronic infection refers to patients in whom airway samples were *Pa* culture positive in >50% of the explored months. Intermittent infection refers to patients with ⩽50% of *Pa* positive cultures. A patient is defined as free of *Pa* when *Pa* has been isolated in the past, whereas all cultures taken in the last 12 months remain *Pa* negative. Never infected obviously refers to patients in whom *Pa* has never been cultured [7]. This definition was evaluated in the paediatric CF population of LEE *et al.* [7] and has been proved to classify patients appropriately in relation to relevant clinical scores and investigations.

AFFILIATIONS
Depts of *Paediatric Pulmonology, ¶Adult Pulmonology, +Laboratory Medicine, Immunology, and §Microbiology, University Hospital Gasthuisberg, Leuven, Belgium. #Dept of Paediatrics, Allergology and Cardiology, Wroclaw Medical University, Wroclaw, Poland. [f]Dept of Microbiology, Rigshospitalet, University of Copenhagen, Denmark.

CORRESPONDENCE
K. de Boeck
University Hospital Gasthuisberg Leuven
Paediatric Pulmonology
Herestraat 49
3000 Leuven
Belgium
Fax: 32 16343842
E-mail: christiane.deboeck@uz.kuleuven.ac.be

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Acquisition of *Pa* can occur at an early age, but may remain undetected depending on the frequency of airway sampling and the site where the sample is taken [8]. Diagnosis of *Pa* acquisition is based on sputum microbial culture or is replaced by cough swabs for patients unable to expectorate sputum, mainly young children. The negative predictive value of a cough swab for *Pa* is known to be as high as 95% with a low positive predictive value of only 44% [9].

It has been shown that the *Pa* antibody response is related to the degree of inflammation and lung tissue damage [10, 11]. Therefore, the level of antibody against *Pa* may distinguish between chronic and intermittent infection.

The aim of the present study was to evaluate the definition by LEE *et al.* [7] (further referred to as the Leeds criteria) for chronic *Pa* infection in the paediatric, as well as adult, CF population at the Leuven clinic (Belgium). The profile of the patients in the different groups of *Pa* status were compared with clinical and biochemical parameters, as well as by measuring the level of *Pa* antibodies in the four groups, as in the study by LEE *et al.* [7].

PATIENTS AND METHODS

Patients and controls

For 162 out of the 193 patients in follow-up (120 aged $\leqslant$18 yrs and 73 aged >18 yrs) at the Leuven CF centre, at least four airway culture results in different months spread over the year, as well as *Pa* antibody measurements, were available. Patients with less than four sputum cultures were excluded as well as CF patients after lung transplants (n=13). In all patients, the diagnosis of CF was in accordance with the Rosenstein diagnostic criteria [12]. The patient characteristics are presented in table 1. CF transmembrane regulator (CFTR) gene mutation analysis was performed in all patients. A mutation was classified as class I–V, as reported in the CFTR mutation consortium [13]. For the different *Pa* groups, the CF genotype was classified in these different classes (I–II–III or IV–V) [14–16].

Routinely, patients attend the centre every 3 months. When exacerbations or complications occurred, extra visits were scheduled. In total, 162 patients with at least four sputum results spread over different months of the year were available for inclusion. In patients without chronic *Pa* infection, isolation of *Pa* from sputum or the upper airway was followed by initiation of treatment according to Danish protocol [6]: inhaled colistin for 3 months together with oral ciprofloxacin for 3 weeks to 3 months. At every clinic visit, an airway sample was obtained (expectorated sputum sample or cough swab). When clinically indicated, a sputum induction or bronchoscopy was performed. Based on the results from airway cultures obtained in the previous year, patients were divided into four groups according to the Leeds criteria: never, free of *Pa*, intermittent or chronic *Pa* infection.

For chronically infected patients, the total number of days receiving *i.v.* antibiotic therapy was calculated from 2001 to 2003. The dates of the first isolation of *Pa*, as well as age at onset of chronic infection according to the European definition, were taken from the database.

For validation of *Pa* antibody testing, a control group consisted of 60 non-CF patients with a mean (range) age of 22.4 yrs (1.8–48.9) who attended the outpatient clinic because of respiratory diseases, such as bronchial asthma, recurrent cough or recurrent infections. In none of these patients was *Pa* isolated from airway cultures.

The current study was approved by the hospital's ethics committee and consent was obtained from the patients or parent(s).

TABLE 1 Patient characteristics

Subjects n	162
Males n	92
Females n	70
Age yrs	14.4 (1.3–52.8)
Aged $\leqslant$18 yrs	100
Aged >18 yrs	62
Two known mutations	148
Two class I, II or III mutations	137/148
One class IV, V mutation	11/148
CFRDM	13
PS	13

Data are presented as n or mean (range). CFRDM: cystic fibrosis-related diabetes mellitus; PS: pancreatic sufficiency.

Airway cultures

Sputa were collected in sterile plastic disposable containers. Sputa and cough swabs were stored at ambient temperature and normally processed within 4 h from collection. Sputa and swabs were inoculated and incubated onto several media for the isolation of *Pa* and other potential pathogens using Columbia Agar Base (CMO331; Oxoid, Basingstoke, UK) with 5% defibrinated horse blood, MacConkey agar (CM0007; Oxoid) and mannitol salt agar (CM0085; Oxoid). All media were incubated aerobically at 37°C for 48 h. All different phenotypes from the sputum of each patient were identified as *Pa* employing a combination of conventional identification methods (growth at 42°C, positive oxidase, pigment production), as well as the Vitek 2 system (BioMérieux, Marcy l'Etoile, France) and cellular fatty acid analysis by gas chromatography.

ELISA for the detection of anti-pseudomonas antibodies

A maxisorp (Nunc, Kamstrupvej, Denmark) ELISA 96-well plate was coated for 60 min at room temperature with a Pseudomonas extract (6.66 $mg \cdot L^{-1}$ in PBS Dulbecco). The antigen extract was kindly provided by one of the authors (N. Høiby) and consisted of water-soluble extracts obtained by sonication of the 17 most common O-groups of *Pa* (standard antigen (St-Ag) 1–17) as previously described [17]. After coating, the plate was washed three times with 200 μL PBS containing 0.1% TWEEN 20. Thereafter, the plate was blocked for 1 h at room temperature with blocking dilution buffer (PBS Dulbecco + 0.1% TWEEN + 15$g \cdot L^{-1}$ NaCl). Serum was diluted 1:40,000 with blocking dilution buffer, which was added to the wells, and incubated for 1 h at room temperature. After washing three times with 200 μL in PBS containing 0.1% TWEEN 20, peroxidase-conjugated anti-human immunoglobulin (Ig)G (Nordic Immunological Laboratories, Tilburg,

the Netherlands) in a dilution of 1:5,000 (in blocking dilution buffer) was added to the wells. The plate was incubated for 1 h at room temperature. Thereafter, 3.3′-5.5′-tetramethylbenzidine was added for colour development. After 60 min, the reaction was stopped by acid stock solution containing 1N HCl and 3N H_2SO_4. Plates were read at 450 nm.

A pool of serum obtained from Danish CF patients colonised with *Pa* 100 AU was assigned as a reference. The ELISA method has been described in detail previously. The predictive value of a positive test to diagnose chronic *Pa* infection was 92% (diagnostic specificity) and the predictive value of a negative test to rule out chronic infection was 93% (diagnostic sensitivity) [18, 19].

Lung function

From the age of 6 yrs, lung function tests are performed every 3 months. Forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) are measured with a Master Screen spirometer (Jaeger, Hoechberg, Germany) according to American Thoracic Society guidelines [20]. Values are expressed as percentages of the predicted normal values according to the modified Knudson reference equation [21, 22]. The best value from 2003 was taken for analysis. The nutritional state was expressed as weight for height percentage (W/H %) [23].

Statistical methods

Data were tested for normal distribution with the Kolmogorow–Smirnov test. For variables with a normal distribution, a t-test was used; otherwise, nonparametric statistical tests were used. For evaluation of categorical data, Fisher's exact or Chi-squared test were used. A p-value of $\leqslant 0.05$ was considered significant.

RESULTS

Validation of the different colonisation groups according to the Leeds criteria

Of the CF patients, 55 (34%) had never been infected by *Pa*, 27 (17%) were free of *Pa*, 29 (18%) were intermittently infected and 51 (31%) were chronically infected. The mean age of the patients was 11.3, 15.2, 13.8 and 21.5 yrs for the never, free of *Pa*, intermittent and chronic groups, respectively (tables 2–4). For the patients aged $\leqslant$18 yrs, only 12% were chronically infected while 63% of adult patients (aged >18 yrs) had chronic *Pa* infection. The prevalence of chronic infection increased with age. The number of infected patients for different age groups is shown in figure 1.

Different disease severity markers such as FVC, FEV_1, total IgG and W/H % differed between the groups and were significantly worse in the chronic group (table 2). The differences between the means for FEV_1 were statistically significant for all groups except free of *Pa versus* the intermittent group (unpaired t-test; table 2). W/H % was lower in the chronic group. This was significantly different from the never ($p<0.005$) and intermittent ($p<0.001$) group, but the difference did not reach significance in comparison with the free of *Pa* group ($p=0.09$; unpaired t-test). In the paediatric age group ($\leqslant$18 yrs) differences in disease severity parameters were more pronounced (table 3), whereas in patients aged >18 yrs these differences were no longer significant (table 4).

When looking at comorbidities, the number of pancreatic insufficient patients was similar in the paediatric and adult group (91 out of 100 pancreatic insufficient in those aged $\leqslant$18 yrs *versus* 58 out of 62 aged >18 yrs; Chi-squared p=nonsignificant). As can be expected, the number of patients with CF-related diabetes mellitus (CFRDM) was higher in the adult group (12 out of 62 *versus* one out of 100 for the paediatric group; Chi-squared $p=0.001$).

Co-pathogens were analysed for each *Pa* group. Overall, 66% of patients had an isolation of *Staphylococcus aureus*. *S aureus* infection was significantly lower ($p<0.01$) in the chronic group (25 out of 51) compared with the other groups (40 out of 55 for never, 19 out of 27 for free of *Pa*, and 24 out of 29 in the intermittent group). Infection with other Gram-negative bacteria (*Stenotrophomonas maltophilia, Alcalygenes xylosoxidans* and *Pseudomonas* spp.) was found in 20% of patients. The incidence was significantly lower in the never group ($p<0.01$) compared with the other groups (four out of 40 for the never

TABLE 2 Patient characteristics according to *Pseudomonas aeruginosa* (*Pa*) colonisation status for the whole patient population[#]

Group (number)	Subjects n	Age yrs	FEV_1 % pred	FVC % pred	W/H %	IgG	*Pa* IgG AU	*i.v.* therapy in 2003 days
Never (1)	55	11.3±1.0	98.0±2.3	101.8±2.2	98.1±2.0	966±47	1.8±0.6	0 (0–0)
Statistical differences		2, 4[¶]	2, 3, 4[¶]	2, 3, 4[¶]	4[¶]	2, 4[¶]	2, 3, 4[¶]	2, 3, 4[+]
Free of *Pa* (2)	27	15.3±1.4	83.8±4.8	93.1±3.8	95.4±2.2[¶]	1339±94	5.3±1.4	0 (0–44)
Statistical differences		1, 4[¶]	1, 4[¶]	1[¶]		1, 3[¶]	1, 4[¶]	1[+]
Intermittent (3)	29	13.4±1.8	89.1±3.4	95.4±2.9	100.1±2.0	1001±61	8.4±2.8	13 (0–26)
Statistical differences		4[¶]	1, 4[¶]	1, 4[¶]	4[¶]	2, 4[¶]	1, 4[¶]	1, 4[+]
Chronic (4)	51	21.5±0.8	67.3±3.1	87.3±2.5	90.4±1.8	1426±54	55.4±5.5	28 (0–42)
Statistical differences		1, 2, 3[¶]	1, 2, 3[¶]	1, 3[¶]	1, 3[¶]	1, 3[¶]	1, 2, 3[¶]	1, 3[+]

Data are presented as mean±SEM and median (interquartile range), unless otherwise stated. FEV_1: forced expiratory volume in one second; % pred: per cent predicted; FVC: forced vital capacity; W/H: weight for height; Ig: immunoglobulin. [#]: different disease severity markers differ between the groups and are significantly worse in the chronic group; [¶]: using unpaired t-test; [+]: using the Mann–Whitney U-test.

TABLE 3 Patient characteristics according to *Pseudomonas aeruginosa* (*Pa*) colonisation status for paediatric patients aged ⩽18 yrs[#]

Group (number)	Subjects n	Age yrs	FEV1 % pred	FVC % pred	W/H %	IgG	*Pa* IgG AU	*i.v.* therapy in 2003 days
Never (1)	48	9.1±0.7	101.2±2.2	104.6±2.2	97.8±2.2	906±54	2.1±0.7	0 (0–0)
Statistical differences		4[¶]	3, 4[¶]	3, 4[¶]		4[¶]	4[¶]	3, 4[+]
Free of *Pa* (2)	16	10.0±1.1	96.6±4.3	103.6±3.1	95.8±2.3	1121±97	3.3±1.2	0 (0–26)
Statistical differences		4[¶]	1, 4[¶]	1, 4[¶]		4[¶]	4[¶]	
Intermittent (3)	24	10.4±0.9	90.9±3.9	95.8±3.4	99.6±1.8	956±72	5.6±1.9	8 (0–14.5)
Statistical differences		4[¶]	1, 4[¶]	1, 4[¶]	4[¶]	4[¶]	1, 4[¶]	1[+]
Chronic (4)	12	13.7±1.2	73.3±4.7	84.6±3.0	90.1±3.9	1484±137	38.5±10.2	27 (0–39)
Statistical differences		1, 2, 3[¶]	1, 2, 3[¶]	1, 2, 3[¶]	3[¶]	1, 2, 3[¶]	1, 2, 3[¶]	1[+]

Data are presented as mean±SEM and median (interquartile range), unless otherwise stated. FEV1: forced expiratory volume in one second; % pred: per cent predicted; FVC: forced vital capacity; W/H: weight for height; Ig: immunoglobulin. [#]: differences in disease severity markers were more pronounced in the paediatric age group; [¶]: using unpaired t-test; [+]: using Mann–Whitney U-test.

TABLE 4 Characteristics according to *Pseudomonas aeruginosa* (*Pa*) colonisation status within the age group aged >18 yrs[#]

Group (number)	Subjects n	Age yrs	FEV1 % pred	FVC % pred	W/H %	IgG	*Pa* IgG AU	*i.v.* therapy in 2003 days
Never (1)	7	26.7±2.7	80.6±8.0	86.9±4.7	99.5±3.2	1272±125	0.2±1.4	0 (0–7)
Statistical differences						2[¶]	4[¶]	3, 4[+]
Free of *Pa* (2)	11	22.6±1.1	66.3±7.4	82.0±5.9	94.7±4.6	1720±138	6.4±2.6	12 (0–67)
Statistical differences						1[¶]	4[¶]	
Intermittent (3)	5	28.3±6.2	71.3±5.2	85.9±6.5	102±8.5	1505±60	27.3±13.9	28 (14–46)
Statistical differences								1[+]
Chronic (4)	39	23.9±0.6	65.5±3.8	88.1±3.1	90.5±2.0	1449±63	60.8±6.5	27 (0–45)
Statistical differences							1, 2[¶]	1, 3[+]

Data are presented as mean±SEM and median (interquartile range), unless otherwise stated. FEV1: forced expiratory volume in one second; % pred: per cent predicted; FVC: forced vital capacity; W/H: weight for height; Ig: immunoglobulin. [#]: differences in disease severity parameters were no longer significant in patients aged >18 yrs; [¶]: using unpaired t-test; [+]: using Mann–Whitney U-test.

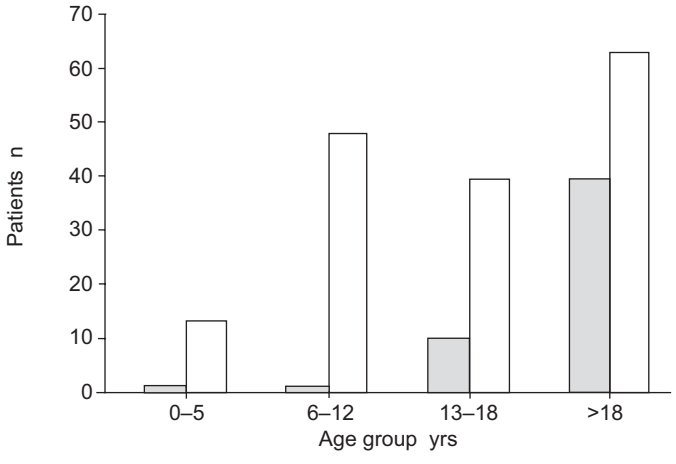

FIGURE 1. Number of chronic *Pseudomonas aeruginosa* (*Pa*) patients per age group. The total number of patients (□) was 13, 48, 39 and 62 for the age groups 0–5, 6–12, 13–18 and >18 yrs, respectively. The number of colonised patients (■) was 1, 1, 10 and 39 for each of the respective age groups.

group, 10 out of 27 for the free of *Pa* group, eight out of 29 for the intermittent group, and 11 out of 51 for the chronic group). Infection with *Burkholderia cepacia* and methicillin-resistent *S. aureus* has a low incidence in the current study population (three and 10 out of 162, respectively).

The present authors evaluated the presence of mucoid strains according to the Leeds criteria (defined as ⩾50% of isolates mucoid). As expected, chronic infection was strongly associated with mucoid infection (65% mucoid patients within the chronic group), whereas the intermittent group had only 10% of mucoid *Pa* strains.

Changes of colonisation state between 2002 and 2003

The majority of interchanges occurred between the intermittent and free of *Pa* group. Out of the intermittent group in 2002, 13 (8%) patients changed to free of *Pa* in 2003 (in most of these patients *Pseudomonas* spp. was intermittently of the mucoid type) and 10 (6.2%) free of *Pa* patients changed to intermittent. Apart from eight (4.9%) patients changing from never to intermittent, only minor exchanges occurred between the other groups: of the intermittent group, one (0.6%) patient in 2002

changed to chronic in 2003, two (1.2%) free of *Pa* patients changed to chronic, one chronic patient changed to free of *Pa*, and one chronic patient in 2002 changed to intermittent in 2003.

Mutation analysis

In the chronic infection group, 48 (94.1%) patients had two class I–II or III mutations and three (5.9%) patients had either one mutation classified as IV–V (n=2) or both mutations unknown (n=1). In the other groups, a combined total of 89 (79.5%) patients had two class I–II–III mutations, 23 (20.5%) patients had either one IV–V mutation (n=9), and one or both mutations unknown (n=8 and n=5, respectively; Fisher's exact p=0.01).

Specific Pseudomonas aeruginosa *antibodies*

The specific serum IgG to whole-cell *Pa* antigen was measured by ELISA at the turn of 2003–2004. The serum sample was taken outside respiratory exacerbations and during the routine visit to the CF Centre while the patient required other routine blood tests, including total serum IgG.

Pa IgG antibodies differed strongly between the groups (fig. 2). The antibodies were very high (mean±SEM 55.4±5.5) and highly statistically significant from all other groups in the chronic group. The antibodies were also very low (1.8±0.6) and different from all other groups in the never group and the control group (1.37±0.22). *Pa* IgG antibodies did not differ between the free of *Pa* group (5.3±1.4) and the intermittent group (8.4±5.5; unpaired t-test; tables 2–4).

A cut-off level for specific *Pa* IgG values was determined in the current study population using a receiver operating curve. A value of 17 AU had the best combination of sensitivity (88%) and specificity (96%) to distinguish chronically infected patients from the rest of the cohort. Positive predictive value was 92.1% and negative predictive value was 93.9%.

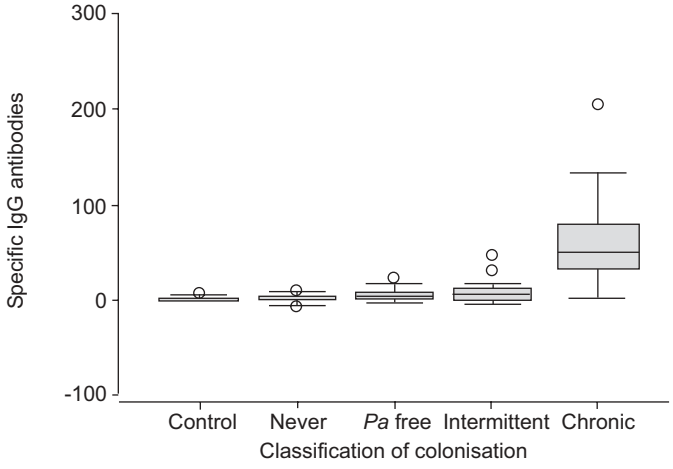

FIGURE 2. Box and whisker plot for *Pseudomonas aeruginosa* (*Pa*) antibody values for control patients and cystic fibrosis patients with different *Pa* status. Horizontal line represents median value. Box and whisker represent the SE and minimum/maximum values, respectively. Ig: immunoglobulin. o: outliers.

Duration of chronic infection and total days on i.v. *antibiotics treatment*

The duration of *Pa* infection influenced the level of specific *Pa* antibodies, but the correlation was weak (Pearson r=0.47) although significant (p<0.001).

The total numbers of days treated with *i.v.* antibiotics from 2001 until 2003 also correlated significantly, but weakly, with the level of specific IgG antibodies to *Pa* (Pearson r=0.432; p<0.001). Similar correlations were found between *i.v.* therapy and total IgG (Pearson r=0.413; p<0.001).

DISCUSSION

A uniform definition of *Pa* status reflecting the clinical and microbiological status of CF patients is important for: 1) decisions concerning patient cohorts in order to prevent cross-infection; 2) the intensity of treatment (eradication of *Pa versus* maintenance treatment with inhaled antibiotics); 3) evaluation of prognosis; and 4) research or centre evaluation purposes (*e.g.* effect of patient cohorts on the incidence of chronic *Pa* infection). In the study by LEE *et al.* [7], a new classification of *Pa* infection in CF was introduced and four different groups of *Pa* infection status were defined based on the sputum cultures obtained over the last 12 months. As most of the airway cultures in the current patients were carried out every 3 months, this definition is more applicable than the European consensus definition [4], where three positive sputa over 6 months with a ⩾1-month interval are evaluated. Moreover, the present data confirm that these four distinct states of *Pa* status are in agreement with the clinical status of paediatric CF patients followed at the present authors' centre. Lung function values, total IgG and nutritional status differed significantly between the chronic and other *Pa* groups. However, for adult CF patients, the above parameters did not show similar differences between the four *Pa* groups when looking at most of the parameters used. This may be explained by the smaller number of adult patients in the current analysis and by the fact that the sicker patients in the chronic group had received transplants (n=13) or were deceased, improving the data for the remaining patients in the chronic group (median FEV_1 % pred for the transplanted group just before transplantation was 21.3% for a median age of 22.5 yrs). The lower mean age in the chronic group of adult patients (although not statistically different) compared with the other groups may support this explanation. However, after long-standing *Pa* infection, other factors may become as important for the severity of illness, such as CFRDM, modifier genes, and long-standing infection with other pathogens, *etc.* Finally, for this older cohort, treatment strategies for early *Pa* infection and chronic lung infection in general were different in early childhood when most patients were not yet cared for in a specialised CF centre. More data on larger numbers of adult CF patients are needed to evaluate whether the Leeds criteria are a valuable tool in this CF population.

The intermittent and free of *Pa* groups did not differ significantly according to age, lung function, W/H %, total IgG and specific *Pa* antibodies in the current study population. The most likely reason was that patients interchanged between these groups in the course of the years. This idea is supported by the observed interchanges of infection status between 2002 and 2003, mostly observed between the free of *Pa* and intermittent group. Eradication of long-standing and even

mucoid *Pa* is exceptional but has been previously reported [24]. However, it can be argued whether the interchange between the groups is a real change in *Pa* infectious status or, at least in some patients, an artefact due to the intrinsic weaknesses of the Leeds criteria. The minimum sample number needed to use the Leeds criteria is not defined by LEE *et al.* [7], although they state that sampling should be taken every 3 months as a minimum. Monthly sampling would make the classification more accurate, but it is not realistic for most CF centres. In the study by LEE *et al.* [7], the frequency of sampling ranged 1–12 months·yr^{-1}, with a mean number of samples of more than seven per year. The present authors obtained samples from the patients at least every 3 months during routine clinic together with unscheduled visits for exacerbations, as is recommended in the European consensus document [4]. Patients were included only if at least four sputum results from different months were available.

Apart from the number of cultures, the origin of the sample may influence the classification of the *Pa* status (expectorated sputum/bronchoscopic sample *versus* cough swab). The lower positive predictive value of cough swabs may be the explanation for the presence of mucoid *Pa* in patients classified as intermittently infected [9]. In the two patients with an ''unexpected'' shift from chronic to free of *Pa* and chronic to intermittent almost all cultures were cough swabs. In the first patient, sputa grew *A. xylosoxidans* on every occasion in 2003, possibly pointing towards a shift in pathogens.

To further support the categories defined by the Leeds CF centre, a previously described *Pa* whole-cell antibody ELISA measurement [18] was introduced into the current authors' clinic. A *Pa*-positive culture combined with a negative *Pa* antibody titre suggests early superficial infection rather than advanced chronic infection [4]. For *Pa* antibody measurements, several pseudomonal antigens and a variety of techniques have been reported in the literature. Most studies using *Pa* whole-cell lysate as an antigen show a rise in antibody titre early in the course of *Pa* infection, a relationship between antibody level and duration of infection, and a decrease of antibody level with intensive antibiotic treatment [1, 8, 3, 19, 25–29]. Due to these characteristics, the sonicated extract of 17 *Pa* serotypes (St-Ag 1–17) was used as the antigen.

The level of specific *Pa* antibodies was high in the chronic group and significantly different from all other groups, confirming that the definition for chronic *Pa* groups is CF patients with severe *Pa* lung infection leading to inflammation and lung tissue destruction. Despite the absence of significant different clinical parameters in the adult group, the *Pa* antibody level was the highest in the chronic group and this difference was significantly raised compared with the never and free of *Pa* group.

Although the correlation was weak, the duration of chronic *Pa* infection and the number of days receiving *i.v.* therapy correlated with the *Pa* antibody level in the current study population. The *Pa* antibody level can be followed-up to evaluate the success of anti-*Pa* treatment [29]. In patients chronically colonised with *Pa*, a large and sudden increase in *Pa* antibody level is a poor prognostic factor [19, 30]. Unfortunately, the present data do not include serial measurements of *Pa* antibody values, but the present authors plan to perform this assay at least yearly for follow-up of individual patients.

The Leuven CF centre has a very low chronic *Pa* infection rate, especially in the paediatric age group (amounting to only 12% compared with 18% in the study by LEE *et al.* [7]). This is in agreement with a favourable clinical condition. In fact, children classified to every nonchronic group have relatively low total IgG levels and a very low level of specific *Pa* antibodies, thus confirming the proper classification.

Conclusion

This is the first validation of the Leeds criteria in another cystic fibrosis clinic and the first evaluation in adult patients. The present authors confirmed an agreement between *Pseudomonas aeruginosa* status and clinical status as well as with the level of *Pseudomonas aeruginosa* antibodies, thus confirming it is a workable classification. While clinical parameters are significantly different for children with chronic *Pseudomonas aeruginosa* infection compared with the other groups, differences in adult patients are smaller and no longer significant. Chronic *Pseudomonas aeruginosa* infection in the current paediatric patients is low and agrees with the low *Pseudomonas aeruginosa* antibody levels within this population.

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