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Chronic *Pseudomonas aeruginosa* infection definition: EuroCareCF Working Group report

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Abstract

Chronic pulmonary infection with *P. aeruginosa* develops in most patients with cystic fibrosis (CF); by adulthood 80% of patients are infected and chronic *P. aeruginosa* infection is the primary cause of increased morbidity and mortality in CF. Chronic infection is preceded by an intermittent stage of infection. The initial stage is characteristically followed by the gradual emergence of mucoid variants of the colonizing strains and a rise in anti-*Pseudomonas* antibodies. In addition to optimizing existing therapeutic strategies, effective new agents need to be identified. Studies in patients with CF are particularly challenging: the progressive nature of the disease and the wide variation in severity influence considerably the outcome of drug testing. A validated, universally accepted, and clinically useful classification of patients infected with *P. aeruginosa*, particularly those chronically infected, is needed that can be used as both a criterion for patient selection for clinical trials and as a study endpoint.

Keywords: Cystic fibrosis; *Pseudomonas aeruginosa*; Chronic infection; Definitions

1. Introduction

Improvements in clinical care have resulted in a dramatic increase in the life expectancy of patients with cystic fibrosis (CF) over the last 40 years [1]. In several European countries, the median survival is now between 30 and 40 years. The aggressive treatment of lung disease and improvements in nutrition are the major factors responsible. However, 95% of people with CF still die from respiratory failure. *Pseudomonas aeruginosa* (*P. aeruginosa*) is the most common pathogen causing chronic infection in people with CF. Chronic pulmonary infection with *P. aeruginosa* develops in most patients with CF; by adulthood 80% of patients are infected in most CF centres and chronic *P. aeruginosa* infection is the primary cause of increased morbidity and mortality in CF [2–11]. In addition to optimising existing therapeutic strategies, effective new agents need to be identified. Studies in patients with CF are particularly challenging because of the progressive nature of the disease and the wide variation in severity and number of organs involved, which influences considerably the outcome of drug testing. Confounding factors for which patients may be stratified include their class of mutation, age, gender, bacterial lung infection, lung function, pancreatic function, hepato-biliary disease and nutritional status. The most relevant and robust endpoint for interventional studies in CF patients is survival. However, the use of survival as an endpoint is no longer appropriate as it would take enormous numbers of patients and many years to see an

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effect. Thus, surrogate endpoints and biomarkers are needed. One such endpoint is the prevalence of chronic *P. aeruginosa*. A validated, universally accepted, and clinically useful classification of patients infected with *P. aeruginosa*, particularly those chronically infected, is needed that can be used as both a criterion for patient selection for clinical trials and as a study endpoint. For studies evaluating different treatments for chronically infected patient populations, the proper definition of chronic infection is crucial for the interpretation of results, as well as when assessing different isolation policies. Without a universally accepted definition of chronic *P. aeruginosa* infection comparison of results from different CF centres or the clinical effectiveness of different anti-*Pseudomonas* strategies will not be possible.

2. Definition of chronic *P. aeruginosa* infection

Currently there is no universally accepted definition of chronic *P. aeruginosa* infection. Most of the currently used definitions are based on microbiological results from sputum cultures. However, modern CF care, particularly the success of early *P. aeruginosa* eradication protocols, makes the microbiological definition less appropriate. Moreover, the accuracy of any definition using culture results will depend on the frequency of microbiological assessment of secretions from the respiratory tract of CF patients.

Chronic infection can be defined as an infection, which persists in spite of therapy, and in spite of the host's immune and inflammatory response. Moreover, in contrast to bacterial colonization, chronic infection is characterized by persistent pathology and immune responses. This definition of chronic infection necessitates the measurement of immune responses, the measurement of specific anti-*Pseudomonas* antibodies.

The following definitions of chronic *P. aeruginosa* infection in CF have been published and used either in clinical settings or for research purposes:

1. The Copenhagen definition of chronic *P. aeruginosa* infection [2] was formulated 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* precipitating antibodies and
   - Intermittent *P. aeruginosa* colonization was defined as a culture of *P. aeruginosa* at least once and the presence of normal levels of precipitating antibodies against *P. aeruginosa*.

   All patients were controlled on a regular monthly basis and each patient had an average of 10 sputum cultures per year.
2. The steps in the course of *P. aeruginosa* colonization were defined by Ballmann et al. [11] as:
   - first detection of *P. aeruginosa* (PA1),
   - chronic *P. aeruginosa* colonization (PAc),
   - first detection of mucoid *P. aeruginosa* strains (PAm), and
   - chronic mucoid *P. aeruginosa* colonisation (Pacm).

   For transition to the chronic stages (PAc and PAcm) more than 50% of cultures in a 12 month period had to be positive for *P. aeruginosa* and the related phenotype (mucoid or non-mucoid).

   Patients were seen regularly one to four times a year.

   At each visit a sputum sample or a deep throat swab was collected.
3. In 2003, Lee et al. [12] formulated the Leeds definition of different stages of *P. aeruginosa* infection and colonization as:
   - 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 infected: *P. aeruginosa* never cultured from sputum or cough swab.

   The recommendation for sampling of sputum or cough swabs is at least every three months. The mean number of samples taken is between 7 and 10 per year.
4. In 2006, Proesmans et al. [13] evaluated the definition of Lee et al. [12] (Leeds criteria) for chronic *P. aeruginosa* infection in the paediatric and adult CF populations at the CF centre in Leuven, Belgium. The profile of patients with different stages of *P. aeruginosa* infection and colonization were compared with clinical and biochemical parameters. In addition, the levels of *P. aeruginosa* antibodies in the four groups were measured.

   No differences in *P. aeruginosa* antibody levels were found between the intermittent infection and free of infection groups. The authors confirmed the agreement between *P. aeruginosa* status according to the Leeds definition and both clinical status and levels of *P. aeruginosa* antibodies. At least 4 airway cultures were required in different months spread over the year.
5. The publications of Pressler et al. [14,15] demonstrated the potential diagnostic value of specific antibody measurements as a prognostic tool to identify CF patients at risk of establishing chronic *P. aeruginosa* infection.

   On average, sputum culture covered 10 months per year (range 4–12).
6. Ratjen et al. [16] evaluated the usefulness of specific serum antibodies in defining the status of *P. aeruginosa* infection as well as the response to early intervention treatment in CF patients. Serum antibody titers were on average low at the time of first *P. aeruginosa* isolation from respiratory specimens and they decreased during treatment. The data of Ratjen et al. [16] suggest that serum antibody titers might be used to monitor the response of CF patients to therapy because they detect the presence of *P. aeruginosa* in respiratory cultures of CF patients with high sensitivity and specificity.

3. Availability and limitations

1. The accuracy of any definition will depend on the frequency of sampling. Different numbers of sputum cultures have been used and recommended by different authors. The UK Cystic Fibrosis Trust suggests sampling sputum at least every 2 months (six times per year) in children and every 3 months (four times per year) in adults [17]. In Ballmann et al. [11], patients were seen regularly one to four times a year, while in Copenhagen, sputum is cultured on average 10 times per year from each patient (once per month) [14]. Both Proesmans et al. [13] and Lee et al. [12] recommended that sputum or cough swabs should be taken at least every three months (four times per year), but the mean number of samples taken was between 7 and 10 per year.
2. Acquisition of *P. aeruginosa* 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. Diagnosis of *P. aeruginosa* acquisition is based on sputum microbial culture or is replaced by cough swabs for patients, mainly young children, unable to expectorate sputum. The negative predictive value of a cough swab for *P. aeruginosa* is known to be as high as 95% with a low positive predictive value of only 44% [18]. Specific *P. aeruginosa* IgG values have a high sensitivity (88–97%) and specificity (83–96%) measured by different assays to distinguish chronically infected patients from the rest of the cohort. Positive predictive value varies between 80% and 92.1% and negative predictive value at 93.9–95% [13,15].

4. Can chronic *P. aeruginosa* infection be used as an outcome measurement in clinical studies?

The introduction of cohort isolation and early intensive treatment following the initial isolation of *P. aeruginosa* resulted in a reduced incidence and prevalence of chronic *P. aeruginosa* infection [5,19–21].

Furthermore, the time from acquisition of first *P. aeruginosa* to development of chronic *P. aeruginosa* infection increased significantly, from approximately 1 year to almost 4 years after introduction of cohort isolation [5]. After introduction of early intensive treatment, the probability of still not having developed chronic *P. aeruginosa* infection 15 years after the first isolation of *P. aeruginosa* was above 80% [22]. Management strategies reported by other CF centres were also associated with both reduced prevalence and an increase in the mean age of onset of chronic *P. aeruginosa* infection [19]. The yearly prevalence of patients having chronic *P. aeruginosa* infection fell significantly during the study period from 24.5% in 1990 to 18.1% in 2000 ($p < 0.05$), despite an increase in mean age of CF patients from 7.7 to 9.4 years. The number of patients aged less than 11 years who had chronic *P. aeruginosa* infection fell from 23.8% in January 1990 to only 4.3% by December 2000 [19].

For over 15 years, strict segregation of patients on a bacteriological basis and early, often "prophylactic" use of inhaled antibiotics have been progressively implemented to treat *P. aeruginosa* infection. At the end of 2003, 94.4% of patients aged younger than 18 years were on intermittent or continuous inhaled antibiotic therapy and the prevalence of *P. aeruginosa* among 116 patients was 20.7%. The chronic colonization rate was 19.8%, but only 2.8% in patients aged less than 18 years ($n = 72$). Serologic data strongly support these results [20].

5. Do we have surrogate markers of chronic *P. aeruginosa* infections in CF?

As anti-*Pseudomonas* management strategies are associated with both reduced prevalence and an increase in the mean age of onset of chronic *P. aeruginosa* infection, there is need of surrogate markers for study end-points [5,19,23]. The mucoid phenotype and increasing levels of specific anti-*Pseudomonas* antibodies are risk factors for the development of chronic *P. aeruginosa* infection. Mucoid *P. aeruginosa* plays a much greater role in CF lung disease progression than non-mucoid *P. aeruginosa* [3,14].

The greatest risk factor for development of chronic *P. aeruginosa* infection is increasing levels of specific anti-*Pseudomonas* IgG antibodies 3 years prior to the onset of chronic infection, with odds 7.4, ($p < 0.005$), and growth of mucoid *P. aeruginosa* strains with OR of 7.4, $p = 0.006$) [14].

Regular determination of serum antibodies has been reported to be useful in CF patients intermittently infected and free of infection by *P. aeruginosa* [13–15,24]. Longitudinal assessment of antibody titers assessed before and after inhaled antibiotic therapy in patients with first *P. aeruginosa* isolation showed a significant decrease in antibody titers against *P. aeruginosa* antigens in patients clearing *P. aeruginosa* infection, whereas titers increased in patients in whom antibiotic therapy failed to eradicate the organism [16].

6. Conclusions and recommendations from the chronic *P. aeruginosa* infection definition Working Group

Because the definition of infection is based on results from microbiological investigations of sputum or cough swab samples, the results will depend on the number of investigated samples. We therefore recommend that over the course of one year either (i) a minimum of 6 samples – if sputum – in separate months should be examined or (ii) a minimum of 8 samples in separate months should be examined – if other samples (cough swab, nasopharyngeal aspirate) are used. The negative predictive value of cough swab is reported to be 95% [18]. The definition of chronic *P. aeruginosa* infection is proposed to be defined as 50% or more of these samples being positive in the preceding 12 months. This definition can be useful as an entry criterion in studies where chronically infected patients are a target group. As the sensitivity and positive predictive value of cultures from respiratory tract secretions are poor [18], the definition of intermittently colonised CF patients should be based on both culture results

and levels of anti-*Pseudomonas* antibodies below the defined chronic infection cut-off level (taken within the last three months). There are only two commercially available assays for the detection of anti-*Pseudomonas* antibodies [14,16] as well as a number of other tests used in one or a few CF centres. There is therefore a need to evaluate and compare the assays used by the majority of CF centres to detect anti-*Pseudomonas* antibodies. In the future, the establishment of a reference laboratory to evaluate assays for anti-*Pseudomonas* antibodies might be a solution.

The group of CF patients who are always negative for *P. aeruginosa* infection are extremely interesting. However, the definition of this group of CF patients is problematic. One definition proposed is: Patients must have documented at least 6 sputum or 8 cough swab samples per year negative for *P. aeruginosa* and this for all years since diagnosis, together with level of specific anti-*Pseudomonas* antibodies below the defined cut-off level.

7. Recommendation of Endpoints for studies

1. Chronic infection with *P. aeruginosa* (as defined above) can be used as an endpoint in future clinical trials.
2. to next positive sample for *P. aeruginosa* can represent a useful additional endpoint.
3. Specific anti-*Pseudomonas* antibodies can provide a surrogate marker for infection, but needs further evaluation before being used as a trial endpoint.
4. Using mucoid/non-mucoid phenotype as an endpoint in clinical studies cannot be recommended at the present time because of the need to standardise methodology.

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Conflict of interest

None declared.

References

[1] Frederiksen B, Lanng S, Koch C, Hoiby N. Improved survival in the Danish center-treated cystic fibrosis patients: results of aggressive treatment. Pediatr Pulmonol 1996;21(3):153–8.
[2] Hoiby N. *Pseudomonas aeruginosa* infection in cystic fibrosis. Diagnostic and prognostic significance of *Pseudomonas aeruginosa* precipitins determined by means of crossed immunoelectrophoresis. A survey. Acta Pathol Microbiol Scand Suppl 1977;262:1–96.
[3] Li Z, Kosorok MR, Farrell PM, et al. Longitudinal development of mucoid *Pseudomonas aeruginosa* infection and lung disease progression in children with cystic fibrosis. JAMA 2005;293(5):581–8.
[4] Doring G, Conway SP, Heijerman HG, et al. Antibiotic therapy against *Pseudomonas aeruginosa* in cystic fibrosis: a European consensus. Eur Respir J 2000;16(4):749–67.
[5] Frederiksen B, Koch C, Hoiby N. Changing epidemiology of *Pseudomonas aeruginosa* infection in Danish cystic fibrosis patients (1974–1995). Pediatr Pulmonol 1999;28(3):159–66.
[6] Koch C, Cuppens H, Rainisio M, et al. European Epidemiologic Registry of Cystic Fibrosis (ERCF): comparison of major disease manifestations between patients with different classes of mutations. Pediatr Pulmonol 2001;31(1):1–12.
[7] Kosorok MR, Zeng L, West SE, et al. Acceleration of lung disease in children with cystic fibrosis after *Pseudomonas aeruginosa* acquisition. Pediatr Pulmonol 2001;32(4):277–87.
[8] Schiotz PO, Hoiby N, Juhl F, Permin H, Nielsen H, Svehag SE. Immune complexes in cystic fibrosis. Acta Pathol Microbiol Scand C 1977;85(1):57–64.
[9] Kerem E, Corey M, Gold R, Levison H. Pulmonary function and clinical course in patients with cystic fibrosis after pulmonary colonization with *Pseudomonas aeruginosa*. J Pediatr 1990;116(5):714–9.
[10] Henry RL, Mellis CM, Petrovic L. Mucoid *Pseudomonas aeruginosa* is a marker of poor survival in cystic fibrosis. Pediatr Pulmonol 1992;12(3):158–61.
[11] 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.
[12] Lee TW, Brownlee KG, Conway SP, Denton M, Littlewood JM. Evaluation of a new definition for chronic *Pseudomonas aeruginosa* infection in cystic fibrosis patients. J Cyst Fibros 2003;2(1):29–34.
[13] Proesmans M, Balinska-Miskiewicz W, Dupont L, et al. Evaluating the "Leeds criteria" for *Pseudomonas aeruginosa* infection in a cystic fibrosis centre. Eur Respir J 2006;27(5):937–43.
[14] Pressler T, Frederiksen B, Skov M, Garred P, Koch C, Hoiby N. Early rise of anti-pseudomonas antibodies and a mucoid phenotype of *Pseudomonas aeruginosa* are risk factors for development of chronic lung infection – a case control study. J Cyst Fibros 2006;5(1):9–15.
[15] Pressler T, Karpati F, Granstrom M, et al. Diagnostic significance of measurements of specific IgG antibodies to *Pseudomonas aeruginosa* by three different serological methods. J Cyst Fibros 2009;8(1):37–42.
[16] Ratjen F, Walter H, Haug M, Meisner C, Grasemann H, Doring G. Diagnostic value of serum antibodies in early *Pseudomonas aeruginosa* infection in cystic fibrosis patients. Pediatr Pulmonol 2007;42(3):249–55.
[17] *Pseudomonas aeruginosa* infection: prevention and infection control. Report of the United Kingdom Cystic Fibrosis Trust's Infection Control Group. 2001.
[18] Rosenfeld M, Emerson J, Accurso F, et al. Diagnostic accuracy of oropharyngeal cultures in infants and young children with cystic fibrosis. Pediatr Pulmonol 1999;28(5):321–8.
[19] Lee TW, Brownlee KG, Denton M, Littlewood JM, Conway SP. Reduction in prevalence of chronic *Pseudomonas aeruginosa* infection at a regional pediatric cystic fibrosis center. Pediatr Pulmonol 2004;37(2):104–10.
[20] Lebecque P, Leal T, Zylberberg K, Reychler G, Bossuyt X, Godding V. Towards zero prevalence of chronic *Pseudomonas aeruginosa* infection in children with cystic fibrosis. J Cyst Fibros 2006;5(4):237–44.
[21] Knudsen PK, Olesen HV, Hoiby N, et al. Differences in prevalence and treatment of *Pseudomonas aeruginosa* in cystic fibrosis centres in Denmark, Norway and Sweden. J Cyst Fibros 2009;8(2):135–42.
[22] Hansen CR, Pressler T, Hoiby N. Early aggressive eradication therapy for intermittent *Pseudomonas aeruginosa* airway colonization in cystic fibrosis patients: 15 years experience. J Cyst Fibros 2008;7(6):523–30.
[23] Frederiksen B, Koch C, Hoiby N. Antibiotic treatment of initial colonization with *Pseudomonas aeruginosa* postpones chronic infection and prevents deterioration of pulmonary function in cystic fibrosis. Pediatr Pulmonol 1997;23(5):330–5.
[24] Kappler M, Kraxner A, Reinhardt D, Ganster B, Griese M, Lang T. Diagnostic and prognostic value of serum antibodies against *Pseudomonas aeruginosa* in cystic fibrosis. Thorax 2006;61(8):684–8.