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Sequential genotyping of *Pseudomonas aeruginosa* from upper and lower airways of cystic fibrosis patients

A. Jung*, I. Kleinau*, G. Schönian#, A. Bauernfeind¶, C. Chen*, M. Griese+, G. Döring§, U. Göbel#, U. Wahn*, K. Paul*

Sequential genotyping of Pseudomonas aeruginosa *from upper and lower airways of cystic fibrosis patients. A. Jung, I. Kleinau, G. Schönian, A. Bauernfeind, C. Chen, M. Griese, G. Döring, U. Göbel, U. Wahn, K. Paul. ©ERS Journals Ltd 2002.*

ABSTRACT: A controversy exists concerning the adequate specimen to characterise colonisation of cystic fibrosis (CF) airways by *Pseudomonas aeruginosa*. Oropharyngeal, sputum and bronchoalveolar lavage samples were evaluated from 38 stable CF patients for the detection of *P. aeruginosa*, genetically different isolates within the same host and longitudinal variations in the genotype during repeated examinations.

Bacterial isolates were typed by pulsed-field gel electrophoresis of deoxyribonucleic acid macrorestriction fragments.

Sensitivity, negative and positive predictive values and specificity to detect *P. aeruginosa* were 35.7, 73.5, 83.3 and 96.2% for oropharyngeal cultures in nonexpectorating patients and 91.7, 94.1, 100 and 100% for sputum cultures from expectorating patients, respectively. Genotypes of Pseudomonas isolates recovered from oropharyngeal swabs and sputum differed to the strains recovered by bronchoscopy in 55% and 40%, respectively. In 62% longitudinal variations in the genotype occurred. One-half of these alterations were detectable by bronchoscopy only.

In conclusion, sputum samples were of equal value as specimens from bronchoalveolar lavage to detect *Pseudomonas aeruginosa* colonisation. Cultures from the oropharynx are not suitable for characterising bacterial conditions in the cystic fibrosis lung. Different genotypes within the same host and longitudinal genetic alterations are common and may be detectable in the bronchoalveolar lavage fluid exclusively.

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*Children's University Hospital and #Institute of Microbiology and Hygiene, Medical Faculty Charité, Humboldt-University, Berlin, ¶Max-von-Pettenkofer-Institute for Microbiology and Hygiene and +Children's University Hospital, Ludwig-Maximilians-University, Munich, and §Institute of Hygiene, Eberhard-Karls-University, Tübingen, Germany.

Correspondence: K. Paul, Universitätsklinikum Charité, Campus Virchow-Klinikum, Klinik für Pädiatrie mit Schwerpunkt Pneumologie/Immunologie, Augustenburger Platz 1, 13353 Berlin, Germany.
Fax: 49 3045066983
E-mail: karl.paul@charite.de

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As chronic lung infection leads to persistent inflammation and irreversible lung damage in cystic fibrosis (CF) patients [1, 2], it is a major concern to detect *Pseudomonas aeruginosa* colonisation of the CF lung as early as possible and to start proper antimicrobial treatment. Isolates from oropharyngeal swabs and sputum specimens are recovered routinely for identification of bacterial colonisation. Patients at an early disease state may be unable to provide adequate lower respiratory tract samples for microbiological examination, while others with advanced disease do not seem to respond favourably to antibiotic choices made on the basis of their sputum cultures. Consequently, previous studies, comparing bronchoalveolar lavage (BAL) to oropharyngeal cultures from CF patients [2–6], have suggested that BAL is the most specific method for obtaining samples of the lower respiratory flora. However, comparative data are contradictory depending on the patients' age and clinical background. Moreover, most epidemiological studies used oropharyngeal or sputa samples, even though these specimens may not adequately reflect the bacterial conditions within the lungs.

Even if there is evidence of *P. aeruginosa* in various respiratory compartments, this does not mean that these organisms are identical or derive from the same bacterial clone. Deoxyribonucleic acid (DNA) macrorestriction analysis (restriction fragments length polymorphism (RFLP)) is a very sensitive, specific and currently perhaps the most reliable genetic typing technique available to determine clonal relationships of various bacterial species [7–13]. Digestion of genomic DNA by the restriction endonuclease Spe I and subsequent separation of the restriction fragments by pulsed-field gel electrophoresis (PFGE) has been found to be a useful method for the typing of *P. aeruginosa* isolates from CF patients [3, 8–10, 14–17].

The aim of this study was to evaluate samples

recovered from the oropharynx, sputum and BAL of clinically stable CF patients for the detection of *P. aeruginosa*, for the appearance of genetically different isolates within the same host and for assessing longitudinal variations in the genotype during repeated examinations.

Material and methods

Subjects

A total of 38 CF patients from the Children's University Hospitals in Berlin and Munich, Germany, were investigated between June 1996 and July 1999. All patients took part in the Bronchoalveolar Lavage for Evaluation of Anti-inflammatory Treatment (BEAT) study, a multicentre trial designed to prospectively evaluate the natural course of bronchial inflammation in stable CF patients with normal lung function and the influence of dornase alpha [18]. Patients were eligible for this study if they were $\geq$5 yrs of age and had normal lung function, defined by forced expiratory volume in one second (FEV_1) >80% predicted. Patients receiving anti-inflammatory treatment (ibuprofen, systemic or inhaled corticosteroids and α_1-antitrypsin) as well as patients with evidence of allergic bronchopulmonary aspergillosis were not included in this study. All patients had to be free of acute respiratory infections and pulmonary exacerbations for at least 6 weeks prior to the first BAL.

Specimens were recovered from two investigations (t_1 and t_2), with an interval of ~18 months between them, from the upper and lower respiratory tract by oropharyngeal swabs, sputum collection and bronchoscopy (BAL). Oropharyngeal deep cultures were taken by experienced staff in each centre, wiping off root of tongue, tonsils and pharynx. At the time of their first investigation, the patients' age ranged 5.2–34.2 yrs (mean 14.2 yrs) and FEV_1 80–120% pred (mean 95%). All patients were clinically free of acute respiratory infections and pulmonary exacerbations for at least 6 weeks before the BALs were performed, and intravenous antibiotics and quinolones had to be withheld for 5 weeks before each BAL. Lung function tests were performed every 3 months during the observation time. In cases of confirmed new infection with *P. aeruginosa*, inhalation antibiotic therapy with tobramycin, if sensitive, or colistin was given for a period of 1 yr (n=4). Intravenous cephalosporins and aminoglycosides were given in cases of pulmonary exacerbation (n=6).

The study was approved by the ethics committees of the participating centres; written informed consent from both parents and/or the patients was obtained in all cases.

Bronchoscopy and BAL were performed as previously described [18]. To standardise the BAL procedure bronchoscopy was performed by the same investigator in each centre. BAL was performed *via* a flexible bronchoscope with an outer diameter of 3.5 mm in patients aged <10 yrs and 4.9 mm in older patients. Topical anaesthesia was achieved *via* inhalation of 2–4 mL of a 4% lidocaine solution prior to BAL and 1–4 mL of 1% lidocaine as needed while introducing the bronchoscope into the airways. Patients were sedated with a combination of midazolam (0.2–0.3 mg·kg body weight^{-1}) and propofol (loading dose of 2 mg·kg body weight^{-1} and repeated doses of 10 mg). The bronchoscope was wedged in the lingula or one of its segments in all patients. BAL was performed with 3 mL·kg body weight^{-1} normal saline warmed to body temperature. In children weighing <20 kg, 3×1 mL·kg^{-1} of normal saline was instilled and immediately withdrawn by gentle manual suction. In children weighing >20 kg, BAL was performed in aliquots of 20 mL syringes up to a total volume of 3 mL·kg body weight^{-1}. Bacterial cultures were obtained on 2 mL of the first BAL fluid sample and inoculated onto Columbia agar prior to filtration. Rather than using 1×10^5 bacteria as a cut-off point, the authors considered any pathogens in BAL fluid to be of relevance regardless of bacterial counts, which is similar to the approach used in previous studies of CF patients [2].

The bacterial cultures were diluted on selective media and *P. aeruginosa* was identified with the API 20 NE system (Bio Mérieux, Marcy-Etoile, France). A positive culture was defined as growth of the organism at any density. Single colonies of any phenotypically different isolates, if obtained from the same culture, were chosen for further investigations. Antimicrobial susceptibility testing was performed by micro-broth dilution. The isolates were frozen at -80°C, recultured on McConkey agar overnight at 37°C and characterised by genotyping techniques.

DNA macrorestriction and PFGE of *P. aeruginosa* isolates were carried out as previously described [14], with a few modifications. Bacterial isolates were grown overnight at 37°C in tryptone soya broth agar to an optical density of 0.7 (λ=590 nm). The suspensions were incubated with 0.5 M ethylenediamine tetraacetic acid (EDTA) and centrifuged for 3 min at 10,000 rpm (Eppendorf Centrifuge 5415D; Eppendorf, Hamburg, Germany). After washing and resuspending the pellets in 100 μL of a sodium chloride/EDTA (SE) buffer (75 mM NaCl, 0.5 M EDTA, pH 7.4), the suspensions were heated to 55°C, and 100 μL of a 2% low melting agarose (Bio-Rad Laboratories, Hercules, CA, USA) was added. The suspensions were pipetted into a plug mould and hardened at 4°C. Plugs were incubated in 1 mL of sodium chloride/N-lauryl-sarcosine (SLS) buffer (0.5 M EDTA, 1% SLS, pH 9.5) and proteinase K was added to a final concentration of 0.5 mg·mL^{-1}. The mixtures were incubated overnight at 55°C with gentle shaking. After washing with Tris/EDTA (TE) buffer (5 times over a period of 10 h), 2×5 mm pieces were cut from the plugs and incubated overnight at 37°C with 15 U of the restriction enzyme Spe I (Boehringer, Mannheim, Germany). The reaction was stopped by adding TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). Plugs were loaded onto a 1% agarose gel (Seakem Gold Agarose for PFGE; Bio-Products, Rockland, ME, USA). A clinical *P. aeruginosa* reference strain, obtained from the authors' laboratory, had been added to each gel for internal control.

A molecular size standard (DNA size λ ladder; Boehringer, Mannheim, Germany) was also included. The slots were filled up with 2% low-melting agarose, and the electrophoresis (Chef-DRII System; Bio-Rad Laboratories, Richmond, CA, USA) was run in 0.5×Tris/borate/EDTA (TBE) buffer using the following ramp conditions: 1–25 s for 24 h at 12°C, with amperage=0.1 ampere (6 volt (V)/cm), voltage=200 V, included angle 120°C. Thereafter, the gels were stained with ethidium bromide, photographed with a Polaroid camera and compared by eye.

All samples were typed in duplicate by reculturing isolates from the frozen stocks. The relationship between typing patterns and interpretation of the results was determined using criteria suggested by TENOVER *et al.* [17]. Thus, isolates were considered to be closely related if their PFGE patterns differed in not more than three restriction bands. Isolates that showed fragment differences from four to six bands were judged to be possibly related, whereas strains with more than six different restriction fragments were considered to be genetically unrelated.

Data from both investigations (t1 and t2) were pooled for statistical analysis and sensitivity, specificity and predictive values of respiratory specimens were calculated.

Results

Eighteen of the 38 subjects (prevalence 47.4%) yielded growth of *P. aeruginosa* in at least one of the investigations (table 1). Seven patients had quit the study at the time of the second investigation (data of patients who were negative for *P. aeruginosa* are not shown). Two of the *P. aeruginosa*-positive patients underwent only one bronchoscopy. In the first investigation, 13 of these children could expectorate sputum and five were nonexpectorators, whereas in the second collection only five patients were expectorators and 11 were not, due to a generally improved pulmonary condition. In addition to patients who were unable to produce sputum and had oropharyngeal swabs collected instead, nine (t1) and four (t2) patients, respectively, had both oropharyngeal and sputum cultures, as these subjects were initially unable to expectorate sputum but expectorated after the oropharyngeal sample was taken. Altogether, 80 isolates of *P. aeruginosa* were collected. A total of 77 of these isolates were characterised by DNA macrorestriction, followed by PFGE. For three isolates, genotyping results were not available.

Every single patient harvesting *P. aeruginosa* in BAL fluid is depicted in table 2. Between 1×10^2–10^9 colony forming units·mL^{-1} of *P. aeruginosa* were identified in each of the positive samples.

Regularly performed lung function tests showed a decline in the lung function of the *P. aeruginosa*-positive patients over the observation time of ~18 months. Mean FEV1 decreased from 95% to 87% pred.

Sensitivity, specificity and predictive values to detect *P. aeruginosa* colonisation of the lungs are shown in table 3.

DNA macrorestriction, followed by PFGE, proved to be a highly reliable and discriminative tool in the present investigation. Typing ability and reproducibility were 100%. In patient nos. 6, 7 and 9, DNA macrorestriction was able to detect genetic alterations of the initial colonising strain in the form of loss or gain of single bands (RFLP-types 7b, 8b and 8d, respectively; fig. 1). To exclude the possibility that these changes in the macrorestriction patterns were due to artefacts, bacteria were recultured and retyped a second time.

Epidemiological typing results of the *P. aeruginosa*-positive patients are shown in table 2. Two of these patients did not take part in the second lavage and in one case no genotyping results were available. Each patient harboured at least one host-typical *P. aeruginosa* strain. Patient nos. 4 and 5 carried one identical strain at the time of the second investigation. It is not clear whether this was a result of cross-over colonisation or due to a common source of infection. In the second BAL, patient no. 7 was colonised with the host-typical strain of patient no. 8 in addition to the major colonising strain, suggesting a cross-over infection. Patient nos. 7 and 9 harboured related strains in their airways. However, the origin of these isolates remains unknown. Patient nos. 14 and 15, who were sisters, showed evidence of one identical strain.

Genotypes of isolates recovered by bronchoscopy differed from oropharyngeal swabs and sputum in up to 55.6% of the investigations (table 4). In patient nos. 7, 11 and 12, bronchoscopy specimens detected additional strains which were epidemiologically unrelated to the major colonising isolates. Patient no. 5 had acquired a new strain in the oropharynx at t2, while the initial strain persisted in the lung over the evaluation period. Genotyping of these isolates

Table 1.–Number of respiratory specimens recovered from patients who yielded growth of *Pseudomonas aeruginosa* (Pseudomonas+) and from all patients

	t1		t2	
	Pseudomonas+	Total	Pseudomonas+	Total
Oropharynx	14 (36.8)	25 (65.8)	15 (48.4)	29 (93.5)
Sputum	13 (34.2)	22 (57.9)	5 (16.1)	6 (19.4)
Bronchoscopy	18 (47.4)	38 (100)	16 (51.6)	31 (100)

Data are presented as n (%). t1: first specimens (n=38); t2: second specimens (n=31). Seven patients had quit the study at the time of the second investigation.

Table 2. – Patient data and genotyping results of *Pseudomonas aeruginosa* isolates

Patient data				*Pseudomonas aeruginosa* RFLP types					
Patient no.	Age at first evaluation yrs	Sex	Time between t1 and t2 months	First specimens (t1)			Second specimens (t2)		
				Oropharynx	Sputum	BAL	Oropharynx	Sputum	BAL
1	34.2	F	22.5	ND	-	-	-	ND	1
2	17.4	F	23.0	ND	-	-	-	-	2
3	21.3	F	21.5	-	ND	3	-	ND	4
4	12.3	M	21.0	-	-	-	-	ND	5
5	11.1	M	20.5	6	6	6	5	ND	6
6	15.3	M		ND	7a	7a, 7b	ND	ND	ND
7	14.9	F	22.0	-	8a	8a, 8b	8a	ND	8a, 9
8	11.0	F	19.5	-	ND	9	-	ND	9
9	15.3	M	21.0	8c	ND	8c	8c	ND	8c, 8d
10	12.9	F	19.5	ND	-	-	10	ND	-
11	15.3	M		11	ND	11, 12	ND	ND	ND
12	14.4	F	14.5	-	13	13, 14	13, 15, 16	13, 15, 16	13, 14, 15, 16
13	9.1	M	20.0	-	ND	17	-	ND	-
14	6.4	F	19.0	-	18	18	-	ND	18
15	8.7	F	16.0	18	18	18	-	18	18
16	16.3	M	19.0	-	-	-	-	19	19
17	5.2	F	18.0	-	-	-	-	ND	20
18	14.4	F	21.5	21	21	21	ND	+	+

RFLP: restriction fragments length polymorphism; BAL: bronchoalveolar lavage; F: female; M: male; ND: not determined; -: sample negative for *P. Aeruginosa*; +: sample positive for *P. aeruginosa*, but no data of genotyping available. RFLP (deoxyribonucleic acid macrorestriction) types are indicated in Arabic numerals; clonal relationships (subtypes) are described by affixed small letters.

Table 3. – Sensitivity, specificity and positive and negative predictive values for respiratory specimens to detect *Pseudomonas aeruginosa* colonisation of the lungs

	Sensitivity %	Specificity %	PPV %	NPV %
Oropharynx				
Nonexpectorators	35.7	96.2	83.3	73.5
Expectorators	40.0	100	100	45.5
Sputum				
Expectorators	91.7	100	100	94.1

The bronchoscopy samples were defined as the standard. PPV: positive predictive value; NPV: negative predictive value.

suggested that this was due to coinfections with new strains rather than mutations of the initial strains, as the number of fragment differences in PFGE pattern was >6. Loss or gain of single restriction bands in the PFGE patterns was observed for some strains isolated from the BAL fluid of patient nos. 6, 7 and 9 (fig. 1), indicating that these isolates were closely related to their initial colonising strain.

In eight patients, possible longitudinal genetic variability could be assessed (table 5). DNA macrorestriction showed the appearance of additional strains in four investigations. Strain replacement occurred in patient no. 3 (fig. 2), where the host-typical *P. aeruginosa* strain had been lost but had acquired a new isolate at the time of the second investigation. In four cases, longitudinal variations in the genotype were detectable by bronchoscopy only (patient nos. 3, 7, 9 and 12; figs 1 and 2).

Results of antibiotic susceptibility testing and of genotyping showed poor correlation (data not shown). Resistance patterns from bacterial isolates

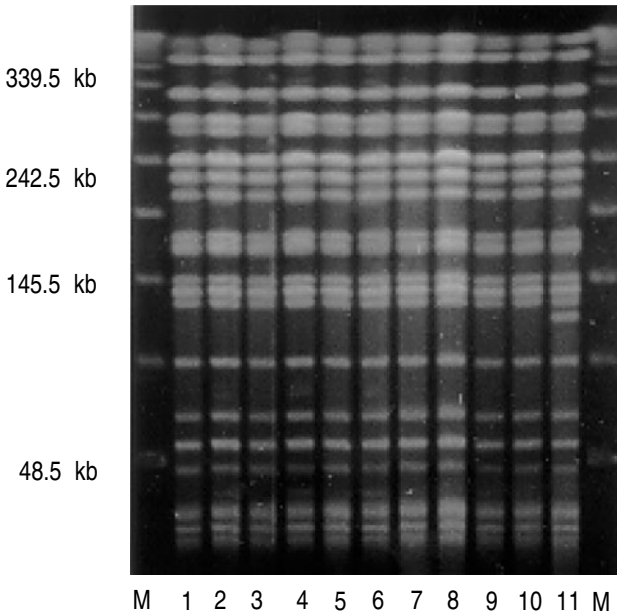

Fig. 1. – Pulsed-field gel electrophoresis (PFGE) fingerprints of *Pseudomonas aeruginosa* isolates, recovered from patient no. 9. Lanes 1–5: oropharyngeal swab at specimen 1 (t1); lanes 6 and 7: bronchoalveolar lavage (BAL) fluid at t1; lanes 8 and 9: oropharyngeal swab at t2; lanes 10 and 11: BAL fluid at t2. A new band (~125 kilobases (kb)) appeared in the PFGE pattern of one of the two BAL isolates in second specimens (lane 11). A molecular size marker was run in lane M.

which had identical genotypes differed in up to three antibiotics. Conversely, sometimes distinct *P. aeruginosa* genotypes showed a very similar resistance profile.

Table 4. – Comparison of genotypes yielded from different respiratory samples within one patient

	Bronchoscopy		
	Investigations n	Identical strains n	Different strains n
Oropharynx	9	4	5
Sputum	10	6	4

Different strains could be related or unrelated.

Table 5. – Longitudinal genetic variations in the pulsed-field gel electrophoresis genotype for all respiratory specimens

	Patients n
Patients with strain persistence	8
+ No changes	3
+ Additional strain	
Related	1
Unrelated	3
+ Loss of strain	0
+ Strain replacement	1

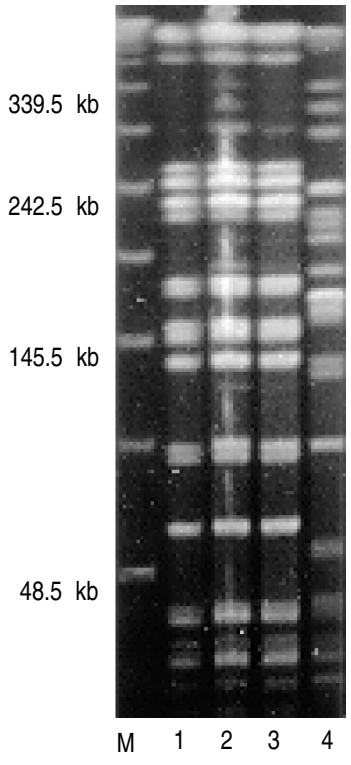

Fig. 2. – Pulsed-field gel electrophoresis (PFGE) fingerprints of *Pseudomonas aeruginosa* isolates, recovered from patient no. 3. Lanes 1, 2 and 3: bronchoalveolar lavage (BAL) fluid at specimen 1 (t1). A new strain appeared in the BAL fluid at specimen 2 (t2) (lane 4), whereas the initial colonising strain was no longer detectable. A molecular size marker was run in lane M.

Discussion

The results of this study indicate that, in order to obtain reliable results from microbiology in CF, samples from different compartments of the respiratory tract should be cultured. Evaluation of isolates recovered by BAL, oropharyngeal swab and sputum showed that genetic variability frequently occurs by modifications within the same strain, loss of strains, strain replacement and additional strains possibly acquired by coinfection within a period of 18 months. Most importantly, some of theses variations could be detected by BAL only.

In the present study, sensitivity and negative predictive values for a single oropharyngeal culture to detect *P. aeruginosa* colonisation of the lungs were low (table 3). In contrast, negative sputa samples in the presence of *P. aeruginosa* in the BAL fluid were obtained only once in 18 investigations, and sensitivity was high. However, sputum samples were collected in only 28 investigations (40.6%), as a number of patients were unable to expectorate.

Previous studies have compared BAL, sputum and oropharyngeal cultures from CF children [3, 5, 6] and adolescents/adults [2, 4] with contradicting results. ARMSTRONG *et al.* [3] prospectively collected oropharyngeal smears and BAL fluids from 75 infants with CF and mild lung disease at the age of 1–52 months over a period of 30 months. Absence of *P. aeruginosa* from oropharyngeal cultures in symptomatic children did not exclude the presence of this pathogen in the lower respiratory tract. These data were confirmed by RAMSEY *et al.* [5] in their investigation of 43 young CF patients (mean age 8.2 yrs) with good respiratory status.

In a multicentre study, ROSENFELD *et al.* [6], including patients of the above-mentioned investigations of ARMSTRONG *et al.* [3] and RAMSEY *et al.* [5], obtained cultures from 141 CF patients aged <5 yrs without experience of pulmonary exacerbations. In this age range, specificity and negative predictive values for detection of *P. aeruginosa* were high. However, poor sensitivity and positive predictive values of oropharyngeal cultures indicated that a positive specimen does not reliably predict lower airway colonisation.

Good agreement between oropharyngeal cultures and BAL was obtained by KONSTAN *et al.* [2]. They performed BAL on 18 CF patients aged $\geqslant$12 yrs with moderate lung disease who were stable and appeared clinically well. However, disease was more advanced in the investigated subjects compared to the present patients and those of ARMSTRONG *et al.* [3], RAMSEY *et al.* [5] and ROSENFELD *et al.* [6], and the frequency of *P. aeruginosa* colonisation was much higher (88.9% compared to 47.4% in this study).

BAUGHMAN *et al.* [4] tried to determine the utility of bronchoscopy and semiquantitative BAL to identify pathogens, mainly *P. aeruginosa*, in the lower respiratory tract of 11 adult CF patients with severe obstructive disease. Indication for bronchoscopy was a clinically unsatisfactory outcome despite antibiotic therapy guided by antimicrobial susceptibility patterns. In 41%, *P. aeruginosa* identified in the BAL fluid

was not found in a concurrent sputum sample. A total of 46% of the bronchoscopies led to a change in antimicrobial therapy, either because a new pathogen was identified in the BAL fluid or as a result of sensitivity testing. Consequently, BAUGHMAN *et al.* [4] suggested BAL as a useful tool in CF patients in whom therapy guided by sputum sensitivity testing fails.

The application of BAL was well tolerated by all patients participating in the study. However, bronchoscopy is not a standard diagnostic tool to screen for bacterial pathogens. Induced sputum was not collected in this study. Since sensitivity for sputa samples to detect *P. aeruginosa* colonisation of CF patients was about the same as for bronchoscopy specimens and predictive values were high, it can be speculated that induced sputa samples from patients unable to spontaneously produce sputum would show results comparable to cultures obtained by BAL. This suggestion is corroborated by the results of a recent study using induced sputum [19], although the authors did not compare induced sputum specimens to BAL fluids.

Furthermore, genetic alterations of *P. aeruginosa* were assessed in CF patients. The results demonstrate that when *P. aeruginosa* is cultured from different sites of the airways, a high proportion is genetically different within the individual host. In particular, genotypes of isolates recovered from oropharyngeal swabs and sputum may differ to the strains recovered by bronchoscopy in a high percentage of the investigations. In contrast to previous studies in CF patients, which evaluated oropharyngeal or sputa samples, this study is the first to sequentially genotype *P. aeruginosa* isolates recovered from the upper and lower respiratory tract including BAL fluids. Although major and minor changes in the genotype were visible within all compartments, in a considerable number of cases longitudinal variations in the genotype were detectable by bronchoscopy only. Due to the small number of patients and the relatively short period of investigation, relationships between changes in the *P. aeruginosa* genotype and clinical presentation were not visible. Also, there was no correlation between genotyping and antibiotic susceptibility testing. In future studies, addressing the clinical significance of longitudinal changes in the genotype bronchoscopy samples from different sites of the bronchial tree may be valuable.

Genomic typing analyses of *P. aeruginosa* CF isolates have demonstrated that most CF patients are chronically colonised with isolates of one or a few lineages for prolonged periods of time [10, 11, 16, 20, 21] However, a high frequency of hypermutable Pseudomonas strains in CF patients has been suggested in recent work by OLIVER *et al.* [20]. Coinfection with one or more strains and strain replacement have also been reported [11, 16, 21]. ARMSTRONG *et al.* [3] were the first and only to perform genotyping of *P. aeruginosa* isolates from BAL fluids of CF patients. Comparing randomly selected paired oropharyngeal and BAL cultures from nine of the 75 children participating in the study, concordance in the genotype was observed in eight patients. However, the authors did not look for longitudinal persistence or changes of the genotype.

These data raise the question "how many sites of airway secretions should be routinely collected from?" Sputum samples have proven to be superior to oropharyngeal swabs, even if these are carried out in a standardised way and with the highest precision possible. In nonexpectorating patients, induced sputum might be a more reliable alternative to oropharyngeal cultures. In contrast to bronchoalveolar lavage, induced sputum has the theoretical advantage of reflecting the microbiological flora of more than just one region of the bronchial tree. These questions should be the subject of further investigations. Bronchoalveolar lavage should be performed in case of therapy-resistant pulmonary exacerbation. In addition to its value as a diagnostic tool, the application of bronchoalveolar lavage is necessary to answer epidemiological questions properly and to reliably detect longitudinal alterations in the genome of bacterial isolates within a host's respiratory tract.

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