

Epidemiology of chronic *Pseudomonas aeruginosa* infections in the airways of lung transplant recipients with cystic fibrosis

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Abstract

Background – The source of airway colonisation with *Pseudomonas aeruginosa* is not well defined in patients with cystic fibrosis after lung transplantation. Using a DNA-based typing system a study was undertaken to investigate whether lung transplant recipients acquired new strains of *P aeruginosa* or retained those they had before transplantation.

Methods – Seventy four *P aeruginosa* isolates taken before and after transplantation were analysed from 11 patients with cystic fibrosis who had undergone lung transplantation in the Medical School of Hannover between 1988 and 1994. The genetic relatedness of the 74 *P aeruginosa* strains was evaluated from macrorestriction fragment pattern similarity.

Results – Each of the 11 lung transplant recipients harboured one identical *P aeruginosa* clone before and after transplantation. The airways of four of the 11 patients were preoperatively colonised by two or three different clones, but six months after transplantation only one clone was detectable.

Conclusions – These results show that there is no change in the *P aeruginosa* population in the airways of lung transplant recipients before and after transplantation and it is assumed that the chronic drainage of *P aeruginosa* into the lung allografts is caused by the bacterial reservoir in the paranasal sinuses and the trachea.

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Keywords: lung transplantation, cystic fibrosis, *Pseudomonas aeruginosa*.

Double lung transplantation has become a recognised and established treatment for adults and children in terminal respiratory failure resulting from cystic fibrosis. One of the major problems in the early postoperative period is infectious bronchopulmonary complications caused by viruses, bacteria, and fungi.¹ Although the cystic fibrosis specific epithelial membrane defect does not recur in lung transplants,² it seems that the upper airways, particularly the sinuses and the trachea, remain a bacterial reservoir after surgery.^{3,4} Using macrorestriction fragment pattern analysis as a DNA-based typing system we investigated whether

lung transplant recipients in our clinic infected with *Pseudomonas aeruginosa* acquired new strains or retained their strains which they harboured before lung transplantation. The results might have direct implications for the clinical and perioperative management.

A previous study by Tsang *et al*⁵ showed that three of the four lung transplant recipients with cystic fibrosis who harboured *P aeruginosa* were colonised postoperatively by genotypes which were different from those carried before the transplantation. Only in one transplant recipient with cystic fibrosis were the preoperative and postoperative isolates identical. Analysis of the origin of *Burkholderia cepacia* infection after lung transplantation in patients with cystic fibrosis indicated that the upper airways could be a source of bacterial colonisation or reinfection of the lung allografts.⁶

Methods

PATIENT POPULATION, BRONCHOALVEOLAR LAVAGE, AND BACTERIAL STRAINS

P aeruginosa strains were isolated from 11 lung transplant recipients with cystic fibrosis (five men) of mean age 28.8 years (range 23.5-32.1) who had undergone lung transplantation in the Medical School of Hannover between 1988 and 1994 (table 1). With the exception of patient 2 who had a heart-lung transplant, all other patients received bilateral lung transplantation with bronchial anastomosis. All patients were colonised before and after lung transplantation with *P aeruginosa*.

Preoperative isolates were retrieved from sputum samples and postoperative isolates from bronchoalveolar lavage (BAL) fluid. Sputum samples were homogenised by vortexing with sterile glass beads for one minute. An aliquot was diluted 1:1000 in phosphate buffer saline (PBS). Undiluted and diluted specimens (50 µl each) were plated onto Columbia blood agar (Unipath, Wesel, Germany), MacConkey agar (Unipath), and chocolate agar (Unipath). BAL fluid samples were taken by fiberoptic bronchoscopy from the middle lobe. After placing the bronchoscope in a wedge position, six aliquots of 0.9% (w/v) sodium chloride solution (20 ml each) were instilled into segment 4 or 5. The recovery of the first aliquot was discarded to make sure that the lavage was taken from the alveolar compartment rather than the small airways. The mean recovery rate was 55%. Seventy four isolates of *P aeruginosa* with different phenotypes one week before, one week

Table 1 Clinical characteristics of lung transplant recipients with cystic fibrosis (CF)

Patient no.	Sex	Age at diagnosis of CF	CFTR genotype	Age at onset of chronic <i>P. aeruginosa</i> infection (years)	Age at transplantation (years)	First <i>P. aeruginosa</i> infection after transplantation (days)	No. of <i>P. aeruginosa</i> infections after transplantation ¹	Cooper index ¹	Survival time after transplantation (days) ¹
1	M	5 years	Rare unknown	21	21	30	1	BOS 0a	438
2	M	2 years	dF508/dF508	9	22	1431	2	BOS 0a	2643
3	M	12 years	dF508/dF508	17	21	–	0	BOS 0a	462
4	M	10 months	dF508/R553X	12	30	136	4	BOS 2a	746
5	F	12 years	dF508/2789+5G-A	18	25	–	0	BOS 0a	1319
6	F	12 years	dF508/dF508	13	21	145	3	BOS 2a	1604
7	F	18 years	dF508/dF508	18	28	687	2	BOS 2b	1312
8	M	9 months	dF508/unknown	17	21	–	0	BOS 0a	1512
9	F	12 years	dF508/dF508	15	23	317	4	BOS 0a	1359
10	F	19 years	dF508/dF508	19	27	–	0	BOS 0a	1321
11	F	10 months	dF508/2184insA	14	19	795	3	BOS 3b	1452 ²

Cooper index: BOS=bronchiolitis obliterans syndrome; stages 0=no abnormality, 1=mild, 2=moderate, 3=severe; subcategory a=absence of pathological evidence of bronchiolitis obliterans; b=presence of bronchiolitis obliterans pathologically.¹²

¹ Referring to 28 February 1996.

² Deceased.

after, and six months after transplantation were characterised by macrorestriction analysis. Based on the results in previous studies, the BAL fluid was considered to be not contaminated, or only marginally so, if the specimen contained $<10^2$ colony forming units (CFU)/ml of oropharyngeal flora, no ciliated epithelial cells, and $<1\%$ squamous epithelial cells.^{7–9}

MACRORESTRICTION ANALYSIS

The genotyping of the *P. aeruginosa* strains was carried out as described previously.¹⁰ Briefly, the agarose-embedded *P. aeruginosa* DNA was digested by *Spe* I and the resulting fragments were separated using a CHEF DRII or CHEF DRIII apparatus (BioRad, Richmond, California, USA). Running conditions in 1% agarose gels were electric field strength 6 V/cm, linear ramping 5–25 seconds for 20 hours and 5–60 seconds for 17 hours in $0.5 \times$ Tris-borate-EDTA (TBE) buffer. λ *Bst* EII digest, λ phage DNA oligomers, *Spe* I digested *P. aeruginosa* C,¹¹ and PAO (DSM 1707) were used as size markers and restriction digestion fingerprints of reference strains. Fragment patterns on the same gel were compared visually side by side. The relatedness of patterns of the strains was then evaluated by computerised cluster analysis.¹⁰ Two *P. aeruginosa* isolates are members of a clonal lineage if they share $>75\%$ of *Spe* I bands.¹⁰ Of the isolates from a specimen of sputum or BAL fluid with identical *Spe* I fragment patterns, one representative strain was selected for a second macrorestriction analysis. In total, 33 isolates were rerun to ascertain the pairwise fragment pattern similarity in quantitative terms. Clones and clonal variants that were identical were indicated with the same letters.

Results

MICROBIOLOGICAL RESULTS

The genotyping indicated that each of the 11 lung transplant recipients harboured at least one identical clone or a clonal variant of a *P. aeruginosa* strain preoperatively and post-operatively but each patient harboured a different clone (table 2). Two of the 11 patients were preoperatively colonised with three

different genotypes. One week after transplantation one of these was colonised with two different and the other with only one genotype. Six months after transplantation only one clone persisted in each of these patients. Of the two patients who were carrying two different genotypes before transplantation, one harboured two different clones one week after transplantation and the other retained only a single strain. Six months after transplantation only one genotype was isolated in the airways of both patients (fig 1). The isolates of *P. aeruginosa* of the lung transplant recipients after lung transplantation were mostly mucoid LPS-rough strains specific for chronic respiratory infections of patients with cystic fibrosis.¹⁰ They did not change their cystic fibrosis specific morphology in the lung allografts.

CLINICAL RESULTS

Clinical characteristics of the studied patients are summarised in table 1. Cystic fibrosis was diagnosed at a mean age of 8.6 years (range 9 months to 19 years). The mean age at onset of the chronic *P. aeruginosa* infection was 15.7 years (range 9–21) and at lung transplantation it was 23.5 years (range 19–30).

The Cooper index^{12,13} was used as a standardised method for assessing functional results after lung transplantation. In this staging system four grades of bronchiolitis obliterans syndrome (BOS), with (a) or without (b) histo-

Table 2 Molecular epidemiology of *Pseudomonas aeruginosa* in lung allografts of lung transplant recipients with cystic fibrosis

Patient no.	<i>P. aeruginosa</i> genotypes*		
	Before transplantation ¹	After transplantation ²	Six months after transplantation ²
1	ab, ac, ad	ab, ac	ab
2	C, af, ag	C	C
3	ah	ah	ah
4	ai	ai	ai
5	aj	aj	aj
6	ak	ak	ak
7	al, am	al, am	al
8	an	an	an
9	ao, ap	ao	ao
10	aq	aq	aq
11	ar	ar	ar

¹ Sputum or throat swab; ² Bronchoalveolar lavage fluid.

* Clones and clonal variants already identified in recent studies¹⁰ are indicated with the same capital letter. Novel genotypes were given two small letters.

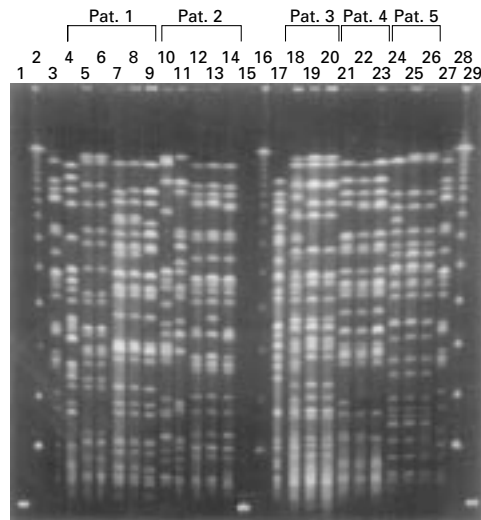


Figure 1 Genotyping of *Pseudomonas aeruginosa* isolates of five of the 11 lung transplant recipients with cystic fibrosis. *Spe I* fragment patterns of patient strains one week before (*p*), one week after (*po*), and six months after transplantation (*sma*) (lanes 4–14, 18–26). Lanes 1, 15, 29: λ Bst EII digests; lanes 2, 16, 28: λ oligomers; lanes 3, 27: *Spe I* digests of *P. aeruginosa* C (11) chromosome; lane 17: *Spe I* digest of *P. aeruginosa* PAO (DSM 1707) chromosome. Genotypes of patient strains: lanes 4–9: patient 1, lane 4 *ad* (*p*), lanes 5–6 *ac* (*p*, *po*), lanes 7–9 *ab* (*p*, *po*, *sma*), lane 9 clonal variant of strain *ab*; lanes 10–14: patient 2, lane 10 *af* (*p*), lane 11 *ag* (*p*), lanes 12–14 *C* (*p*, *po*, *sma*), lane 13 clonal variant of strain *C*; lanes 18–20: patient 3, *ah* (*p*, *po*, *sma*), lane 18 clonal variant of strain *ah*; lanes 21–23: patient 4, *ai* (*p*, *po*, *sma*); lanes 24–26: patient 5: *aj* (*p*, *po*, *sma*), lane 24 clonal variant of strain *aj*.

pathological evidence of obliterative bronchiolitis (BOS grades 0–3, subcategories a and b), are defined using the forced expiratory volume in one second (FEV₁) as an indicator of graft dysfunction. In our population of patients with cystic fibrosis who had undergone lung transplantation seven of the 11 lung recipients (64%) had a Cooper index of BOS 0a (no significant abnormality), correlating to the results of the Washington University Lung Transplant Group,¹² and a mean present survival time of 1293 days (range 438–2643). Three patients were graded as BOS 2 (moderate obliterative bronchiolitis syndrome) with a mean survival time of 1220 days (range 746–1604). Seven of the 11 patients experienced acute episodes of infections with *P. aeruginosa* defined by expectoration of mucopurulent secretions, with or without decline in FEV₁, and *P. aeruginosa* colony forming units (CFU) of >10⁴/ml in the BAL fluid and were treated with antipseudomonal chemotherapy. One patient (no. 11) died 1452 days after the lung transplantation due to a chronic progressive *P. aeruginosa* infection; a second patient (no. 4) who has survived for 746 days since lung transplantation has recurrent mucopurulent bronchitic *P. aeruginosa* infections requiring intravenous antibiotic treatment every three months; and a third patient (no. 9) who has survived for 1359 days since lung transplantation has frequent infections but has not needed regular antibiotic treatment so far.

Discussion

Lung transplantation or combined heart-lung transplantation has emerged as a therapeutic intervention for cystic fibrosis patients with end stage respiratory failure.¹⁴ Undoubtedly, lung transplantation improves the quality of life of most of the patients and all efforts should be made to optimise this treatment.

Our genotype analysis shows that all 11 lung transplant recipients harboured different *P. aeruginosa* strains, but each of the patients remained colonised with the same strain as was cultured before lung transplantation. These results are in conflict with those of Tsang *et al*⁶ who used the pCMTox probe for strain typing.¹⁵ This probe recognises restriction fragment length polymorphisms in a region of high genomic variability^{11,16,17} (T Heuer, personal communication) and it is tempting to assume that the shift of the habitat by transplantation is associated with genome rearrangements in the *P. aeruginosa* clone. Hence, a probe such as pCMTox which scans a single hypervariable locus may indicate a change, whereas the global genome-wide fragment pattern may undergo no or minor shifts.

Our genotype data support the hypothesis that a reservoir of *P. aeruginosa* remains in patients with cystic fibrosis who undergo lung transplantation, presumably in the upper respiratory tract or trachea. Recolonisation by these potentially multiresistant pathogens may cause infectious complications of the lung allografts and, combined with the impaired mucociliary clearance after lung transplantation and the immunosuppression, this creates a high risk of graft infection following lung transplantation.¹⁸ Some transplantation groups therefore recommend surgical drainage of the sinuses before transplantation,^{19–22} while other centres prescribe lifelong inhalation of antimicrobial agents to reduce the risk of reinfection or colonisation with *P. aeruginosa* from the upper airways.^{14,23}

The Stanford group developed a management strategy which combined endoscopic surgery with an initial topical antimicrobial treatment course and serial long term monthly sinus antimicrobial lavage.^{19–21} They reduced the need for reoperation for chronic sinusitis and reported that eight long term survivors required only one seven-day admission to hospital and parenteral antimicrobial agents against *P. aeruginosa* infections during a follow up period of 3200 days after lung transplantation. Davidson *et al* introduced a similar protocol²² in which sinus surgery was followed by daily saline nasal irrigation with fluid containing tobramycin. With this regimen the sputum of all patients with cystic fibrosis who had undergone lung transplantation was free of *P. aeruginosa* and bacterial pneumonia was not reported. The Harefield group prescribed lung transplant recipients with a lifelong inhalation containing colistin sulphate via a face mask at a dose of 1 mega unit twice a day in the first three postoperative months reduced thereafter to 1 mega unit daily.^{14,23} In spite of these measures, and an additional course with antipseudomonal antibiotics for the first 10

postoperative days, the 79 lung transplant recipients studied had 43 *P aeruginosa* respiratory infections during the first three months after transplantation and 11 infections during each of months 4–6 and 7–12.

However, most lung transplantation groups do not carry out preoperative sinus surgery, either because they do not feel that sinus disease adversely affects the long term outcome of their patients²⁴ or because of the complications involved in sinus surgery. Stammberger and Wolf,²⁵ however, reported only two cases of cerebrospinal fluid rhinorrhoea and no intracranial or ophthalmic complications in a series of over 4000 cases of functional sinus surgery.

As it is important to protect lung allografts from acquiring *P aeruginosa* from the upper airways, we plan to modify our perioperative management of lung transplant recipients. A prospective study is planned in which we will investigate whether sinus surgery combined with antimicrobial lavage or long term inhalation of nebulised antimicrobial agents via a face mask reduces the risk of postoperative infection in lung transplant recipients with cystic fibrosis.

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