



Pseudomonas infection and mucociliary and absorptive clearance in the cystic fibrosis lung

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ABSTRACT Airway surface liquid hyperabsorption and mucus accumulation are key elements of cystic fibrosis lung disease that can be assessed *in vivo* using functional imaging methods. In this study we evaluated experimental factors affecting measurements of mucociliary clearance (MCC) and small-molecule absorption (ABS) and patient factors associated with abnormal absorption and mucus clearance.

Our imaging technique utilises two radiopharmaceutical probes delivered by inhalation. Measurement repeatability was assessed in 10 adult cystic fibrosis subjects. Experimental factors were assessed in 29 adult and paediatric cystic fibrosis subjects (51 scans). Patient factors were assessed in a subgroup with optimal aerosol deposition (37 scans; 24 subjects). Paediatric subjects (n=9) underwent initial and 2-year follow-up scans. Control subjects from a previously reported study are included for comparison.

High rates of central aerosol deposition influenced measurements of ABS and, to a lesser extent, MCC. Depressed MCC in cystic fibrosis was only detectable in subjects with previous *Pseudomonas aeruginosa* infection. Cystic fibrosis subjects without *P. aeruginosa* had similar MCC to control subjects. Cystic fibrosis subjects had consistently higher ABS rates.

We conclude that the primary experimental factor affecting MCC/ABS measurements is central deposition percentage. Depressed MCC in cystic fibrosis is associated with *P. aeruginosa* infection. ABS is consistently increased in cystic fibrosis.



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Depressed mucus clearance in cystic fibrosis was only detectable in subjects with *Pseudomonas aeruginosa* infection <http://ow.ly/YggZy>

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Introduction

Cystic fibrosis is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which encodes an epithelial anion channel [1]. In the cystic fibrosis airway, absent or dysfunctional CFTR limits chloride and bicarbonate secretion into the airway surface liquid (ASL). Hyperabsorption of sodium ions through the epithelial sodium channel also occurs, creating osmotic gradients that favour ASL and mucus dehydration [2–4]. The accumulation of dehydrated mucus is thought to lead to patterns of chronic infection, inflammation, bronchiectasis and premature respiratory failure. Measurements of mucociliary clearance (MCC) using nuclear imaging techniques are used in cystic fibrosis therapeutics development [5–9]. Our centre has developed a variation of this technique that includes a parallel measurement of small molecule absorption (ABS) [10–13]. *In vitro* studies have demonstrated a proportional relationship between ABS and ASL absorption rate [11]. ABS is increased in the cystic fibrosis lung, and the inhalation of hypertonic saline has been shown to decrease ABS (ASL absorption) and increase MCC (mucus clearance) [12]. In this study we evaluated experimental factors affecting measurements of MCC and ABS and patient factors associated with abnormal absorption and mucus clearance in adult and paediatric subjects with cystic fibrosis.

Materials and methods

Experimental factors affecting measurements of MCC/ABS

We assessed measurement repeatability in a group of 10 adult cystic fibrosis subjects who performed two MCC/ABS imaging measurements separated by 25–35 days. We determined the effects of aerosol deposition pattern using a combined analysis group including 51 scans from 29 cystic fibrosis subjects. This included measurements from: 1) the repeatability group (both measurements used); 2) a paediatric group of nine subjects who performed two MCC/ABS scans separated by a period of 22–26 months; and 3) a therapy group of 14 adult cystic fibrosis subjects who performed two MCC/ABS study days separated by 5–24 days (only baseline measurements were used). The first measurement from the paediatric study has been previously reported, along with outcomes from the therapy group [12]. Imaging procedures were identical during all studies. Approval was granted by the University of Pittsburgh institutional review board.

Patient factors associated with abnormal MCC/ABS

We assessed correlations between MCC/ABS and patient factors in the combined analysis group and in a subset of this group that had $\leq 50\%$ deposition in central lung zones, the optimal deposition group, which included 37 scans from 24 cystic fibrosis subjects. In addition, comparisons were made with healthy control subjects from a previous study ($n=9$). Patient factors included forced expiratory volume in 1 s (FEV₁), forced vital capacity (FVC) and forced expiratory flow at 25–75% of FVC (FEF_{25–75%}), which were assessed immediately prior to each imaging study. For the paediatric cohort, the slopes of FEV₁, FEF_{25–75%} and FVC % predicted were also calculated by linear regression using available study and clinical pulmonary function tests recorded over the period from 2 years prior to the first imaging visit through the second imaging visit. The slopes are represented as a percentage change in predicted value per year. Sputum and deep oropharyngeal culture results, obtained for routine cystic fibrosis longitudinal care, were surveyed for all subjects. We also assessed whether subjects had previously demonstrated colonisation with *Pseudomonas aeruginosa*. Subjects were considered to be *P. aeruginosa*-positive if they had a single positive culture over the 2-year period before each imaging study.

Radiopharmaceutical aerosol inhalation and imaging protocol

Evaluation of ABS and MCC was performed as previously described [12]. Briefly, a liquid aerosol containing a nonabsorbable radiolabelled particle probe (technetium 99m sulfur colloid (Tc-SC), 296 MBq) and an absorbable radiolabeled small molecule (indium 111 DTPA (In-DTPA), 55 MBq) was inhaled by the subjects using a fixed breathing pattern to achieve predominant airways deposition [9]. (More detail is included in the online supplementary material.) Tc-SC is only cleared by MCC, while In-DTPA is cleared by both MCC and absorption. Subtracting the MCC clearance rate from the total DTPA clearance rate provides an estimate of the absorptive component of DTPA clearance (ABS) [10, 12, 13]. Studies in epithelial cell cultures have demonstrated proportional relationships between ABS and ASL absorption. However, the transport of DTPA is mediated by paracellular liquid flows across the epithelium [11], and other factors such as changes in tight junction permeability or epithelial damage may also affect ABS. During the baseline measurement technique, subjects inhaled isotonic saline from $t=10$ min through $t=20$ min, followed by 60 additional minutes of recumbent imaging.

Statistical analysis

Stata 12.1 (StataCorp, College Station, TX, USA) was used to perform all analysis. Nonparametric testing was used for group comparisons. For matched-pair data, the Wilcoxon matched-pairs signed-rank test was used (SIGNRANK in Stata). For unmatched data, Mann–Whitney testing was used (RANKSUM).

Intraclass correlation (ICC) was used to compare measures in the repeatability portion of the study. A one-way random-effects model was used. Linear regression was used to compare potentially correlated continuous variables (REGRESS).

Results

Experimental factors affecting measurements of MCC/ABS

Characteristics of the 10 adult cystic fibrosis subjects who performed repeatability studies are summarised in table 1. Subjects performed two studies within a 25–30-day window, except for one subject whose studies were separated by 44 days due to illness. Measures of pulmonary function were similar between testing days (FEV₁ % pred p=0.30; FVC % pred p=0.26; and FEF_{25–75%} % pred p=0.20; using the Wilcoxon test), although three subjects did have a >5% change in FEV₁ % pred. There were no statistical differences in indicators of deposition pattern between study days: central deposition percentage (p=0.24) and central/peripheral deposition ratio (p=0.33).

The mean±SD ABS including all 20 measurements was 23.7±11.0%·80 min⁻¹ and MCC was 32.7±15.8%·80 min⁻¹. ABS and MCC values are compared in figure 1. Bland–Altman plots are shown in online supplementary figures S1 and S2. The mean±SD intrasubject difference between ABS measurements (day 2–day 1) was 5.4±8.4%·80 min⁻¹ (ICC 0.63). Excluding data from the three subjects with a >5% change in FEV₁ between testing days reduced the mean difference to 3.4±5.2%·80 min⁻¹ and increased ICC to 0.85. The mean intrasubject difference between MCC measurements was -1.5±12.0%·80 min⁻¹ (ICC 0.75). Excluding data from the subjects with a >5% FEV₁ change between visits changed the mean difference to -4.2±7.7%·80 min⁻¹ and increased ICC to 0.90.

We analysed a combined dataset including data from three different studies in which 29 patients with cystic fibrosis (nine paediatric and 20 adult) performed one to three measurements each (a total of 51 measurements). As shown in figure 2, there was a positive linear correlation between central lung deposition and MCC and a negative linear correlation between central lung deposition and ABS. Very high central deposition favours rapid MCC and prevents accurate measurement of ABS, as the small molecule probe is cleared through MCC before it can be absorbed. The confounding effect of central deposition on ABS becomes less significant if 14 measurements with central deposition >50% are removed (p-value for linear regression increases to 0.10). The confounding effect on MCC becomes statistically insignificant if studies with central deposition >55% are removed (four measurements). We generated an optimal deposition dataset by applying a conservative 50% central deposition threshold to all 51 imaging measurements. This dataset included 37 measurements from 24 subjects. No healthy control subjects demonstrated central deposition >50%.

Patient factors associated with abnormal MCC/ABS

In figure 3, measurements of ABS and MCC from our combined (51 measurement) and optimal deposition (37 measurement) cystic fibrosis groups are compared to data from control subjects [12]. Our previous studies, which included these control subjects and a portion of the current cystic fibrosis subjects, concluded that ABS was significantly increased in cystic fibrosis and that MCC rates were similar in cystic fibrosis and

TABLE 1 Characteristics, pulmonary function, imaging outcomes and deposition variables from 10 adult cystic fibrosis subjects enrolled in the repeatability study group

	Age years	Sex	FEV ₁ % pred		FEF _{25–75%} % pred		MCC %·80 min ⁻¹		ABS %·80 min ⁻¹		Central lung deposition %		Central/peripheral deposition ratio %	
			Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
1	20	M	53	60 [#]	24	28	16.6	31.2	26.0	33.4	50.4	42.9	2.6	2.0
2	35	M	61	55 [#]	30	28	28.3	45.6	10.4	7.9	53.7	57.7	3.1	3.4
3	30	M	81	78	56	52	14.4	11.4	40.2	43.3	39.7	37.8	1.8	1.7
4	57	F	93	93	89	112	44.8	28.8	27.1	36.8	33.8	41.2	1.4	2.0
5	32	M	50	50	28	26	51.1	49.7	13.6	20.9	51.7	53.0	2.4	2.5
6	24	F	79	80	67	69	38.1	26.4	24.0	24.5	44.5	46.1	2.0	2.1
7	24	M	70	73	38	43	18.7	16.3	16.3	16.7	45.9	43.2	1.8	1.7
8	24	M	92	101 [#]	80	91	43.9	26.1	9.1	34.1	51.5	42.1	2.5	1.8
9	33	M	54	56	24	27	21.2	18.6	28.0	35.8	56.3	50.1	2.8	2.2
10	25	F	71	71	40	37	57.3	65.2	15.4	10.3	66.8	59.5	4.2	3.4
Mean±SD	30±11		70±16	72±15	48±24	51±30	33.4±15.6	31.9±16.8	21.0±9.7	26.4±12.1	49.4±9.2	47.4±7.4	2.5±0.8	2.3±0.6

Data are presented as n, unless otherwise stated. FEV₁: forced expiratory volume in 1 s; FEF_{25–75%}: forced expiratory flow at 25–75% of forced vital capacity; MCC: mucociliary clearance; ABS: DTPA absorption; M: male; F: female. [#]: change of >5% between testing days.

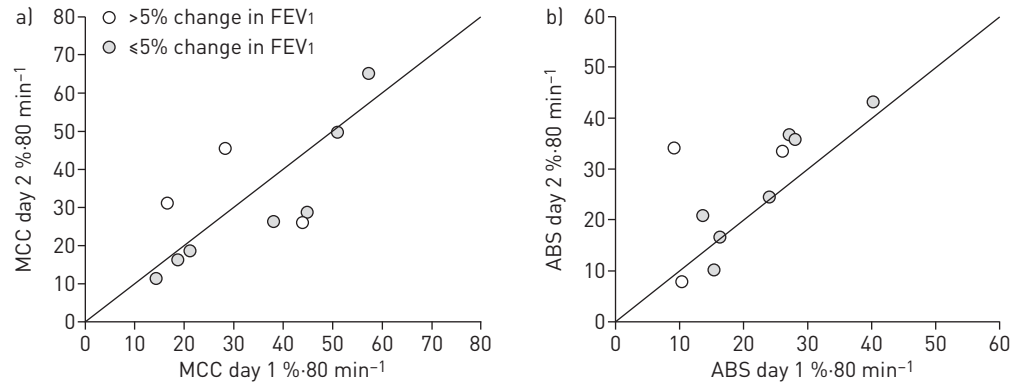


FIGURE 1 Measurement repeatability of a) mucociliary clearance (MCC) and b) DTPA absorption (ABS) in 10 cystic fibrosis subjects. FEV1: forced expiratory volume in 1 s.

control subjects [12]. The present result corroborates that ABS is increased in cystic fibrosis, and with increased significance when the optimised deposition group is considered. MCC differences between cystic fibrosis and noncystic fibrosis approach significance only when the cystic fibrosis group with optimised deposition is considered ($p=0.09$). The mean \pm SD FEV1 in the optimal deposition group was $82\pm 17\%$ pred versus $77\pm 19\%$ pred in the combined dataset.

Based on previous studies linking airway bacterial colonisation to impaired MCC [14], we categorised cystic fibrosis subjects according to whether or not they had had a *P. aeruginosa*-positive (PA^+) sputum or throat culture in the 2 years prior to the imaging study. Of the 37 studies from 24 subjects in the optimal deposition group, 25 were from 16 PA^+ subjects. Table 2 compares *P. aeruginosa*-negative (PA^-) and PA^+ subjects. Figure 4 compares MCC in PA^+ and PA^- groups and healthy controls. PA^+ cystic fibrosis subjects had significantly lower MCC rates than healthy controls ($p=0.04$), especially those who were PA^+ at their last culture ($p=0.03$). PA^- cystic fibrosis subjects had MCC rates similar to healthy controls ($p=0.62$). Comparisons between PA^+ and PA^- cystic fibrosis subjects did not demonstrate significant differences, but trended towards decreased MCC, especially with recent *P. aeruginosa* infection. ABS measurements for the same groups are shown in figure 5. ABS was increased in all cystic fibrosis groups when compared to healthy controls and did not vary significantly with *P. aeruginosa* colonisation.

Table 3 considers other correlations between ABS and MCC and patient-related variables, measures of clinical condition and imaging procedure-related measurements. As previously described, central deposition can influence measurements of both MCC and ABS. The use of the optimal deposition group, which excludes subjects with high central levels of deposition, lessens this influence, but does not completely negate it for ABS ($p=0.10$).

10 paediatric cystic fibrosis patients were recruited to perform two measurements of ABS and MCC separated by 2 years. Subject data are presented in tables 4 and 5. One subject was excluded from all analyses based on poor inhalation technique (not listed). Another subject failed to deposit a sufficient radiopharmaceutical lung dose during their second study visit and data from that visit were excluded (subject 7). One subject (subject 3)

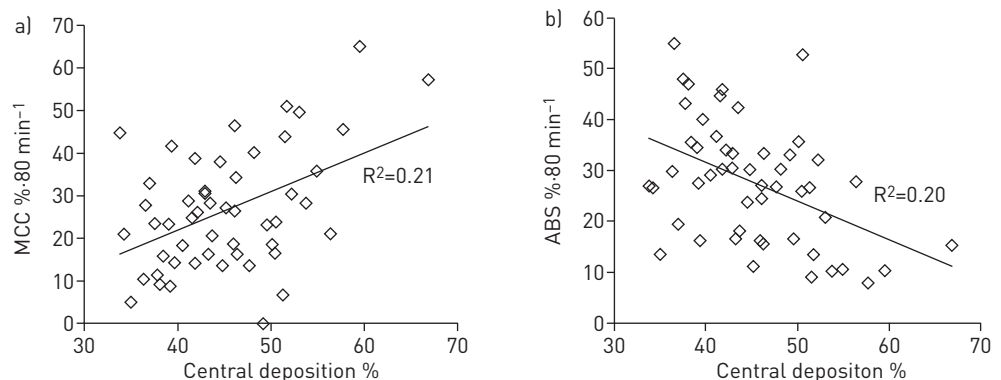


FIGURE 2 Comparison of central lung deposition percentage to measurements of a) mucociliary clearance (MCC) and b) DTPA absorption (ABS).

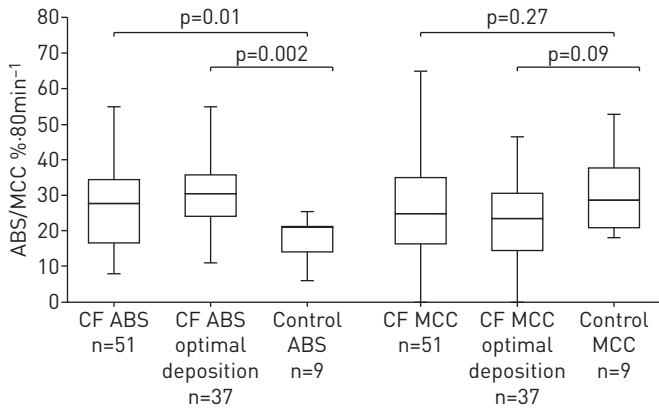


FIGURE 3 Multistudy comparison of measurements of DTPA absorption (ABS) and mucociliary clearance (MCC) in cystic fibrosis (CF) and noncystic fibrosis subjects (p-values calculated using Mann-Whitney test).

was able to perform ABS/MCC tests, but was unable to perform repeatable pulmonary function tests. One subject (subject 4) had >50% central lung deposition on the second day, and day 2 was excluded from the analysis. MCC/ABS data were therefore available from all nine subjects on visit 1 and seven subjects on visit 2.

As shown in figure 6, there were no statistical differences between day 1 and day 2 values for MCC (p=0.87) or ABS (p=0.24). Central lung deposition percentage was similar between visits (p=0.33). On average, FEV₁ % pred declined by 0.9±14% in this group, while FEF_{25-75%} % pred declined by 1.5±27% between study visits.

To determine whether MCC or ABS measurements were associated with subsequent clinical course, a number of associations were evaluated. Day 1 MCC measurements were not predictive of 2- or 4-year slopes in FEV₁, FVC or FEF_{25-75%}, nor were they predictive of the number of oral or intravenous antibiotics received during the 2-year period between visits. Day 1 ABS measurements were not predictive of 2-year slopes in FEV₁, FVC, FEF_{25-75%} or antibiotic treatments. They did predict 4-year slopes in FEV₁ and FEF_{25-75%} (p=0.02 and p=0.04, respectively, by linear regression). Subject age was positively correlated with ABS on study day 1 (p=0.05), but not on study day 2. Age did not correlate with MCC, central deposition percentage or central/peripheral deposition ratio on either study day. Sex was not associated with differences in MCC or ABS.

Average MCC in studies of paediatric subjects with previous *P. aeruginosa* colonisation was 18.4±10.6%·80 min⁻¹ (n=8 measurements from five subjects) versus 26.4±14.6%·80 min⁻¹ in never-infected subjects (n=8 measurements from four subjects; p=0.34 by Mann-Whitney). Average ABS values in these groups were similar (p=0.87). FEV₁ and FEF_{25-75%} were 96±17% pred and 85±32% pred for PA⁺ subjects versus 89±11% pred and 80±35% pred for PA⁻ subjects (p=0.34 and p=0.56 for FEV₁ and FEF_{25-75%}, respectively). Paediatric subject data are included in the results shown in figures 4 and 5.

TABLE 2 Comparison of cystic fibrosis subjects who were *Pseudomonas aeruginosa*-negative (PA⁻), -positive (PA⁺) (during past 2 years), and PA⁺ at last sputum or throat culture

	PA ⁻	PA ⁺	PA ⁺ at last culture	p-value [#]	
				PA ⁺ versus PA ⁻	PA ⁺ at last culture versus PA ⁻
MCC %·80 min ⁻¹	27.9±12.9	21.3±10.2	18.9±10.5	0.16	0.10
ABS %·80 min ⁻¹	30.7±10.5	30.0±11.2	34.8±9.3	0.78	0.33
FEV ₁	90±11	78±18	65±30	0.04	0.03
FEF _{25-75%}	81±30	55±25	46±25	0.02	0.02
Central deposition %	41.6±5.3	41.9±3.8	42.0±3.8	0.82	0.77
Age years	21±17	20±7	21 ± 8	0.31	0.25
Studies	12	25	15		
Subjects	8	16	12		

Data are presented as mean±SD or n, unless otherwise stated. MCC: mucociliary clearance; ABS: DTPA absorption; FEV₁: forced expiratory volume in 1 s; FEF_{25-75%}: forced expiratory flow at 25-75% of forced vital capacity. [#]: Mann-Whitney test.

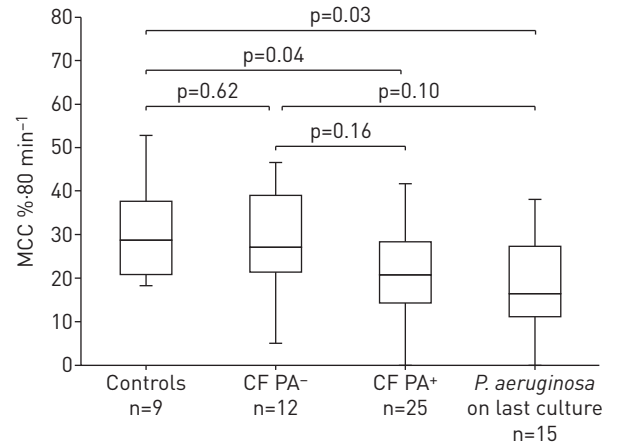


FIGURE 4 Mucociliary clearance (MCC) in healthy controls and cystic fibrosis (CF) subjects with or without *Pseudomonas aeruginosa* infection (PA⁺ or PA⁻, respectively) in the past 2 years.

Discussion

ASL dehydration is a key defect of cystic fibrosis lung disease that is directly linked to CFTR dysfunction. Dehydrated ASL is thought to cause defective MCC, linking CFTR dysfunction to the opportunistic bacterial infections often associated with cystic fibrosis lung disease. The key finding of our study is that defective MCC is present only in cystic fibrosis subjects with a previous history of *P. aeruginosa* infection. Noncolonised subjects demonstrated MCC rates similar to healthy controls. In addition, cystic fibrosis subjects were found to have consistently increased ABS, which may be associated with ASL dehydration. Our studies also indicated potential confounding effects for both MCC and ABS measurements with high central lung aerosol deposition.

Our first goal was to determine the repeatability of the ABS and MCC measurements. Using values for the standard deviation of the intrasubject measurement differences for subjects with similar pulmonary function on both testing days (ABS $\sigma=5.2$ and MCC $\sigma=7.7$) we can consider power. For comparison purposes, the inhalation of 7% hypertonic saline caused a $9.8\% \cdot 80 \text{ min}^{-1}$ decrease in ABS and an $18.8\% \cdot 80 \text{ min}^{-1}$ increase in MCC versus the inhalation of isotonic saline [12]. For a crossover study using parametric statistics to estimate power, a group size of 11 is needed to demonstrate a $5\% \cdot 80 \text{ min}^{-1}$ change in ABS through a paired t-test, assuming that $\sigma=5.2$, $\Delta=5$, $\alpha=0.05$ and $\beta=0.2$. Group sizes of seven and 21 are needed to demonstrate $10\% \cdot 80 \text{ min}^{-1}$ and $5\% \cdot 80 \text{ min}^{-1}$ changes in MCC, respectively, assuming $\sigma=7.7$, $\Delta=10$, $\alpha=0.05$ and $\beta=0.2$ [15]. These group sizes are very attractive and feasible for proof-of-concept studies with ASL-modulating drugs.

Aerosol deposition pattern has been previously shown to affect MCC measurements [9], and most current techniques use controlled breathing to promote consistency between studies and deliver aerosols primarily to the large airways [8]. However, obstructive lung disease may cause extremely high rates of central lung aerosol deposition in some subjects [16]. Excessive deposition in the highly ciliated proximal airways will result in very high MCC measurements that make accurate measurement of ABS impossible as the In-DTPA probe clears through MCC too rapidly to absorb. Here, in order to determine the specific patient and disease factors affecting baseline MCC/ABS, we utilised an analysis group with a central deposition

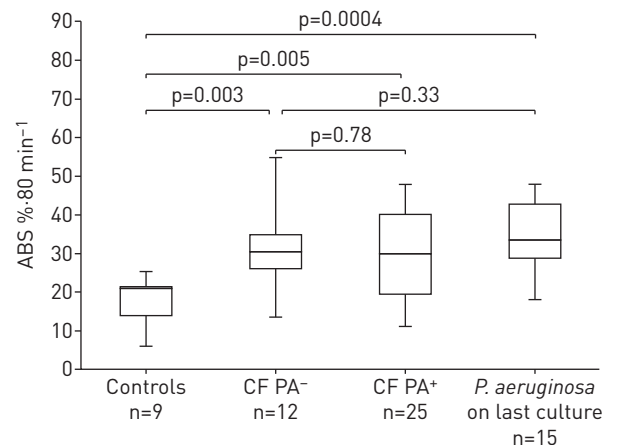


FIGURE 5 DTPA absorption (ABS) in healthy controls and cystic fibrosis subjects (CF) with or without *Pseudomonas aeruginosa* infection (PA⁺ or PA⁻, respectively) in the past 2 years.

TABLE 3 Sex comparison and linear regression analysis of imaging outcomes

	Male	Female	Coefficient (95% CI)	p-value [#]
Subjects n	22	15		
MCC %·80 min⁻¹	21.0±11.0	27.0±11.4		0.14
ABS %·80 min⁻¹	31.4±11.9	28.4±9.1		0.26
Univariate linear regression				
<i>MCC versus</i>				
ABS			-0.18 [-0.53-0.18]	0.32
c/p ratio			-2.74 [-13.86-8.38]	0.62
Central deposition [¶] %			0.20 [-0.71-1.11]	0.66
Age			0.22 [-0.12-0.56]	0.20
FEV ₁			0.078 [-0.159-0.316]	0.51
FEF _{25-75%}			0.087 [-0.051-0.224]	0.21
Starting technetium-99m counts			-0.0013 [-0.005-0.002]	0.44
Starting indium-111 counts			-0.000084 [-0.02-0.02]	0.99
<i>ABS versus</i>				
c/p ratio			-7.47 [-17.1-2.16]	0.12
Central deposition [¶] %			-0.7 [-1.54-0.13]	0.10
Age			0.06 [-0.28-0.39]	0.73
FEV ₁			0.09 [-0.14-0.31]	0.44
FEF _{25-75%}			0.1 [-0.03-0.23]	0.14
Starting technetium-99m counts			0.0015 [-0.002-0.005]	0.32
Starting indium-111 counts			0.0041 [-0.01-0.02]	0.57

Data are presented as n or mean±SD, unless otherwise stated. Based on 37 measurements from 24 adult and paediatric cystic fibrosis subjects. MCC: mucociliary clearance; ABS: DTPA absorption; c/p: central/peripheral deposition; FEV₁: forced expiratory volume in 1 s; FEF_{25-75%}: forced expiratory flow at 25-75% of forced vital capacity. [#]: p-values for sex comparison calculated using Mann-Whitney test; other p-values based on linear regression; [¶]: subjects with high central deposition percentages (>50%) are not included in this group.

percentage <50%. This is not intended to be a general limit to be applied to other studies. In particular, crossover studies involving only MCC measurement, where deposition is well matched between study days, may allow for the evaluation of therapeutic effect even with high central deposition percentages.

We generated a combined baseline dataset of MCC/ABS measurements that included measurements from several recent and previous studies [12]. Previously reported trends were confirmed in this larger group. Specifically, baseline ABS is significantly increased in cystic fibrosis subjects *versus* healthy controls and baseline MCC is not detectably lower in cystic fibrosis. When analysis was restricted to subjects with optimal deposition (central deposition <50%, 37 out of 51 measurements), MCC differences between cystic fibrosis and control subjects approached significance and the significance of ABS differences increased.

TABLE 4 Characteristics of paediatric subjects participating 2-year longitudinal studies

Subject	Age years		Sex	FEV ₁ % pred		FEF _{25-75%} % pred		FEV ₁ % pred	
	Day 1	Day 2		Day 1	Day 2	Day 1	Day 2	4-year [#]	2-year [¶]
1	13.4	15.6	M	88	105	65	86	-1.1	8.4
2	9.1	11.2	F	106	126	82	135	-2.8	6.1
3	9.6	11.8	F	NA	NA	NA	NA	NA	NA
4	12	14.1	F	103	94	134	120	2.1	0.6
5	11.6	13.7	M	78	68	45	35	-3.0	-3.9
6	10.6	12.6	M	94	89	91	63	-4.0	-2.8
7	9.2	11.2	F	100	104	111	113	-1.3	-2.1
8	12.3	14.3	M	80	76	56	53	-0.3	-2.6
9	11.1	13.3	M	93	73	80	47	-2.4	-3.7
Mean±SD	11.0±1.5	13.1±1.5		93±10	92±20	83±29	82±38	-1.6±1.9	0.0±4.7

Data are presented as n, unless otherwise stated. FEV₁: forced expiratory volume in 1 s; FEF_{25-75%}: forced expiratory flow at 25-75% of forced vital capacity; M: male; F: female; NA: not available. [#]: 4-year slope of FEV₁ % pred; [¶]: 2-year slope of FEV₁ % pred.

TABLE 5 Imaging outcomes of paediatric subjects participating in 2-year longitudinal studies

Subject	MCC %·80 min ⁻¹		ABS %·80 min ⁻¹		Central deposition %	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
1	24.9	16.4	44.8	33.4	41.6	46.3
2	33.0	13.7	19.5	30.4	37.0	44.8
3	10.5	20.7	30.0	18.2	36.3	43.7
4	27.9	EX	55.0	EX	36.5	51.3
5	13.6	5.1	26.8	13.6	47.7	5.0
6	38.8	46.5	30.3	27.2	41.9	46.1
7	40.2	EX	30.4	EX	48.2	EX
8	0.0	28.3	33.3	42.5	49.2	43.5
9	15.9	23.4	35.7	34.6	38.4	39.0
Mean±sd	22.8±13.7	22.0±13.1	34.0±10.4	28.6±9.9	41.9±5.3	43.7±4.9

Data are presented as n, unless otherwise stated. MCC: mucociliary clearance; ABS: DTPA absorption; EX: excluded (see text).

The effect of *P. aeruginosa* colonisation on MCC was previously considered by LAUBE *et al.* [14]. In that study, MCC with included cough clearance was depressed in children with *P. aeruginosa* colonisation when compared to those without. Controversy has existed about whether cystic fibrosis subjects demonstrate baseline deficits in 60–90 min measurements of MCC, with some studies demonstrating no differences *versus* healthy controls [6, 12] and others demonstrating clear deficits [17, 18]. These differences are probably related to *P. aeruginosa* status, since the latter group of studies enrolled only PA⁺ subjects. (This was also the case for several other studies utilising MCC to evaluate therapeutics [7, 19].) One of these studies did identify two PA⁻ subjects as having normal clearance, but did not include them in the final analysis [18]. Some studies have noted an MCC defect in cystic fibrosis using techniques designed to assess peripheral lung MCC, such as 24-h measurements [6]. Shorter measurement periods, including those used here, are dominated by large airway clearance. Our results indicate that PA⁻ cystic fibrosis subjects have MCC rates very similar to healthy controls while PA⁺ cystic fibrosis subjects have MCC rates that are significantly decreased *versus* controls. MCC differences between the PA⁺ and PA⁻ cystic fibrosis groups were not statistically significant, but trends indicated a likely association between MCC depression and recent *P. aeruginosa* infection.

MCC may be depressed by *P. aeruginosa* through multiple mechanisms. Ciliary beat frequency can be reduced after exposure to *P. aeruginosa* [20, 21], potentially mediated by virulence factors such as pyocyanin [22] and rhamnolipids [23]. Previous studies have demonstrated that mucus production, composition and rheology could be affected by *P. aeruginosa* [24], and pyocyanin in particular is associated with goblet cell hyperplasia and mucus hypersecretion [25]. As a host response to pathogens in healthy airways, CFTR facilitates ASL secretion, increasing the transportability of mucus. The failure of this mechanism in cystic fibrosis, compounded by infection-driven increases in mucus production, could

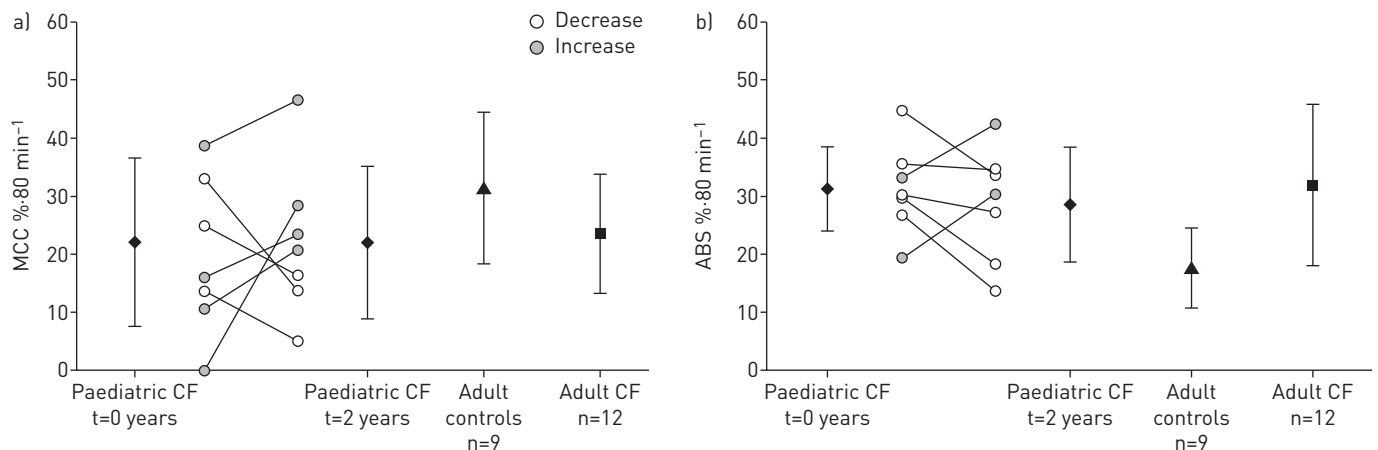


FIGURE 6 Longitudinal measures of a) mucociliary clearance (MCC) and b) DTPA absorption (ABS) in paediatric cystic fibrosis (CF) patients over a 2-year period. Previous data from adult cystic fibrosis and adult control subjects are included for comparison [12]. Data are presented as mean±sd.

defeat otherwise normal rates of MCC in the cystic fibrosis lung. Acute impairments of clearance may extend beyond *P. aeruginosa*. We can hypothesise that other bacterial or viral infections may depress MCC in cystic fibrosis leading to vulnerability to secondary infections, including *P. aeruginosa* or other cystic fibrosis pathogens. In support of this, previous studies have associated initial *P. aeruginosa* infection with respiratory syncytial virus and other upper respiratory infections [26, 27]. However, the cause-and-effect relationship between *P. aeruginosa* and depressed clearance cannot be clearly determined from our data. Depressed MCC could be the direct cause of *P. aeruginosa* infection, in line with classic descriptions of cystic fibrosis lung pathophysiology. Loss of this host defence mechanism would certainly make subjects more vulnerable to infection. Another possibility is that airway conditions, such as mucus plugging and associated local hypoxia, could independently predispose subjects to both depressed MCC and *P. aeruginosa* infection.

In longitudinal studies of MCC/ABS in paediatric subjects, there was no indication that ABS or MCC predicted clinical outcomes over a 2-year interval. Infection status and age may have confounded our ability to determine these relationships. The role of *P. aeruginosa* infection has already been described and trends within the paediatric group matched those of the larger analysis group in this regard. Ultimately the small sample size and the use of only a single follow-up measurement probably decreased our ability to detect any subtle effects with these measurements.

Many of the limitations of the current studies are associated with the inherent variability of the aerosol-based techniques applied. We have confirmed the previously described potential for confounding based on changes in aerosol deposition pattern [9]. In addition, our techniques rely on two-dimensional planar imaging of an inherently complex three-dimensional organ that contains large and small airways and alveoli. Our aerosol delivery techniques are intended to target deposition primarily to large airways, but some alveolar contribution to outcomes is likely. Limitations to consider with regard to our analysis of baseline relationships between MCC/ABS and disease state include the use of multiple measurements from some subjects. Our primary analysis group included 37 measurements from 24 subjects obtained through several different protocols that utilised identical techniques. The time between measurements varied from a minimum of ~30 days to ≥ 2 years. Since each of these studies was associated with a unique set of variables describing patient condition and ABS/MCC, we opted to utilise all measurements. The study was also limited by having only a small group of control subjects for comparison.

In summary, measurements of MCC and ABS demonstrated good repeatability. The primary experimental factor affecting the measurements is the extent of central deposition. Excluding subjects with high central deposition appeared to increase the statistical significance of observed trends. The primary patient factor associated with depressed MCC is *P. aeruginosa* infection. PA⁻ cystic fibrosis subjects had MCC rates similar to healthy controls while PA⁺ cystic fibrosis subjects had depressed MCC, apparently more so with recent infection. Neither ABS nor MCC predicted the clinical course of disease in a small number of paediatric cystic fibrosis subjects though a 2-year follow-up. Multiple longitudinal measurements may provide more comprehensive information on disease evolution and the factors leading to initial *P. aeruginosa* colonisation. We hypothesise that defects in ASL secretion in the cystic fibrosis airway result in clearance that is not depressed at baseline, but rather is vulnerable to depression by factors such as infection that in turn might lead to cycles of secondary infection and disease progression.

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