

Nasal mucociliary clearance and ciliary beat frequency in cystic fibrosis compared with sinusitis and bronchiectasis

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ABSTRACT Nasal ciliary function and mucociliary clearance were studied in patients with cystic fibrosis and in three control groups. Ciliary beat frequency and nasal clearance time were measured in groups of 10 subjects with cystic fibrosis, sinusitis and bronchiectasis and age and sex-matched control subjects. Ciliary beat frequency was also measured in normal subjects matched as bronchiectasis controls. Cystic fibrosis patients and their controls, patients with sinusitis, and the bronchiectasis controls did not differ in ciliary beat frequency, but it was slower in the patients with bronchiectasis ($p < 0.05$). Nasal mucociliary clearance in cystic fibrosis and bronchiectasis was slower than in the cystic fibrosis controls ($p < 0.001$) and in patients with sinusitis ($p < 0.01$). The finding of a normal beat frequency in cystic fibrosis cilia studied *in vitro* together with abnormal nasal mucociliary clearance measured *in vivo* in the same patients suggests the existence of an abnormality of mucus *in vivo*. The innate function of cystic fibrosis cilia, as measured *in vitro* by beat frequency, is normal.

Pulmonary mucociliary clearance in patients with cystic fibrosis has been shown to be decreased¹⁻³ although some studies have reported patients with normal^{2,3} or even increased clearance rates.⁴ It seems likely that abnormalities in the mucous aspects of mucociliary clearance, rather than an innate defect in ciliary structure or function, underlie defective clearance. Several possibilities exist for such a defect. Abnormal glycoproteins may result in mucus with suboptimal visco-elastic properties.^{5,6} Another possibility is the existence in serum and other body fluids of a "cystic fibrosis factor" which can disorganise ciliary function by a direct effect,^{5,6} or by stimulating release of abnormal mucus resulting in disruption of the sol phase in which cilia beat. Ciliary function in isolation from mucus has not been quantitated in patients with cystic fibrosis.

This study was designed to determine whether ciliary beat frequency, one aspect of ciliary function, is abnormal in patients with cystic fibrosis. Nasal mucociliary clearance was determined to obtain an *in vivo* measurement of the effectiveness of the interaction between cilia and mucus in these patients. Sinusitis is almost universal in patients with cystic fibrosis although often asymptomatic.^{7,8} Because of this, the measurements were made in

normal subjects and in a group of patients with sinusitis but no chest disease. The intrathoracic pathology which develops in patients with cystic fibrosis usually includes the changes of bronchiectasis.⁹ A group of patients with bronchiectasis not caused by cystic fibrosis was therefore included in the study.

Methods

Nasal mucociliary clearance was measured with a saccharin method as described by Andersen *et al.*^{10,11} Before starting the procedure subjects spent at least one hour in a stable environment (temperature 21-24°C; relative humidity 30-50%). The subject blew his nose gently to remove any excess secretions and a saccharin particle, 0.5 mm diameter, was gently placed on the medial surface of the inferior turbinate of one nasal cavity at least 7 mm behind the turbinate's anterior end to avoid the area of mucosa where cilia beat in an anterior direction. The time from particle placement until the subject reported the first sensation of a sweet taste was measured with a stop-watch. Unlike Andersen we did not ask patients to swallow repeatedly and we did not measure the distance from nasal tip or mucocutaneous junction to posterior nasopharynx. Our results are therefore expressed as a clearance time, not a rate.

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After this measurement, ciliated epithelium from the contralateral inferior turbinate was obtained with a brushing technique not requiring local anaesthesia. This technique and the photometric method of measuring ciliary beat frequency used in this study have been described previously.¹² In brief, strips of ciliated epithelium are placed in a vial of nutrient medium from where they can be transferred to a sealed microscope preparation. Beating cilia can be seen easily and positioned to obstruct intermittently the passage of light through a small diaphragm and into a photometer which transduces light energy into an electrical signal. This is recorded and the rate of beating calculated. Ten consecutive measurements of vigorously beating cilia were made from each sample and the result expressed as a mean \pm standard deviation (SD).

Ten patients aged between 15 and 29 years with

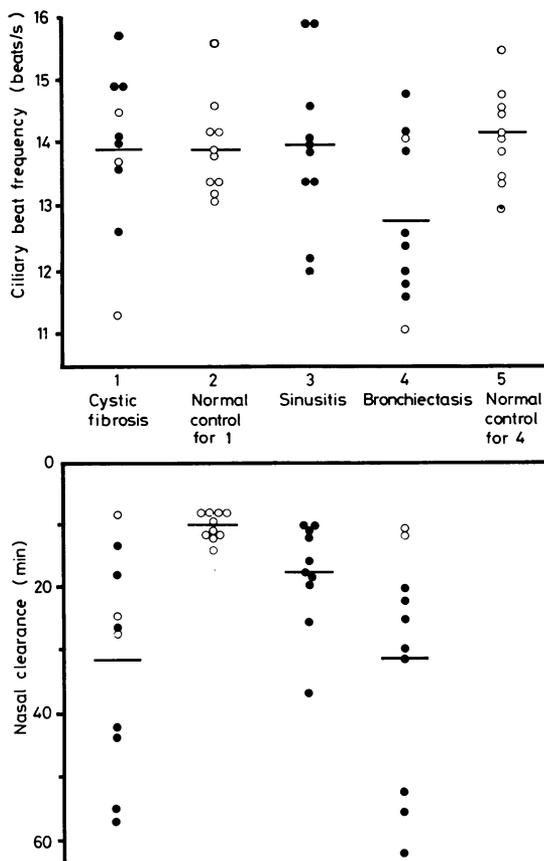


Figure Ciliary beat frequencies (above) and nasal clearance times (below) in patients with cystic fibrosis and controls with (●) and without (○) sinusitis. Horizontal lines indicate group means.

well-documented cystic fibrosis of varying severity were studied (group 1). All had a sweat sodium concentration of >80 mmol/l (normal <70 mmol/l). All patients gave informed consent as did a parent of those younger than 21 years. Eight of the patients were studied in hospital. Where this was for an acute respiratory infection the study was performed immediately before discharge when the patient was clinically stable. None was studied at a time of acute exacerbation of nasal symptoms. Therapy included antibiotics and bronchodilators where appropriate. The control group (2) for the cystic fibrosis patients consisted of 10 age- and sex-matched normal subjects none of whom was a smoker or had a history of nasal or pulmonary disease.

Group 3 comprised 10 patients with chronic or recurrent sinusitis. All had a continual or recurrent mucopurulent post-nasal drip and abnormal sinus radiographs. None had a history of respiratory disease and two were smokers. The fourth group (4) of 10 patients had bronchographically proven bronchiectasis not caused by cystic fibrosis. All were clinically stable when studied and none had an acute exacerbation of nasal symptoms. All 10 were non-smokers. The final group (5) was formed in the same way as group 2 but were age- and sex-matched with the patients with bronchiectasis. None had a history of nasal or pulmonary disease; none had ever smoked. Ciliary beat frequency only was measured in this group.

The differences between the patient groups' ages and the nasal clearance times were compared by means of the Kruskal-Wallis one-way analysis of variance.^{13 14} The differences in beat frequency were examined by an analysis of variance using the 10 replications made on each patient sample.^{13 14}

Results

The nasal mucociliary clearance times for groups 1 to 4 and the ciliary beat frequencies are illustrated in the figure. The table shows mean (± 1 SD) for age, nasal clearance times, and beat frequency of the five patient groups. Ciliary beat frequency for the bronchiectasis group (4) was slower than its control group (5) of normal subjects as well as being slower than the cystic fibrosis (1) and cystic fibrosis control (2) groups. The beat frequency of the patients with cystic fibrosis did not differ from that of the controls.

In patients with sinusitis (3) clearance was slower than in the cystic fibrosis control group (2) but beat frequency was not significantly different. Despite the age difference, beat frequency for the bronchiectasis control group (5) did not differ significantly from that of the younger cystic fibrosis control group. The clearance of the cystic fibrotic patients was signifi-

Table Nasal ciliary beat frequency and mucociliary clearance time in cystic fibrosis, sinusitis, and bronchiectasis

Group	Age (yr)	Nasal CBF (beats/s)	Nasal clearance time (min)
1 Cystic fibrosis	22.6 ± 3.9	13.9 ± 1.3	31.6 ± 16.9
2 Normal subjects (CF controls)	23.3 ± 3.9 (NS)	13.9 ± 0.8 (NS)	10.1 ± 2.1 (p < 0.001)
3 Sinusitis	32.3 ± 13.6 (p < 0.01)	14.0 ± 1.3 (NS)	17.6 ± 8.3 (p < 0.01)
4 Bronchiectasis	42.7 ± 14.3 (p < 0.001)	12.8 ± 1.3 (p < 0.05)	31.8 ± 18.4 (NS)
Normal subjects (Bronchiectasis controls)	41.2 ± 13.6 (NS)	14.2 ± 0.7 (p < 0.025)	—

All results are means ± one standard deviation.

NS = not significant.

p values for 2, 3, and 4 are for differences from CF (1); for 5 from bronchiectasis (4).

cantly slower than that of the controls and of the patients with sinusitis.

Discussion

Mucociliary clearance is one of the major defences of the tracheobronchial tree, nasal cavity, and its sinuses. Effective clearance depends on the visco-elastic properties of mucus and intact ciliary function to transport this mucus.

This study has demonstrated in a quantitative manner that the beat frequency of cilia in a group of patients with cystic fibrosis is normal. This corroborates a previous visual impression of normal beat frequency in ciliated epithelium covering nasal polyps excised from two patients with this disease.⁸ These polyps were collected and examined in the patient's own serum at 37°C, continuing to beat for 56 hours. Continued beating of normal cilia for several days is common but in these patients this is evidence against the presence of a factor toxic to human cilia being present in their serum. If cystic fibrosis serum does contain a "ciliary dyskinesia factor" perhaps it is not toxic for the patient's own cilia but only for animal or normal human cilia. Alternatively, it is possible that when the amount of tissue is large compared to the volume of serum in which it is bathed an abnormal factor may be in too low a concentration to impair ciliary function.

To ensure effective transport cilia must beat at a normal rate, in a consistent direction and in a properly co-ordinated manner. The total number of cilia present also has a bearing on transport. Although ciliary co-ordination appeared normal in this study it was not possible to measure metachronal wave length or timing. Direction of beating may be inferred from ciliary orientation in ultrastructural studies. In one report orientation of cilia in a bronchus from a patient with cystic fibrosis was normal.³ Transmission electron microscopy has shown the frequency of ultrastructural abnormalities in cystic fibrosis cilia to be similar to that in patients

with chronic bronchitis and bronchiectasis.¹⁵ In patients with Kartagener's syndrome (a form of ciliary dyskinesia) lack of ciliary orientation at the ultrastructural level is well described.^{3,16} In such patients we have seen cilia beating in opposite directions, and at an abnormally slow rate.¹⁷ Altered direction of ciliary beating has been shown in bronchitic rats,¹⁸ and this may be present in patients with cystic fibrosis once chronic infection is established.

It has been suggested that there are decreased numbers of ciliated cells in the trachea and bronchi of cystic fibrosis patients with an increase in mucus secreting cells.¹⁹ Areas of squamous metaplasia are seen more frequently than in normal airways.^{9,19} Any of these three factors might impair mucus transport without the existence of an innate defect in the function of individual cilia.

Since the method of obtaining ciliated epithelium used in this study involved placing small pieces of tissue (< 1 mm diameter) in 2-3 ml nutrient medium any mucus components, including possible ciliotoxic factors, would have been greatly diluted. We aimed to study the function of cilia in the absence of such factors and so have not excluded a possible influence they might have on mucociliary function. Further studies using this model are in progress to evaluate the effect of serum components on human ciliary function. Nasal, rather than pulmonary, ciliary beat frequency and mucociliary function were studied because this can be done in a non-invasive manner. We have shown a positive correlation between nasal and tracheal beat frequencies measured in the same subjects.²⁰ A positive association between nasal clearance determined by the saccharin method and pulmonary clearance measured by an inhaled radiolabelled aerosol has also been shown.¹¹ Although nasal clearance can vary widely between individuals and may be affected by temperature and extremes of humidity it appears to be reproducible in the same subject.^{21,22} We have not attempted to express nasal clearance as a rate but rather as a

clearance time. The exact site at which saccharin particles are tasted is not known and is in any case unlikely to be on the posterior nasopharyngeal wall. Measurements from nasal tip to posterior nasopharynx as made by Andersen^{10 11} are not necessarily related to the distance travelled by the test particles. Furthermore, these particles may not travel by the most direct route.²³ Measurement of nasal clearance by this method gives the most rapid transit time—the leading edge of the saccharin is tasted first to give the endpoint. Because of this the method is more analogous to measurements of pulmonary clearance made in large airways alone (such as is done when insufflating radio-opaque particles¹ or inhaling boluses of a radiolabelled aerosol¹²) rather than pulmonary clearance determined by the inhaled aerosol technique^{3 4} which is affected by the degree of initial particle penetration. Such differences in radioaerosol penetration probably explain much of the conflicting evidence found in studies of pulmonary clearance in cystic fibrosis.¹⁻⁶

Nasal clearance times for patients with cystic fibrosis were slower than for the control group. Such a prolongation was not found by Rossman *et al* who, using radiolabelled albumen, found clearance on the floor of the nose to be no different from that in a control group.²⁴ This type of measurement is more likely to reflect mean clearance than the saccharin method which measures clearance time of the most rapidly moving particles. Rossman *et al* demonstrated decreased nasal clearance in cystic fibrosis patients whose nasal mucosa was subjected to a mild allergic inflammatory reaction induced with anti-IgE. Nasal clearance was also decreased in normal subjects when anti-IgE followed by cystic fibrosis serum was applied to the nasal mucosa.²⁴ Although our patients were clinically stable it is possible that nasal inflammation was more pronounced than in Rossman's patients.

Patients with cystic fibrosis usually develop pathological changes of bronchiectasis,⁹ and sinusitis is common in patients with bronchiectasis.^{25 26} Nasal clearance was slow in our group of patients with bronchiectasis, not differing from that in the cystic fibrosis patients but ciliary beat frequency was slower than in cystic fibrosis. Our controls have made the possibility of an age effect unlikely as an explanation of these differences. Dalhamn *et al* reported no difference between beat frequency in sinus and bronchial mucosa in six patients with bronchiectasis compared with four normal subjects.²⁷

Although the slower beat frequency in our patients with bronchiectasis reached statistical significance it is doubtful whether a reduction of beat frequency of approximately 10% is likely to be biologically significant. Co-ordination of ciliary activity in these

patients appeared normal and none of them had obvious ciliary dyskinesia. A variety of structural abnormalities has been reported in the cilia of patients with bronchiectasis,^{15 28 29} and it is possible that such defects may result in subtle abnormalities of function. The two bronchiectatic patients without sinusitis were the two who had a normal nasal clearance. One of three cystic fibrosis patients without sinusitis had a normal nasal clearance while in the other two it was prolonged (figure).

From this study we conclude that innate ciliary function is normal in cystic fibrosis but an abnormality of mucus or a substance present in mucus results in impaired clearance. This abnormality may be a biochemical one resulting in altered viscoelastic properties or it may be a substance, perhaps also present in serum, which causes ciliary dyskinesia *in vivo*. A number of *in vitro* bioassays are available for determining the effect of cystic fibrosis body fluids on non-human cilia.^{30 31} These are qualitative in nature and the usual endpoint is either dyskinesia (rabbit trachea assay³⁰) or cessation of ciliary beating (oyster gill assay³¹). In the absence of a suitable animal model of this disease the method of measuring beat frequency *in vitro* on human nasal brushings described in this paper is being evaluated as a system for the detection and quantitative measurement of factors toxic to human cilia.

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