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Variability in ciliary beat frequency in normal subjects and in patients with bronchiectasis

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Abstract

***Background*—There is a wide variation in tracheobronchial clearance of inhaled aerosol in normal subjects and in patients with bronchiectasis, but little information is available on the variability in ciliary beat frequency (CBF).**

***Methods*—The variability in CBF was measured in 10 nasal mucosal samples from each of 19 normal controls and 23 stable bronchiectatic subjects.**

***Results*—The CBF varied at different mucosal sites in both normal subjects and bronchiectatic patients. Although the CBF of the fastest beating cilia was similar in both groups, the CBF of the slowest beating cilia was, on average, lower and showed greater within subject variation in bronchiectatic than in normal subjects.**

***Conclusions*—There is a wide variation in CBF in nasal mucosal samples and this is significantly wider in bronchiectatic subjects with some cilia beating slowly. This may be a consequence of chronic inflammation or infection.**

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The mucociliary clearance system provides a major defence mechanism for the respiratory tract.[1] Inhaled particles and endogenous debris are cleared from the airways by mucociliary transport and cough.[2] Abnormality of ciliary function is a recognised cause of chronic bronchial sepsis. Impaired function of the mucociliary system may result from a primary defect in one of its parts[3] or may occur secondary to infection or exogenous agents.[4]

The integrated action of the mucociliary system can be investigated by clearance of radioaerosols[5] while the ciliary component can be assessed by measurement of ciliary beat frequency (CBF),[6] an investigation increasingly performed in patients with chronic bronchial sepsis. Del Donno *et al*[7] have shown that there is wide normal variation in tracheobronchial clearance of an inhaled aerosol. Although standard descriptions of measurement of CBF emphasise the importance of examining different areas of sampled cilia, Rutland and Cole[6] selected only vigorously beating cilia for examination; in other reports details of how the examined areas were selected are often lacking. Furthermore, there is little information available on the variability of CBF within individuals.

We have therefore studied CBF in nasal mucosa in a group of normal subjects and in patients with bronchiectasis to assess the variability in CBF within subjects and between subjects, in health and disease.

Methods

PATIENTS

Twenty three patients with proven bronchiectasis were studied. They were attending respiratory outpatient clinics but were otherwise unselected. The only criteria for study were a stable clinical state with no current exacerbation, and an age of less than 70 years. There were 17 men and six women with a mean age of 53·8 years, all with longstanding bronchiectasis. None of the subjects was a current smoker, although nine were ex-smokers. Two patients had common acquired hypogammaglobulinaemia as the underlying cause of bronchiectasis but no specific cause was identifiable in the others. Twenty patients had undergone bronchography at some time to assess and confirm their condition. The other three patients had a classical history of cough productive of a large volume of sputum, and plain radiography showed unequivocal generalised cystic changes consistent with bronchiectasis. Sixteen of the bronchiectatic patients had some history of sinusitis in childhood or in the past, but none had a recent sinus infection.

The normal control group consisted of 16 men and three women, all non-smokers and in good health. Most were hospital personnel and were aged between 26 and 38 years. None had had a respiratory infection in the week before the study. Samples were taken from these subjects as part of a preliminary assessment of the equipment, and the video recordings were later analysed for comparison with patients undergoing assessment.

STUDY DESIGN

CBF was measured by a method similar to that of Braga *et al*.[8] Samples of cilia were obtained from the nasal cavity by brushing the inferior turbinate with a 2 mm nylon brush and suspending the collected mucosa in medium 199 without Earle's salts (Flow Laboratories Ltd). Several drops of the suspension containing multiple fragments of mucosa were placed in a chamber formed between a microscope slide and a coverslip

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sealed at the edges with silicone grease. The slide was placed on a heated microscope stage (Micro Instruments, Oxford) and equilibrated to a stage temperature of 45°C, which previous studies had shown to give a temperature of 37°C within the chamber containing the suspension. An image of the mucosal fragments was displayed on a monitor screen using a ×200 oil immersion objective on a microscope fitted with a video camera (fig 1). Only strips of mucosa with more than six cells were examined. A video recording of ten strips of intact mucosa was made for the later measurement of CBF.

The use of a pinhole collimator and a photodiode probe allowed examination of the beating of two or three adjacent cilia. The probe was placed against the television screen over the image of beating cilia and the movement of the ciliary image was thus recorded as a waveform on the attached oscilloscope. The trace was frozen when a uniform waveform was evident and from this waveform the CBF was calculated.

Reproducibility of counting method

A preliminary study examining a specific point on the screen repeatedly showed that the minimum number of waveforms required to give a reproducible reading of CBF was four. We therefore chose to read six cycles for each measurement of CBF in the definitive study in order to be certain of reproducible results.

We then examined the reproducibility of the method in one control subject by repeatedly measuring CBF at a single point, on each occasion playing the video recording for long enough to give three consecutive readings at that site. The readings were then repeated 20 times from exactly the same section of video film and the same point on the screen using a grid system attached to the screen. In this individual the CBF ranged from 8·9 Hz to 10·5 Hz ((mean (SD) 9·96 (0·51) Hz) with a coefficient of variation (CV) of three consecutive recordings between 0 and 1·7% (median of the 20 sets 0·7%). The overall CV of the 60 readings was 5·2%.

The inherent variability in measuring the same spot a number of times derives from the fact that the waveform created on the oscilloscope cannot be absolutely reproduced over a number of waveforms on each rerun of the tape. One may start to read the CBF with the photometer a split second later, or the photometer may be displaced 1 mm on the television screen as it is hand held. The readings will therefore have a small degree of variability.

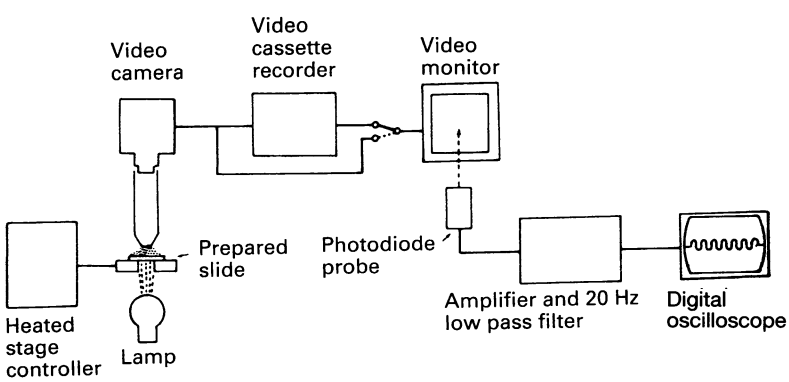

Figure 1 Schematic diagram of videophotometry system.

Effect of time after sampling

In order to assess the long term variation in CBF in an incubated specimen a video recording of a set of uniformly beating cilia was examined every half hour for three hours. At each examination three readings were taken from the same spot on the strip of epithelium. The mean CBF at a single site remained stable for two hours at 13·6 Hz but showed a reduction at three hours from 13·6 to 11·8 Hz, possibly related to drying out of the heated chamber as a newly prepared specimen from the original sample retained CBF for longer.

Variability along a single strip of mucosa

The variation in CBF at eight equidistant points along a single strip of mucosa from a further normal subject was examined by running the video several times and using the grid on the television screen to locate the relevant points in the specimen. Each point was examined three times. The strip studied contained at least six intact mucosal cells.

At each site there was little variation in CBF with the standard deviation of the three readings varying from 0 to 0·17 Hz, but there was appreciable variation from site to site with a range of CBF from 7·6 to 15·2 Hz (mean 9·3 (2·5) Hz) and a CV of 27·1%.

Effect of temperature variability

We examined the effect of temperature on CBF by examining the cilia at a single site as the temperature was adjusted upwards from 21°C to 44°C in the slide. The CBF at a single site increased steadily from a temperature of 21°C to 38°C within the medium in the chamber and then stabilised with no change up to 44°C.

Comparison of patients and normal subjects

The variability within subjects was examined by measuring the standard deviation of CBF values of the apparently fastest and slowest beating cilia in a microscope field taken from 10 strips of mucosa. The measurements at each point were each repeated six times by rerunning the video recording, giving 60 estimates of the fastest and 60 of the slowest CBF in each individual. The average CBF of the fastest and slowest beating cilia was then compared between the normal subjects and patients with bronchiectasis. The fastest and slowest beating areas were examined by direct vision on the television screen, but on every strip of mucosa examined CBF was counted at two random spots on the screen to ensure that a lower or higher CBF was not inadvertently missed.

Within subject variation in ciliary beat frequency (CBF)

	Range of individual mean CBF (Hz)	*Range of within subject coefficient of variation (%)*	*Overall mean (SD) CBF (Hz)*
Fast:			
Controls	10·2–14·6	9·0–27·0	12·7 (1·3)
Patients	8·9–15·0	9·7–39·2	12·2 (1·8)
Slow:			
Controls	7·5–11·2	11·1–58	9·3 (1·2)
Patients	1·6–11·6*	13·1–89*	7·7 (2·5)*

*p < 0·005 between patients and controls.

Results

WITHIN SUBJECT VARIATION

The within subject variation is indicated by the standard deviation of the 60 estimates of CBF from the 10 samples obtained from each individual (fig 2) shown as a range of CV values in the table. There was no statistically significant difference between the CV of the fastest CBF in normal subjects and bronchiectatic patients (unpaired *t* test). Values of CBF for the fastest beating cilia were within the usually quoted normal range of 11–16 Hz.[1]

For the slowest beating cilia in the normal subjects the mean (SD) within subject CV of the CBF was 26% (8·8%). In the bronchiectatic subjects the within subject CV of the CBF was 34·8% (17·3%) which was significantly greater than for normal controls (p < 0·005, unpaired *t* test).

BETWEEN SUBJECT VARIABILITY

Figure 2 shows a similar range of highest CBF in both the normal subjects and the bronchiectatic patients. For the slowest beating cilia, by contrast, the range of CBF was wider and the average values were significantly lower in the bronchiectatic subjects (p < 0·005) (table).

Four subjects with bronchiectasis had lower CBF values than the rest of the group but did not differ with regard to recent exacerbation or severity of clinical condition.

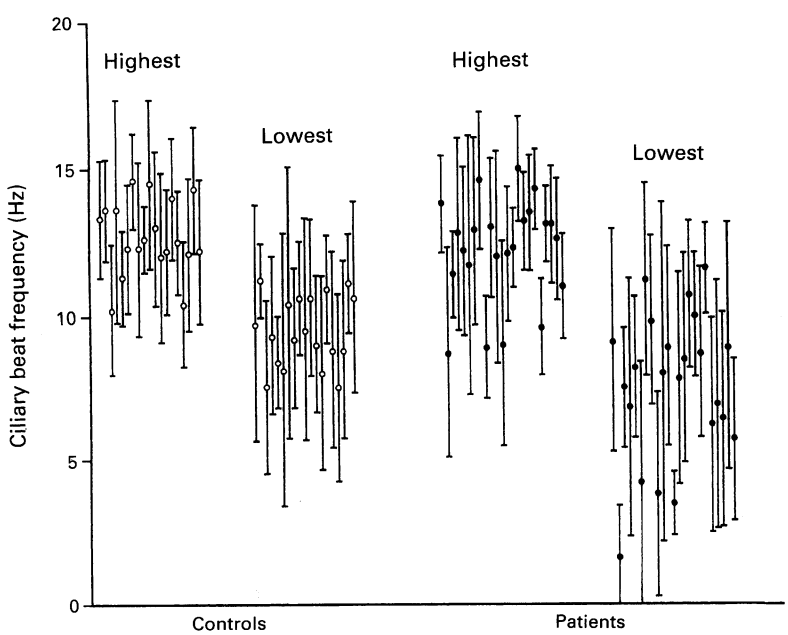

Figure 2 Mean (SD) highest and lowest ciliary beat frequency (CBF) values in 19 control subjects and 23 patients with bronchiectasis. Each symbol represents one subject and the bars indicate ± 1 SD of 60 estimates of fastest and slowest CBF in each individual.

Discussion

We have shown that there is a wide range in CBF with multiple mucosal samples in normal subjects and in patients with bronchiectasis. Although the average CBF of the fastest beating cilia was similar in the two groups studied, the bronchiectatic subjects had lower values and greater variation in CBF of the slowest beating cilia. This might reflect the effects of previous bacterial or viral infection in the epithelium. Although none of the patients had an acute exacerbation at the time of the study, previous infections may have resulted in continued inflammation. Such patients are also frequently colonised with bacteria which may produce ciliary toxins.[9]

The greater variation in CBF in subjects with bronchiectasis than in normal controls is consistent with the reduced tracheobronchial clearance shown by radioaerosols in such patients.[7 10]

The method employed here examines relatively mucus depleted epithelium in a non-physiological environment. It cannot, therefore, assess contributing factors to reduced clearance such as reduction in the total amount of respiratory tract ciliated epithelium or altered rheological properties of mucus.[1]

The variability in CBF seen in our patients may reflect the patchy nature of inflammation in the bronchial mucosa, although none of our subjects had a recent infection. Again recovery from inflammation may be patchy and affect recovery in CBF after any inhaled insult.

We suggest that complete description of ciliary function requires examination of the variation of CBF in several samples rather than a single estimate of "best" CBF.

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