Conflicting results in PCD: the value of respiratory epithelium evaluation

The aim of the present study was to determine whether cilia on disrupted ciliated epithelial edges beat with a normal pattern and frequency, similar to that of cilia on undisrupted edges on nasal brush biopsy samples.

Methods
Nasal brush biopsy samples were obtained from 42 children in whom primary ciliary dyskinesia (PCD) had been excluded. Epithelial strips were categorised into five groups, from intact undisrupted ciliated epithelial edge to single unattached ciliated cells. Ciliary beat frequency (CBF) was measured and beat pattern determined. Ciliated epithelium was observed and video sequences were recorded.

Results
CBFs of different types of ciliated epithelia were 13.4 Hz for normal ciliated edge, 11.4 Hz for ciliated edge with minor projections, 8.7 Hz for ciliated edge with major projections, 9.2 Hz for epithelial strip with an isolated cell and 6.5 Hz for single unattached cells. The dyskinesia scores were 0 (normal beat pattern) for normal ciliated edge, 1 (dyskinetic beat pattern in <25%) for ciliated cells with minor projections, 2 (dyskinetic beat pattern in <50% of the cilia) for ciliated cells with major projections, 2 for epithelial strip with an isolated ciliated cell and 4 (dyskinetic beat pattern in all cilia) for single unattached ciliated cells. The difference in CBF and dyskinesia scores between normal ciliated edge and all the other types of ciliated epithelium was statistically significant (p<0.001).

Conclusions
CBF and beat pattern assessed on cilia not part of an intact undisrupted strip of ciliated epithelium differ significantly compared with an intact uniform ciliated epithelial edge. Disrupted ciliated epithelium showed a slower CBF and increased dyskinesia. Ciliated epithelial strips with major cellular projections and isolated ciliated cells on epithelial strips or single ciliated cells are not recommended for ciliary function studies.

Editorial comment
This is the first study to suggest that only undisrupted strips of ciliated epithelium should be used for diagnostic testing for PCD. When collecting a nasal brush biopsy, it is necessary to suspend all pharmacological and antibiotic therapies, to avoid damage to the respiratory epithelium secondary to infection or inflammation [1]. If secondary damage is present, making diagnosis difficult, or if the patient is suspected of having an unusual PCD phenotype, ciliated cells are cultured. Secondary damage is virtually absent after ciliogenesis in a suspension culture [2]. Some groups have diagnosed PCD in patients who had clinical symptoms consistent with PCD and a slow CBF despite a normal ciliary ultrastructure [3]. It is still to be determined whether such patients have an inherited defect affecting function that cannot be seen on electron microscopy. ZARIWALA et al. [4] described such a situation in 38% of PCD patients, who carried mutations on dynein genes DNAH11. While such patients receive clinical treatment similar to that for PCD, they cannot be confidently diagnosed as having PCD. This is the first study to objectively quantify the ciliary function (in terms of CBF and beat pattern) of ciliated epithelium with varying degrees of disruption. CBF was determined directly: groups of beating cilia were identified by the first observer and the number of frames required to complete 10 beat cycles was recorded and converted to CBF by a simple calculation. To computerise CBF with the help of motion-detection algorithms would be a more accurate way to extract motion gradients from videos, solving two of the principal difficulties always met in this kind of study: time and human error [5, 6]. An unambiguous analysing system that could be easily repeated using the same method from different operators would validate the results obtained from the interpretation of data. The lack of universal guidelines for PCD emphasise the need for objective criteria regarding the quality of the ciliated epithelium studied in protocols used for ciliary function studies.

References

Message
Only undisrupted strips of ciliated epithelium may be used for the assessment of diagnostic testing for PCD.

Competing interests
None declared.