

Ciliary beat frequency and structure of recipient and donor epithelia following lung transplantation

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ABSTRACT: To investigate possible changes following lung transplantation, the structure and *in vitro* ciliary beat frequency (CBF) of airway epithelium from the cytology brushings of 9 heart-lung (HLT) and 5 single-lung (SLT) transplant recipients were examined. The CBF of brushings taken proximal and distal to the anastomosis was measured 2-10 months following transplant.

There was no difference between the measured mean CBF at the two sites or between the two groups; HLT CBF: distal 11.0 ± 0.5 Hz (standard error of mean), proximal 10.5 ± 0.4 Hz, SLT CBF: distal 11.7 ± 0.9 Hz, proximal 12.0 ± 0.6 Hz. Mean CBF of bronchial brushings (except distal brushings from SLT patients) was significantly lower than that from controls: 13.6 ± 0.3 Hz ($n=7$) ($p<0.05$). Transmission electron microscopy of epithelial brushings from 4 patients (3 HLT, 1 SLT) revealed epithelial abnormalities both proximal and distal to the anastomosis, particularly ciliary depletion, mitochondrial abnormalities and death of cells. No significant ciliary ultrastructural abnormalities were seen in any tissue.

We conclude that epithelial abnormalities were observed both proximal and distal to the anastomosis following lung transplantation. These may contribute to impairment of mucociliary clearance.

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Pulmonary infection is a common problem following lung transplantation. Immunosuppression is a likely cause of this, but mucociliary clearance, a first line defence mechanism of the bronchial tree, has recently been shown to be impaired in these patients [1, 2]. Furthermore, following lung transplantation, sensory denervation distal to the suture line could interfere with the cough reflex, which could render the patients more dependent on mucociliary clearance (MCC) to maintain bronchopulmonary toilet. Several factors could impair MCC after transplantation. These include denervation, loss of bronchial arterial supply, inadequate preservation during organ harvesting, immunological damage and repeated infections. All of these mechanisms could act *via* alteration of bronchial epithelial structure or function.

To investigate whether airway epithelium and ciliary function and structure are normal following lung transplantation, we examined bronchoscopic brushings on sites proximal and distal to the anastomosis in transplant recipients. Ciliary beat frequency (CBF) and ciliary activity of strips of epithelium so obtained were measured [3, 4], and the epithelium was examined

by transmission electron microscopy [5]. This procedure was conducted on both heart-lung transplant (HLT) and single-lung transplant (SLT) recipients, to identify any difference between the findings in these two groups

Methods

Subjects

Samples were obtained from 9 HLT and 5 SLT recipients between 2-10 months following transplantation. The primary pathology and time of examination since transplant of each subject is indicated in table 1. Samples were obtained during post-operative flexible bronchoscopy done to detect early signs of tissue rejection on 14 consecutive patients brought up for this procedure. The patients were well at the time of bronchoscopy with no clinical evidence of infection or rejection. Both transplant subjects and controls received premedication with papaveretum (10 mg) and atropine (0.6 mg) and sedation with diazepam (5 mg).

Table 1. — Mean ciliary beat frequency and ciliary activity scores of epithelial strips from sites proximal and distal to the anastomosis in transplant recipients

Patient	Diagnosis	Months since transplant	Proximal brushings			Distal brushings			Controls		
			No. of strips examined	CBF	CAS	No. of strips examined	CBF	CAS	No. of strips examined	CBF	CAS
Heart-lung											
1	Cystic fibrosis	7	20	10.8	30	20	10.3	100	20	13.3	95
2	Cystic fibrosis	4	20	8.8	20	20	11.7	45	20	12.4	100
3	Cystic fibrosis	2	20	8.8	40	20	13.6	100	20	14.7	80
4	Cystic fibrosis	6	20	10.9	30	20	10.4	70	20	14.3	80
5	Cystic fibrosis	4	20	11.1	90	20	11.0	100	20	13.8	80
6	Cystic fibrosis	6	20	11.2	70	20	10.1	50	20	12.6	75
7	PPH	3	20	10.7	100	20	11.3	100	20	14.4	100
8	Bronchiectasis	10	12	10.6	36	12	8.9	66			
9	Emphysema	4	20	12.0	95	20	12.1	100			
Totals				*10.5(0.4)	56.8(10.6)		11.0(0.5)	81.2(7.8)		13.6(0.3)	87.1(4.1)
Single-lung											
10	Bronchiectasis	3	20	12.6	100	20	12.0	100			
11	Bronchiectasis	2	4	13.3	50	6	11.5	80			
12	Sarcoidosis	8	12	9.9	66	12	9.9	75			
13	Fibrosing alveolitis	2	4	11.9	20	18	10.4	70			
14	Lymphangiomas	3	20	12.2	85	20	14.9	95			
Totals				*12.0(0.6)	64.2(14)		11.7(0.9)	84(5.8)			

CBF: ciliary beat frequency in Hz; CAS: ciliary activity score (percentage of strips displaying ciliary activity); PPH: primary pulmonary hypertension. Data expressed as mean±SEM. *: p<0.01, Kruskal Wallis analysis, Mann Whitney test, of patients data cf control.

Bronchoalveolar lavage was conducted in each patient and the lavage fluid examined by routine microbiology. The epithelium was brushed with a bronchoscopic cytology brush 3–5 cm proximal and then 3–5 cm distal to the anastomosis, and the brushings collected into plastic bijoux containers filled with tissue culture medium 199 (Flow Laboratories). The brushings were then immediately transferred to the laboratory for study.

Control samples were taken from the main bronchi of patients undergoing routine hospital investigation and were handled and examined in an identical fashion to the test samples.

Measurement of ciliary beat frequency

The samples were initially incubated within their bijoux containers at 37°C for 30 min. A portion of fluid containing epithelial strips was then aspirated into a Pasteur pipette and transferred to a sealed microscope coverslip-slide preparation, which was then incubated at 37°C for 10 min [3]. This preparation was then mounted on a warm stage at 37°C and inspected under a phase contrast microscope (Leitz) at ×320 magnification. The extent of ciliation of the epithelium was estimated using a method developed to study the effect of viruses on respiratory epithelium [5]. The proportion of the first 20 epithelial strips examined possessing actively beating cilia was calculated (ciliary activity

score). A single reading of CBF was taken from each of the first 10 ciliated strips identified using a photometric technique that has been previously described [3, 4]. Strips consisting of less than 10 cells were never used. Briefly, the position of the strips was adjusted so that the beating cilia interrupted a 1×5 μm area through which light was transmitted to a photometer. An automated ciliary beat frequency processor unit transduced the signal into a CBF reading [4]. When less than 10 of the first 20 strips examined were ciliated, further strips were identified and measured until a total of 10 were counted. If ten ciliated strips could not be found, a mean value was calculated from the total number available. Table 1 shows the number of strips (up to a maximum of 20) that could be identified in each patient sample.

Transmission electron microscopy

The remaining contents of the plastic bijoux tubes were transferred to cacodylate-buffered 2.5% glutaraldehyde and post-fixed in 1% osmium tetroxide. After rinsing, the brushings were embedded in a droplet of 2% agar and gently centrifuged. The agar was allowed to set and the whole processed for transmission electron microscopy by the method described by WILSON *et al.* [5]. Four of the 14 paired specimens had sufficient tissue for adequate examination by electron microscopy. An average of 70 cells in each tissue were

examined, and the percentage of cells that were dead (identified by hypodensity or amorphous cellular contents), and also the percentage of cells that were ciliated was calculated. In addition, the percentage of cells displaying the following features was noted: projection of cells from the surface, loss of cilia from ciliated cells, cytoplasmic blebbing and mitochondrial abnormalities. This allowed comparison of the structural integrity of epithelia from sites distal and proximal to the anastomosis. In addition, changes in ciliary ultrastructure were scored as percentages of cilia displaying abnormalities of microtubules (disarrangement, addition or loss of microtubules, abnormalities in the central pair, compound cilia) and loss of dynein arms (loss of either inner, outer or both dynein arms). The first 600 cilia viewed in each section were examined thus.

Statistics

For all data, means are expressed \pm SEM. Data for CBF and ciliary activity score (CAS) of test and control epithelia were subjected to Kruskal Wallis analysis. Where the null hypothesis was rejected, significance of differences was measured with the Mann Whitney test. Correlation of time since transplant and epithelial abnormalities was conducted by Pearson correlation.

Results

Bronchoscopic examination of the site of anastomosis in each case revealed no evidence of ridging or kinking. In addition, no abnormalities of the airway mucosa or increase in mucus secretion were observed. Bronchoalveolar lavage fluid from all patients was sterile by routine microbiological examination.

The results of the CBF measurements of both sets of patients are summarized in table 1. There was no difference between CBF at the two sites or between the two groups; HLT CBF: distal 11.0 ± 0.5 Hz, proximal 10.5 ± 0.4 Hz; SLT CBF: distal 11.7 ± 0.9 Hz, proximal 12.0 ± 0.6 Hz. CBF of epithelia from proximal brushings of both sets of patients and from the distal brushings of HLT patients, were significantly lower than controls (CBF 13.6 ± 0.3 Hz) (table 1). In the patients who received HLT, 6 out of 9, and 4 out of 9 of the brushings taken proximal and distal to the anastomosis respectively displayed a CBF below the lower limit of the normal range within our laboratory (11 Hz); this compared to 1 out of 5, and 2 out of 5 of the brushings taken proximal and distal to the anastomosis respectively of the SLT patients. None of the strips examined displayed ciliary dyskinesia (incoordinate beating of adjacent cilia) or ciliostasis. In the case of two patients with cystic fibrosis, patients 2 and 3, a very low CBF was found proximally (CBF 8.8 Hz in both patients).

In both groups the ciliary activity score of distal strips of epithelium exceeded that of proximal strips but this was not a statistically significant difference. Bronchoscopic brushings of normal individuals examined at our laboratory have a CAS of $>75\%$, and this was the case for all the control samples examined. In the patients who received HLT, 6 out of 9, and 4 out of 9 of the brushings taken proximal and distal to the anastomosis respectively had a CAS below this figure. This compares to 3 out of 5 and 1 out of 5 of the proximal and distal brushings of the SLT patients. There was a strong negative correlation ($r = -0.9$) between time since transplant and CBF of distal brushings of the HLT group, but this was not the case with the other variables of distal brushings.

The only samples with sufficient tissue for examination of epithelial morphology by electron microscopy were obtained from patients 3, 7, 8 and 14. The results or morphometry of the epithelial tissue are displayed in table 2. A significantly higher frequency of ciliated cells displaying ciliary depletion was observed in proximal compared with control brushings. A higher frequency of dead cells and mitochondrial abnormalities (including swelling and loss of internal structure) was also present in patients epithelia, compared to controls. The percentage of epithelial cells that were ciliated by electron microscopy was approximately equal for proximal and distal brushing and both were significantly lower than controls. Epithelial abnormalities were detected in all proximal and distal brushings (table 2; fig. 1). The number of ultrastructural ciliary abnormalities seen in all sections was well within the normal range [6] with no difference observed between distal and proximal epithelia.

Table 2. - Epithelial ultrastructure of bronchial brushings

Feature (% of cells)	Transplant recipients (3 heart-lung; 1 single lung recipient)		
	Proximal to anastomosis	Distal to anastomosis	Main bronchus
Dead cells	11 \pm 4*	11 \pm 3*	1.3 \pm 0.9
Ciliated cells	72 \pm 5*	72 \pm 3*	85 \pm 1.5
Projecting cells	41 \pm 8	31 \pm 10	16.9 \pm 2.0
Ciliary depletion	33 \pm 6*	19 \pm 2	13.1 \pm 1.6
Blebbing	24 \pm 10	9 \pm 4	4.1 \pm 0.9
Mitochondrial abnormalities	34 \pm 10*	26 \pm 6*	10.1 \pm 2.4

Data shows mean \pm SEM of percentage of cells in sections from 7 control patients and 4 sections each of proximal and distal bronchial brushings from transplant recipients examined by transmission electron microscopy. Numerals refer to percentage of cells that were ciliated, dead, or displayed the following feature of damage; a) projection of cells from the surface, b) loss of cilia (ciliary depletion), c) cytoplasmic blebbing, d) mitochondrial abnormalities. *: $p < 0.05$ cf control. Kruskal Wallis analysis, Mann Whitney test.

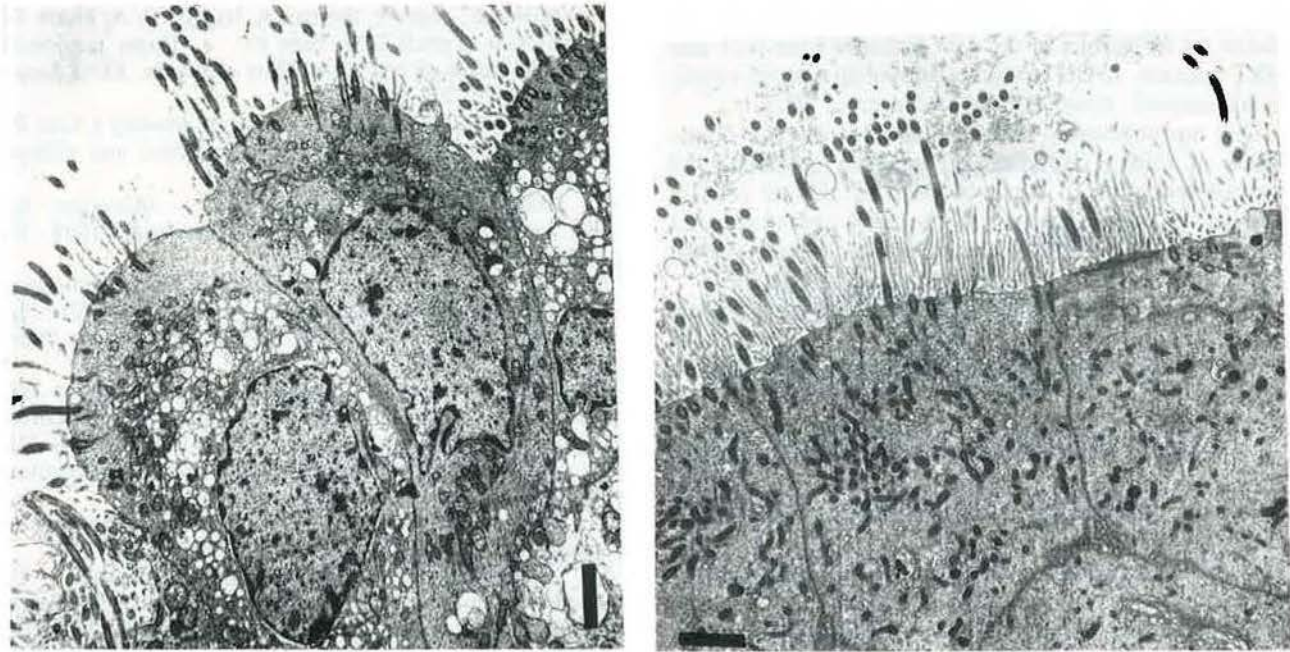


Fig. 1. - Ultrastructure of epithelium of brushings taken (a) proximal and (b) distal to the anastomosis of a patient who underwent heart-lung transplantation for bronchiectasis. Both epithelia display ciliary depletion, projection of cells from the surface, cytoplasmic blebbing and mitochondrial abnormalities. Bar: 2 μ m.

Discussion

Pulmonary infection is a significant cause of morbidity and mortality in HLT and SLT patients largely as a result of immunosuppression [7]. Impairment of mucociliary clearance [1] may be a compounding factor by permitting bacterial colonization and subsequent pulmonary infection [8].

Reduced MCC may be due to airway disease [9], alteration of the rheological properties of tracheobronchial secretions [10, 11], or to abnormal ciliary beating as occurs in primary ciliary dyskinesia [12]. Studies in our laboratory have suggested a relationship between ciliary beat frequency measured *in vitro* and impaired MCC *in vivo* [13]. Secondary ciliary dysfunction due to chronic bronchial infection, asthma, or following viral infection may reduce MCC [14].

We found that ciliary beat frequencies of epithelia of bronchial brushings were lower than expected, but that no difference could be detected between CBF of proximal and distal brushings. The functional and morphological integrity of the epithelia of transplant recipients brushings was inferior to that of control brushings, as demonstrated by the relatively reduced ciliary activity score and increased prevalence of ultrastructural abnormalities particularly in proximal epithelia. The epithelial strips obtained were overall of poorer quality than would be expected from bronchial brushings of normal individuals. The abnormalities found in tissue taken from proximal brushings is not surprising considering the severity of disease that warrants lung

transplantation, but the overall reduction in CBF and the epithelial abnormalities detected in both proximal and distal brushings suggest that the patients may have more widespread ongoing respiratory tract disease.

One possible cause of this would be the presence of bronchial infection, which has been demonstrated to reduce CBF of airway epithelia [15]. A number of patients had chronic bronchial sepsis prior to transplantation. This might explain the low CBF recordings of the proximal brushings from the two cystic fibrosis patients, and if infection spread distal to the anastomosis could affect transplanted tissue. Against this is the fact that bronchoalveolar lavage fluid from all patients at the time of bronchoscopy was sterile. Another possible cause is the occurrence of rejection episodes. Patients were all well at the time of bronchoscopy, but in all cases at least 2 months had elapsed since transplant. Rejection episodes increase as a function of time since transplant; there was a strong negative correlation between time since transplant and CBF of distal brushings in the HLT group, which supports a role for rejection episodes in the epithelial abnormalities observed. Following viral infection, MCC is reduced for prolonged periods [16], an effect which is probably due to epithelial damage [14]; bronchial inflammation due to rejection episodes may result in a similar period of reduced MCC. However, the lack of a difference between proximal and distal samples is against rejection episodes being the only cause of epithelial damage. Another possible cause of epithelial disruption is restriction of

the vascular supply. Against this is the fact that we found no difference in the data gathered from SLT and HLT patients. In SLT patients, bronchial arterial supply is interrupted, whereas it is preserved in HLT.

One morphological feature of interest was the occurrence of ciliary depletion in proximal epithelia, and overall reduction in the proportion of ciliated cells in the recipients airways. This may represent the ciliogenesis of epithelial repair [17] and would explain the impaired ciliary activity compared to controls.

Cilia beat in a co-ordinated metachronal wave pattern in the cephalad direction. Ridging, or kinking of the airway in the region of the anastomosis might lead to reduced MCC. However, this was not observed at bronchoscopic examination. It is possible that loss of local neuronal influences *in vivo* may result in reduced MCC. The autonomic nervous system or its mediators have been shown to affect mucus production [18], mucus rheology [19], and epithelial ion transport [20]. However mucociliary clearance is not impaired in patients with autonomic failure [21] and β_2 adrenergic stimulation does not increase overall bronchial clearance in humans [22]. GREENSTONE [23] found patients following vidian neurectomy, an operation that results in nasal denervation, to have normal nasal mucociliary clearance. The evidence for a direct autonomic effect on ciliary beating is controversial though beta adrenergic stimulants have been observed to increase CBF of some mammalian epithelia, including human [24-26].

Transection of nerves during transplantation could explain a reduction in mucociliary clearance in patients following lung transplantation. However, this study suggests that significant epithelial abnormalities are present both in recipient airways and in transplanted airways with reduction in ciliary beat frequency, and abnormalities of epithelial structure. These changes, whatever their cause, could reduce mucociliary clearance *in vivo*.

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References

- Shankar S, Fulsham L, Read RC, Theodoropoulos S, Cole PJ, Madden B, Yacoub M. - Mucociliary function after lung transplantation. *Transplantation Proceedings*, 1991, (in press).
- Dolovich M, Rossman C, Chambers C, Grossman RF, Newhouse M, Maurer JR. - Mucociliary function in patients following single-lung or heart-lung transplantation. *Am Rev Respir Dis*, 1987, 135, A363.
- Rutland J, Cole PJ. - Non invasive sampling of nasal cilia for measurement of beat frequency and study of ultrastructure. *Lancet*, 1980, 2, 564-565.
- Greenstone MA, Logan-Sinclair R, Cole PJ. - An automated method of recording ciliary beat frequency. *IRCS Med Sci*, 1984, 12, 715-716.
- Wilson R, Alton E, Rutman A, Higgins P, Al Nakib W, Geddes DM, Tyrrell DAJ, Cole PJ. - Upper respiratory tract viral infection and mucociliary clearance. *Eur J Respir Dis*, 1987, 70, 272-279.
- Greenstone M, Rutman A, Dewar A, Mackay I, Cole PJ. - Primary ciliary dyskinesia: cytological and clinical features. *Q J Med*, 1988, 253, 405-430.
- Rubin RH, Tolkoff-Rubin NE. - Infection: the new problems. *Transplantation Proceedings*, 1989, 21, 1440-1445.
- Johanson WG, Pierce AK, Sandford JP. - Nosocomial respiratory infections with gram negative bacilli. The significance of colonisation of the respiratory tract. *Ann Intern Med*, 1972, 701-706.
- Currie DC, Pavia D, Agnew J, Lopez-Vidriero MT, Diamond PD, Cole PJ, Clarke SW. - Impaired tracheobronchial clearance in bronchiectasis. *Thorax*, 1987, 42, 126-130.
- Litt M. - Mucus rheology: relevance to mucociliary clearance. *Arch Int Med*, 1970, 126, 417-423.
- Dulfano MJ, Adler KB. - Physical properties of sputum. *Am Rev Respir Dis*, 1975, 122, 341-347.
- Mossberg B, Afzelius BA, Eliasson R, Camner P. - On the pathogenesis of obstructive lung disease - a study on the immotile cilia syndrome. *Scand J Respir Dis*, 1978, 59, 55-65.
- Munro NC, Barker A, Rutman A, Taylor G, Watson D, McDonald-Gibson WJ, Towart R, Taylor WA, Cole PJ. - Effect of pyocyanin and 1-hydroxyphenazine on *in vivo* tracheal mucus velocity. *J Appl Physiol*, 1989, 67, 316-323.
- Wilson R. - Secondary ciliary dysfunction. *Clin Sci*, 1988, 75, 115-120.
- Wilson R, Sykes D, Currie DC, Cole PJ. - Beat frequency of cilia from sites of pulmonary infections. *Thorax*, 1986, 41, 453-458.
- Camner P, Jarstrand C, Philipson K. - Tracheobronchial clearance in patients with influenza. *Am Rev Respir Dis*, 1973, 108, 131-136.
- Gaillard DA, Lallement AV, Petit AF, Puchelle E. - *In vivo* ciliogenesis in human foetal tracheal epithelium. *Am J Anat*, 1989, 185, 415-428.
- Phipps RJ, Williams IP, Richardson PS, Pell J, Pack RJ, Wright N. - Sympathomimetic drugs stimulate the output of secretory glycoproteins from human bronchi *in vitro*. *Clin Sci*, 1982, 63, 23-28.
- Leikauf GD, Veki IF, Nadel JA. - Autonomic regulation of viscoelasticity of cat tracheal gland secretions. *J Appl Physiol*, 1984, 56, 426-430.
- Boucher RC, Gatzky JT. - Regional effects of autonomic agents on ion transport across excised canine airways. *J Appl Physiol*, 1982, 52, 893-901.
- Jenkins P, Pavia D, Bateman JRM, Clarke SW, Citron KM, Bannister R. - Mucociliary clearance in patients with chronic autonomic failure. *Thorax*, 1980, 35, 690-693.
- Yeates DB, Spektor DM, Pitt BR. - Effect of orally administered orciprenaline on tracheobronchial mucociliary clearance. *Eur J Respir Dis*, 1986, 69, 100-108.
- Greenstone M. *In: MD Thesis*, University of Bristol, 1985.
- Verdugo P, Johnson NT, Tam PY. - Beta-adrenergic stimulation of respiratory ciliary activity. *J Appl Physiol*, 1980, 48, 868-871.
- Wong LB, Miller IF, Yeates DB. - Regulation of ciliary beat frequency by autonomic mechanism; *in vitro*. *J Appl Physiol*, 1988, 65, 1895-1901.
- Wong LB, Miller IF, Yeates DB. - Stimulation of ciliary beat frequency by autonomic agonists: *in vivo*. *J Appl Physiol*, 1988, 65, 971-981.

Fréquence des battements ciliaires et structure des épithélia du donneur et du receveur après transplantation pulmonaire. R.C. Read, S. Shanker, A. Rutman, C. Feldman, M. Yacoub, P.J. Cole, R. Wilson.

RÉSUMÉ: Nous avons examiné, pour investiguer les modifications possibles après transplantation pulmonaire, les structures et la fréquence des battements ciliaires *in vitro* (CBF) dans l'épithélium des voies aériennes obtenu par brosse cytotologique dans 9 cas de transplantation coeur-poumon (HLT) et 5 cas de transplantation pulmonaire (SLT). Le CBF des brosses a été prélevé de manière proximale et distale par rapport à l'anastomose, de 2 à 10 mois après la transplantation.

L'on n'a pas noté différence entre le CBF moyen mesuré au niveau des deux sites ou entre les deux groupes; HLT CBF: distal 11.0 ± 0.5 Hz (erreur standard de la moyenne), proximal

10.5 ± 0.4 Hz; SLT CBF: distal 11.7 ± 0.9 Hz, proximal 12.0 ± 0.6 Hz. Le CBF moyen des brosses bronchiques (à l'exception des brosses distales des patients SLT), était significativement plus bas que celui des contrôles: 13.6 ± 0.3 Hz ($n=7$) ($p<0.05$). La microscopie par transmission d'électrons des brosses épithéliales de 4 patients (3 HLT et 1 SLT) a révélé des anomalies épithéliales à la fois en amont et en aval de l'anastomose, et en particulier une déplétion ciliaire, des anomalies mitochondriales et des morts cellulaires. Aucune anomalie ultrastructural ciliaire n'a été observée dans aucun tissu.

Nous concluons que les anomalies épithéliales s'observent aussi bien en amont qu'en aval de l'anastomose après transplantation pulmonaire. Elles pourraient contribuer au trouble de la clearance muco-ciliaire.

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