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Scanning electron microscopy findings of human respiratory cilia in chronic sinusitis and in recurrent respiratory infections

ELINA TOSKALA, M.D., PH.D.*, JUHANI NUUTINEN, M.D., PH.D.*, MARKUS RAUTIAINEN, M.D., PH.D.†

Abstract

Acute and chronic infections cause morphological changes in the respiratory mucosa. The ultrastructure of human respiratory mucosa was studied by scanning electron microscopy from the maxillary sinuses of 28 patients, with chronic sinusitis, from middle turbinates of 60 patients, with recurrent respiratory infections, and from healthy sphenoidal sinuses of 31 patients. A loss of ciliated cells and an increasing number of nonciliated columnar cells with microvilli were seen in 62 per cent of the maxillary sinus mucosa. Ciliary disorientation was seen in 81 per cent of the chronically infected sinus mucosa and eight per cent in the healthy sphenoidal sinuses. Also metaplasia and extrusion of epithelial cells were prominent in chronic infections. Compound cilia were seen in 52 per cent of the samples from patients with chronic sinusitis and in 31 per cent of the healthy sphenoidal sinuses. Short cilia were often seen in infected mucosa indicating ciliogenesis.

Key words: Cilia; Epithelium, respiratory; Microscopy, electron, scanning; Sinusitis

Introduction

Many ultrastructural changes in respiratory cilia have been reported from patients with acute and chronic respiratory infections and in bronchial asthma (Jahrsdorfer *et al.*, 1979; Herzon, 1981; Fontelliet and Terrier, 1987). The immotile cilia syndrome with the absence of dynein arms containing adenosine triphosphatase in the cilia, is the best known of all these conditions (Afzelius, 1981).

However, the relationship between morphological changes to cilia and ciliated cells and disorder of ciliary function has only recently been studied (Nuutinen *et al.*, 1993). Long-term respiratory diseases, nasal infections, sinusitis and otitis media impair mucociliary function (Nuutinen *et al.*, 1983; Sakakura *et al.*, 1985; Wilson *et al.*, 1986; Rautiainen *et al.*, 1991). Reimer *et al.* (1978) studied specimens of maxillary sinus mucosa by scanning electron microscopy (SEM) and found large areas of unciliated epithelium in 12 patients. It has been suggested that ultrastructural changes in cilia impair ciliary beating in chronic sinusitis (Ohashi and Nakai, 1983). Morphological changes in respiratory cells and cilia may be the cause of many of the symptoms in atrophic rhinitis (Gray *et al.*, 1980).

Previously most papers were based on transmission electron microscopy (TEM) especially when ciliary cross-sections and dynein arms were evaluated. Subsequently (SEM) has proved to be a very useful tool for evaluation of cilia and infected nasal mucosa (Johnson *et al.*, 1983; Hinni *et al.*, 1992; Read *et al.*, 1992). The present study was carried out in order to evaluate the ultrastructure of respiratory cilia, by SEM, in patients operated on because of chronic sinusitis or other respiratory infections. In addition, the ultrastructural properties of healthy human respiratory mucosa were studied.

Materials and methods

One hundred and nineteen respiratory mucosal specimens were taken from 111 patients suffering from various respiratory infections and treated in the Department of Otorhinolaryngology, Kuopio University Hospital, Finland.

Respiratory mucosa samples were taken from 28 maxillary sinuses in 20 patients (15 females and five males, aged nine to 73 years, with a mean age of 37 years) operated on because of chronic sinusitis, not cured by repeated antral irrigation and antibiotics. Sinus X-ray examination showed changes indicating chronic sinusitis (opacity, fluid level and mucosal thickening). The Caldwell-Luc operation for removal of macroscopically diseased mucosa was performed mostly under local anaesthesia. In children younger than 14 years, endonasal antrostomy was performed under general anaesthesia.

From the Department of Otorhinolaryngology*, Kuopio University Hospital, SF-70211 Kuopio, and the Departments of Clinical Medicine†, and Otorhinolaryngology, Tampere University Hospital, SF-33101 Tampere, Finland.
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Histopathological examination of the sinus mucosa showed chronic sinusitis.

Specimens from the middle turbinate of 60 patients (36 females and 24 males, aged one to 68 years, with a mean age of 21 years) suffering from recurrent respiratory infections were taken under local anaesthesia (lidocaine hydrochloride with epinephrine in cottonoids) using a microcurette. Nineteen of them had recurrent secretory otitis, 10 recurrent sinusitis, 12 bronchitis and 19 recurrent respiratory infections. There were no cases of Kartagener's syndrome, nor dextrocardia or mucovicidosis.

Samples of healthy respiratory mucosa were taken from 31 'normal' sphenoidal sinuses during transphenoidal pituitary surgery because of active acromegalia, prolactinoma, Cushing's disease, panhypopituitarism or hormonally inactive adenoma. These patients (19 females and 13 males, aged 16–72 years, with a mean age of 42 years) did not have any symptoms of acute, or history of chronic, respiratory infections. During the operation, the mucosa was seen to be normal under the operating microscope.

Mucosal specimens were immersed in one per cent glutaraldehyde and four per cent formalin cocktail fixative, and then prepared according to the osmium thiocarbohydrazide procedure (OTOTO-procedure) (Malick *et al.*, 1976). The samples were dehydrated in a graded ethanol series and then dried in a critical point dryer (Balzer). The specimens were coated with gold in a splutter-coater (Polaron II E5100) with 2.5 kV acceleration voltage in an argon atmosphere with a current of 20 mA for one minute.

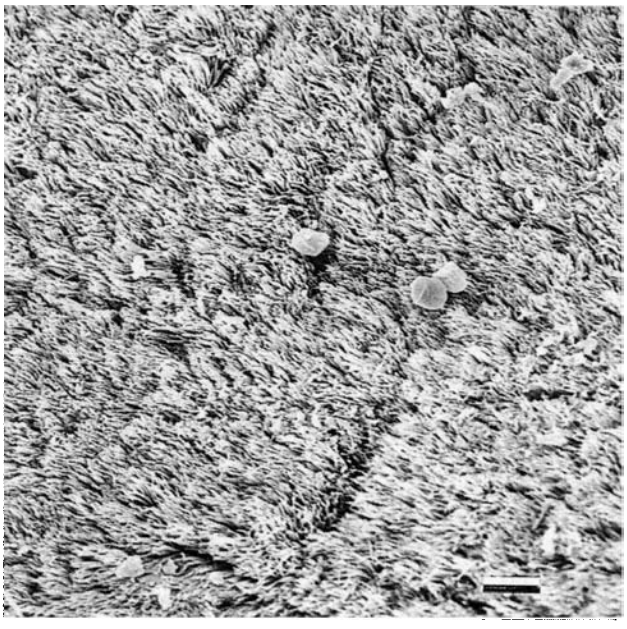
Samples were examined in a Jeol JSM-35 scanning electron microscope. Randomly selected areas were analysed and photographed with magnifications of ×600 and ×6000, avoiding the margins of the specimens where there could be possible mechanical artefacts caused by specimen handling.

Special emphasis was placed on the ciliated cells, ciliary orientation and length of the cilia. Cilia shorter than 5 μm were recorded as short cilia (Afzelius *et al.*, 1985). Any abnormalities such as compound cilia and flattened cilia, ciliary knobs or changes at the ciliary tip were recorded. The presence of microvilli, nonciliated cuboidal epithelial cells and goblet cells were also noted. Fischer's exact test was used for statistical analysis.

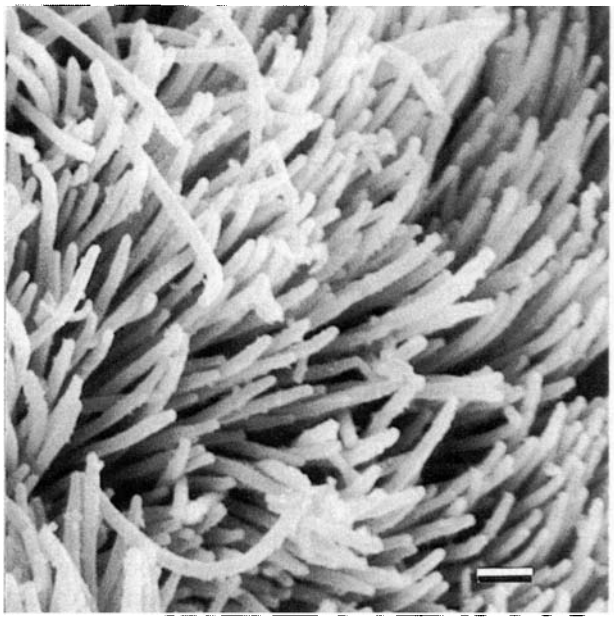
Results

One (three per cent) of the sphenoidal, seven (17 per cent) of the middle turbinate and 10 (25 per cent) of the maxillary sinus specimens could not be studied because of their coverage by an extracellular layer of mucus. In addition to this, four specimens in the hypophysectomy group suffered some degree of mechnical damage. Thus, the numbers of specimens in the final analysis were 21 from the maxillary sinus, 50 from the middle turbinate and 26 from healthy sphenoidal sinuses.

All sphenoidal sinus specimens (Figure 1a and b) and 20 (95 per cent) maxillary sinus specimens showed at least some ciliated cells. Nonciliated columnar cells with microvilli were seen in 35 (70 per cent) middle turbinate specimens compared to nine (35 per cent) in the healthy specimens ($p<0.01$). Also 13 (62 per cent) of the specimens from patients with sinusitis had nonciliated cells and microvilli, but the difference was not statistically significant. Furthermore, no short cilia were seen in the sphenoidal sinuses, but were seen in seven (33 per cent) out of 21 patients with sinusitis and in 19 (38 per cent) middle turbinate mucosa specimens from



(a)



(b)

FIG. 1

Normal ciliated respiratory mucosa in healthy sphenoidal sinus: (a) bar = 10 μm; (b) bar = 1 μm.

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TABLE I

SCANNING ELECTRON MICROSCOPY FINDINGS SEEN IN HEALTHY SPHENOIDAL SINUSES, IN MAXILLARY SINUSES OF THE PATIENTS WITH SINUSITIS, AND IN MIDDLE TURBINATES OF THE PATIENTS WITH RECURRENT RESPIRATORY INFECTIONS. COMPARISONS ARE MADE BETWEEN MAXILLARY SINUS AND MIDDLE TURBINATE SPECIMENS *versus* SPHENOIDAL SINUS SAMPLES

	Sphenoidal sinus (healthy subjects)	Maxillary sinus (sinusitis)	Middle turbinate (recurrent respiratory infections)
No. of specimens (n)	26	21	50
Findings	n (%)	n (%)	n (%)
No cilia	0	1 (5)	5 (10)
Nonciliated columnar cells	9 (35)	13 (62)	36 (70)**
Metaplasia	1 (4)	7 (33)*	4 (8)
Goblet cells	18 (69)	20 (95)*	30 (60)
Short cilia	0	7 (33)***	19 (38)***
Disorientation	2 (8)	17 (81)***	20 (40)**
Compound cilia	8 (31)	11 (52)	4 (8)*
Cilia bunch	8 (31)	5 (24)	10 (20)
Flattened cilia	8 (31)	9 (43)	15 (30)
Ciliary knobs: at shaft	19 (73)	11 (52)	15 (30)***
Ciliary knobs: at tip	12 (46)	10 (48)	5 (10)***

p values *versus* sphenoidal sinus samples: *$p<0.05$; **$p<0.01$; ***$p< 0.001$.

patients with recurrent respiratory infections (Table I; Figure 2a and b).

In all, 17 (81 per cent) of the specimens from patients with chronic sinusitis and 20 (40 per cent) from patients with recurrent respiratory infections showed ciliary disorientation. In the sphenoidal sinuses, only two (eight per cent) of the specimens showed some ciliary disorientation. A further seven (33 per cent) specimens from chronically infected mucosa had metaplasia and extrusion of epithelial cells, compared to one (four per cent) from the sphenoidal sinus group (Table I; Figure 3a and b).

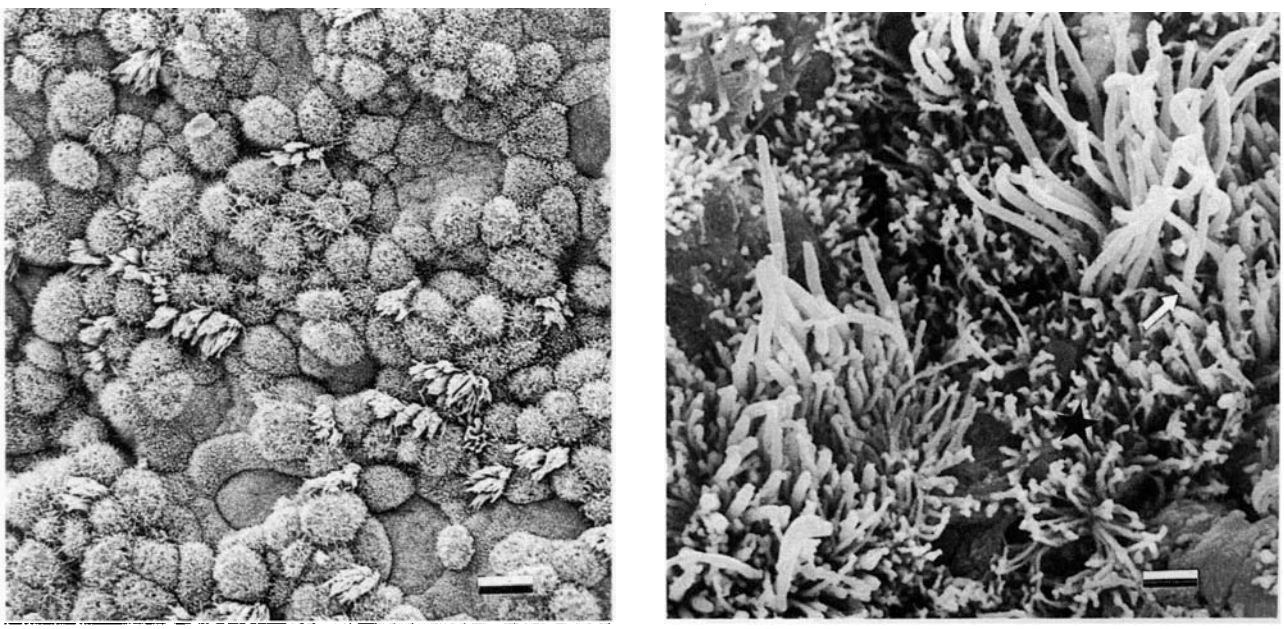
The most common change in ciliary ultrastructure was the presence of compound cilia. They were seen in 11 (52 per cent) of the maxillary specimens, in eight (31 per cent) sphenoidal and in four (eight per cent) middle turbinate samples (Figure 4).

Several ciliary knobs at the shafts and also at the tips of the cilia were seen in all groups (Figure 5).

It was not possible to observe any significant differences in the extent of abnormalities in the middle turbinate specimens by comparing the various respiratory infection specimens with each other.

Discussion

Acute and chronic infections can cause many defects in the ultrastructure of respiratory mucosa. Ohashi and Nakai (1983) reported swelling of the



(a) (b)

FIG. 2

Infected respiratory mucosa: (a) showing loss of ciliated cells (bar = 10 μm); (b) showing short cilia (arrowed) and microvilli (asterik) (bar = 1 μm).

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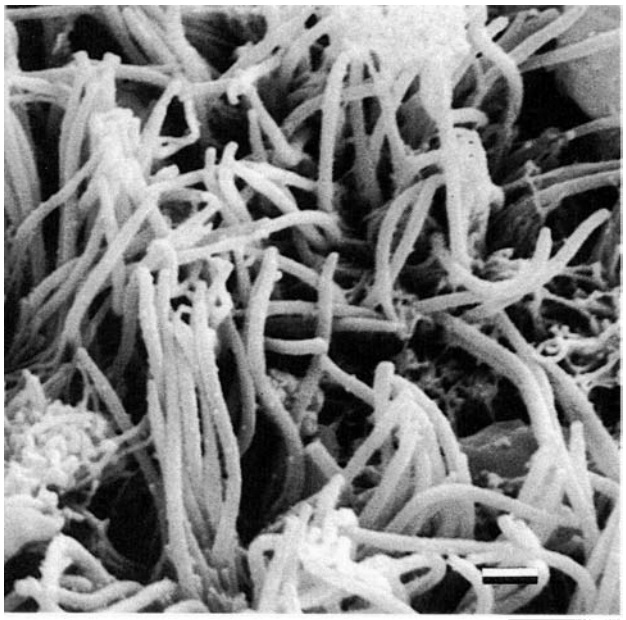
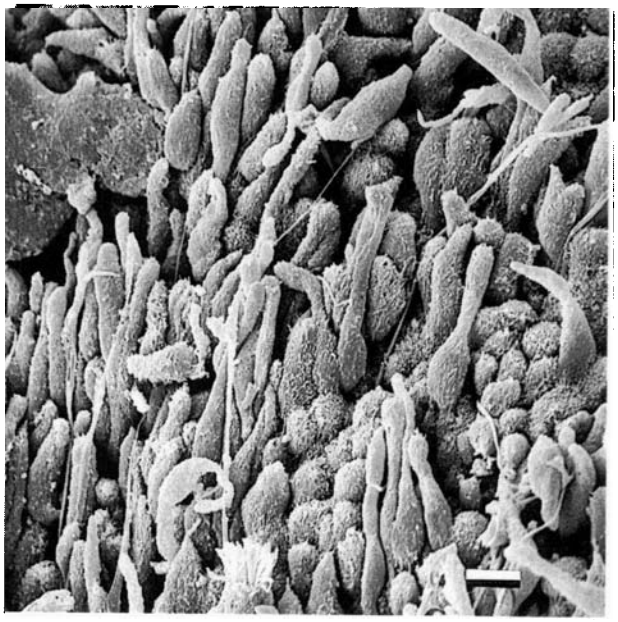


(a)



(b)

FIG. 3

Chronic sinusitis: (a) showing ciliary disorientation and flattened cilia (bar = 1 μm); (b) showing extrusion of epithelial cells and metaplasia (bar = 10 μm).

ciliary membrane, formation of compound cilia, dropping of epithelial cells and metaplasia of squamous epithelium of the maxillary mucosa in patients suffering from chronic infections. Boysen (1982) suggested, in a study of middle turbinate mucosa, that squamous metaplasia of the nasal epithelium can be considered to be a normal finding.

In this study it was observed, that the number of nonciliated cells increased significantly in infected mucosa. In the patient groups many short cilia and microvilli were also seen, which obviously were the result of regeneration of epithelial and ciliated cells. Such signs of ciliogenesis in specimens from these patients suggest that infected mucosa still has the capacity to recover its original activity. Human respiratory cells cultured by monolayer cell culture initially lose their cuboidal shape, then, and more slowly, ciliary function and finally all cilia and cells became squamous cells (Rautiainen *et al.*, 1993). When these squamous cells are cultured in suspension, they regain their cuboidal shape (Jorissen *et al.*, 1989). In rat trachea inoculated with *Haemophilus influenzae*, ciliogenesis was seen three days after inoculation (Johnson *et al.*, 1983). Similar ciliary

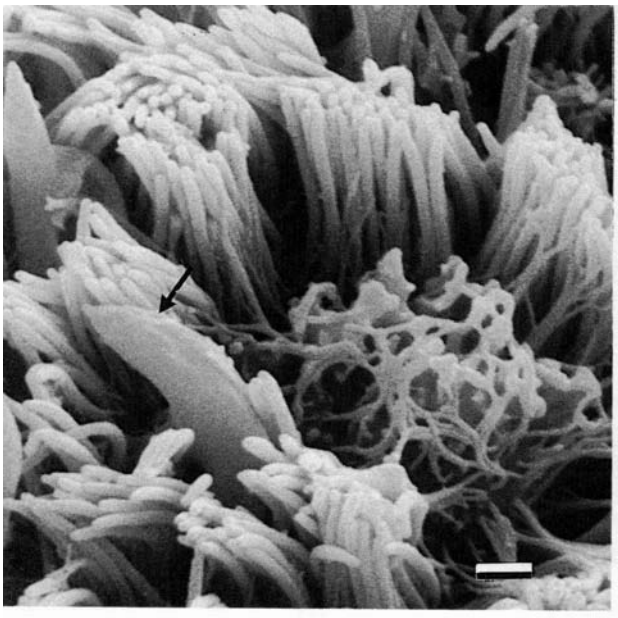


FIG. 4

Compound cilia (arrowed) seen in healthy control patients (bar = 1μm).

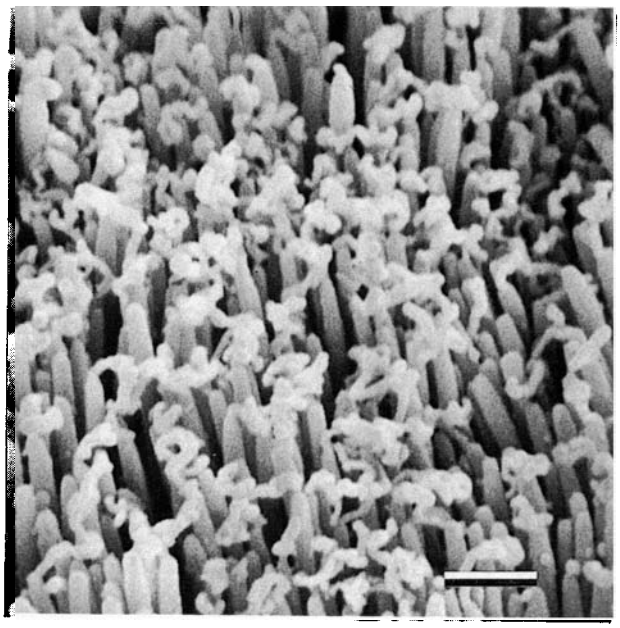


FIG. 5

Several ciliary knobs seen at the tips of the cilia (bar = 1 μm).

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regeneration was seen after one week and up to three weeks from an acute viral respiratory infection in humans (Rautiainen *et al.*, 1992).

Ciliary disorientation was seen much more frequently in specimens from chronic sinusitis than from the normal sphenoidal sinuses and the difference was highly significant. Increased ciliary disorientation has been reported in studies made by TEM of patients with asthma, upper and lower respiratory tract infections and immotile cilia syndrome (Rautiainen *et al.*, 1990). This may partially explain the impaired mucociliary function in chronic sinusitis (Ohashi and Nakai, 1983).

Metaplasia and extrusion of epithelial cells was seen more often in the chronic sinusitis specimens. These findings are considered to be caused by infections (Ohashi and Nakai, 1983), bacterial or viral attack and drying of the nasal mucosa in atrophic rhinitis (Gray *et al.*, 1980).

Compound cilia are thought to be one of the morphological changes in the ultrastructure of respiratory cilia caused by infections (Lungarella *et al.*, 1983). Compound cilia have also been reported in the bronchial epithelium of heavy smokers with bronchial cancer (Veerman *et al.*, 1980). In the present study, the amount of compound cilia in chronic sinusitis was slightly, but not significantly, higher than in the healthy sphenoidal sinuses. On the other hand, middle turbinate specimens from patients with recurrent respiratory infections showed significantly less compound cilia than healthy sphenoidal sinuses. Therefore, the pathological significance of compound cilia is not clear and they have even been reported as a common finding in healthy respiratory mucosa (Fontelliet and Terrier, 1987). In this study, the smoking habits of the patients and control patients were not systematically studied.

Specimens from mucosa with chronic sinusitis had 26 per cent more goblet cells than samples from normal sphenoidal sinuses in this study. This suggests that there is an increase in the number of goblet cells occurring during infections. Ohashi and Nakai (1983) have proposed that, ciliated cells can change into goblet cells in chronic sinusitis. It must be remembered, however, that the number of goblet cells is reliably assessed only by light microscopy or transmission electron microscopy. Furthermore, the number of goblet cells may vary remarkably in different sites of the upper respiratory tract system (Tos, 1982). For practical and ethical reasons it is difficult to collect adequate reference material from healthy respiratory maxillary sinuses or middle turbinate mucosa from symptom-free healthy humans. Theoretically, it is possible that the hormonal activity of these 'normal' patients might have an influence on the sinus mucosa. There was, however no difference between acromegalia or hyperprolactinemia patients and patients with hormonally inactive tumour.

Ciliary knobs, which are local extrusions of the ciliary membrane have been reported to be fixation artefacts in guinea pigs (Dahlen, 1981). In a TEM study of the ciliary ultrastructure in a patient with bronchiectasis this kind of change at the shaft and tip of cilia has been reported (Rautiainen *et al.*, 1984). In this study these knobs were seen in specimens from all patients and therefore they were not considered to show any correlation with respiratory infections.

The high number of specimens covered by extracellular material emphasizes the need to wash specimens before fixation, a change we have now introduced to the current method. In addition, due to the many mechanical artefacts, a gentle biopsy technique is also very important.

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Address for correspondence:
Dr Elina Toskala,
Department of Otorhinolaryngology,
University of Kuopio,
PO Box 1627,
70211 Kuopio,
Finland.

Fax: +358 71 172509