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Effects of heat and moisture exchangers on tracheal mucociliary clearance in laryngectomized patients: a multi-center case–control study

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Abstract After total laryngectomy, inspired air is no longer optimally conditioned by the upper airways. Impaired mucociliary clearance and histological changes of respiratory epithelium, such as loss of ciliated cells, have been described in laryngectomized patients. Heat and moisture exchangers (HMEs) are passive humidifiers that re-condition the inspired air. Aim of this study was to assess the effect of HMEs on tracheal epithelium and tracheal mucus transport velocity (TMV). Tracheal brush biopsies were collected in three groups of TLE patients: 21 long-term HME users, 10 non-HME users, and 16 non-

HME users before and after 4–9 months HME use. Tracheal epithelium biopsies were assessed using a digital high-speed camera mounted onto a light microscope. TMV was determined by scintigraphy in the first two patient groups. Significantly more ciliated cells were found in HME users compared to non-HME users ($p = 0.05$). TMV was higher in HME users (median 2 mm/min; 0–7.9) compared to non-HME users (median 0.8 mm/min; 0–12.3), but this difference was not significant ($p = 0.37$). One-hour breathing without HME in long-term HME users did not measurably decrease TMV ($p = 0.13$). The long-term use of an HME restores/prevents the loss of tracheal ciliated cells. A significant improvement in TMV was not found. Short-term (one hour) detachment of an HME has no measurable effect on TMV.

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Keywords Heat and moisture exchanger · Mucociliary clearance · Total laryngectomy · Cilia · Mucus · Trachea

Introduction

After total laryngectomy (TLE), the upper respiratory tract is bypassed by the newly formed permanent tracheostoma. The air-conditioning role of the upper airways thus is lost and the respiratory epithelium of the lower airways becomes directly exposed to relatively cold and dry inspired (ambient) air. This is the main cause of the pulmonary complaints, such as frequent daily coughing and forced mucus expectoration, which are experienced by the majority of TLE patients [1, 2]. This change in respiratory physiology also leads to histologic changes in the tracheal epithelium, especially near the tracheostoma, such as loss of ciliated cells, goblet cell hyperplasia, and metaplasia, and to excessive mucus production and impaired mucociliary clearance [3–5].

Mucociliary clearance is an important defense mechanism of the respiratory tract and is essential for respiratory health. Particles, viruses and bacteria are trapped in the mucus layer covering the respiratory epithelium and the mucus is cleared upwards from the tracheobronchial tree by ciliary movement [6]. Frequent coughing and mucus hypersecretion are compensatory mechanisms for impaired mucociliary clearance in an attempt to still clear the airways and prevent infections [7]. Moreover, prolonged impaired mucociliary clearance causes damage of the epithelium, and patients with impaired mucociliary clearance are more susceptible to chronic airway inflammation and recurrent infections [6–9].

Prevention and treatment of pulmonary problems play a major role in rehabilitation after TLE. Heat and moisture exchangers (HMEs) are medical devices used for passive re-conditioning of the inspired air. HMEs have proven to be clinically effective and significantly improve pulmonary functioning and quality of life in TLE patients [10–12]. So far, the effect of HMEs on mucociliary clearance has not been studied in TLE patients. Mucociliary clearance depends on the quality of mucus [13] and on ciliary function [14]. Both factors are sensitive to ambient climate changes. Low temperature and/or humidity will immediately slow

the ciliary motion [15] and will change the viscosity of the mucus layer (short-term effect) and may lead to (temporary) impaired mucociliary clearance [6, 8, 16]. Prolonged exposure to these climate changes may cause respiratory epithelium damage and chronic inflammation, leading to chronic impaired mucociliary clearance (long-term effect). In general, the moment that chronic damage occurs, depends not only on the duration of exposure but also on the magnitude of the environmental change [7, 8, 16]. An extreme case is described in a study that found ciliated cell damage in tracheotomized-guinea pigs after only 60 min of breathing completely dry air [9]. With the application of an HME over the tracheostoma, the relative humidity of the inspired air is partially restored [17]. Impaired mucociliary clearance in TLE patients is expected to improve after using an HME on continued 24/7 bases.

This is the first study that combines the evaluation of tracheal ciliated cells collected with brush biopsies and measurements of tracheal mucus transport velocity by means of scintigraphy. The main aims of this study are to understand some of the long and short-term effects of using an HME in TLE patients on tracheal mucociliary clearance. More specifically, does the tracheal ciliated epithelium recover after HME use (long-term effect)? Is tracheal mucus transport velocity in HME users higher/better than in non-HME users (long-term effect)? And finally, is there a measurable effect on mucus transport velocity of one-hour breathing without HME (resulting in a significant decrease in inspired humidity) in HME users (short-term effect)?

Materials and methods

Patients

Three different laryngectomized-patient groups were included: a group of 16 Italian (initial) non-HME users, a group of 10 Dutch non-HME users and a group of 21 Dutch regular (24/7) HME users. In the study design section, the different patient groups are further described in detail and patients' characteristics are shown in Table 1. The Italian patients were treated and in follow-up in Gemelli Hospital and University Hospital la Sapienza in Rome, Italy. The Dutch patients were in long-term follow-up in the Netherlands Cancer Institute–Antoni van Leeuwenhoek, Amsterdam, The Netherlands. Surgery techniques for total laryngectomy were similar in all hospitals.

The HMEs used by the included patients were Provox XtraMoist HME and Provox XtraFlow HME (Atos Medical, Sweden). The study was approved by the local ethical review boards of the corresponding institutes and registered by the national CCMO (Central Committee on Research

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Table 1 Patient characteristics and overview of collected data for the different patient groups

	Non-HME users (Italy)		Non-HME users (Netherlands) Total <i>N</i> = 10	HME users (Netherlands)	
	Total <i>N</i> = 16	Subgroup ^b <i>N</i> = 8		Total <i>N</i> = 21	Subgroup ^c <i>N</i> = 14
HME use	Non-HME	HME <i>t</i> > 4 months	Non-HME	HME	HME/1 h non-HME
Median age years (range)	64 (49–88)	64 (58–79)	67 (54–77)	63 (44–81)	63 (44–81)
Female: male	1:15	0:8	2:8	1:20	0:14
Smoking history	n.a.	n.a.	30 (10–35)	35 (0–80)	35 (20–80)
Median pack/years (range)	n.a.	n.a.	19 (13–27)	9 (0.5–25)	9 (0.5–25)
Median years cessation date (range)					
Radiotherapy PRE TLE (#)	0	0	8	16	10
Radiotherapy post-TLE (#)	11 ^a	6 ^a	2	5	4
Median post laryngectomy years (range)	3 (0–10)	4 (1–10)	20 (9–25)	8 (2–26)	8 (2–15)
Pre-existent lung disease total (#)	7	4	1	3	3
Sarcoidosis				1	1
Lung cancer			1	1	1
COPD/emphysema	7	4		1	1
Brush biopsy	C	C	C	C	n.c.
Mucus transport scintigraphy	n.c.	n.c.	C	C	C
Frequency of complaints per 24 h	C	C	C	C	C

Median and range postlaryngectomy equals approximately HME use

n.a. not available, n.c. not collected, C Collected

^a Missing value for one patient

^b Also measured after 4–9 months HME use (see study design section)

^c Short-term (one hour) HME removal study (see study design section)

involving Human Subjects). Informed consent was obtained from all patients.

Study design

Italian non-HME users

Brush biopsies of tracheal epithelium were obtained in 16 Italian non-HME users prior to their start with HME use. In a subgroup of eight patients tracheal mucosa brush biopsies were repeated after minimally 4 months of HME use. The remaining eight patients could not be included for the post-HME biopsy due to recurrent disease, death, logistical reasons, and/or compliance issues. Patients scored frequency of coughing and mucus expectoration per 24 h on three consecutive days on tally sheets, both pre-HME and after 4 months of HME use.

Dutch non-HME users

Brush biopsy of tracheal epithelium and tracheal mucus transport scintigraphy were performed in 10 Dutch non-

HME users once. These patients also scored the frequency of daily complaints (coughing and mucus expectoration per 24 h) on a tally sheet.

Dutch HME users

Brush biopsies of tracheal epithelium and tracheal mucus transport scintigraphy were performed in 21 Dutch 24/7 HME users. Patients with a minimum mucus transport velocity of 1 mm/min on the initial scintigraphy, received two additional scintigraphy scans within a time period of four weeks and with at least a one-week interval between the consecutive scans. Patients were randomized for the sequence of these additional two scans: one scan after 1-hour non-HME breathing (inside the hospital with stable ambient conditions) and one scan after continuous HME breathing (similar to the initial scan). The subgroup consisted of 14 patients. The other seven patients had a mucus transport velocity of <1 mm/min in the initial scan. A second brush biopsy was obtained from all 21 HME users several weeks after the first scintigraphy as control measurement. For the subgroup of 14 patients with two extra

scans, this second brush biopsy was performed after the last scan. Frequency of daily coughing and mucus expectoration were scored on tally sheets once before and once after each scan and averaged. Available data per patient group are shown in Table 1. For the Italian patients, the number of postlaryngectomy years is equal to the number of non-HME using years. The Dutch HME users have started HME use immediately postlaryngectomy.

Measurements

Brush biopsy of tracheal ciliated epithelium

Collection of ciliated cells was performed by brush biopsy of the tracheal wall without local anesthesia in the outpatient clinic. In patients who underwent a scintigraphy, the brush biopsy was taken immediately after the scan with the patient in supine position. Two intra-tracheal locations were brushed: one biopsy near the tracheostoma (about 1 cm) = “stoma” brush and a second biopsy at a distance of approximately 6 cm from the tracheostoma = “tracheal” brush.

The cells were immediately transferred into a cell culture medium containing an antibiotic solution and subsequently were suspended on a glass slide according to the technique previously described by the research group of the Department of Infection, Immunity and Inflammation, University of Leicester, Leicester Royal Infirmary, Leicester, UK [18], where the first and second author received instructions for use. At least ten different views from two different glass slides were recorded. Ideally, undisrupted ciliated strips of >50 µm in length were studied when possible, otherwise shorter ciliated strips and single cells were also included. In case not enough cells were in focus, a third slide was used to complete the ten recordings. Cilia imaging was performed using microscopy (Olympus microscope BX40F4, Olympus optical LTD, Japan in the Netherlands Cancer Institute; Nikon Eclipse E200 CFI60 Nikon Instruments Inc, USA in Gemelli Hospital; Eurotek MOD T 2000, Eurotek GmbH, Germany, in la Sapienza Hospital, Rome, Italy). A digital high-speed camera (Basler Pilot camera, 648 × 488, 210 fps, Mono, Gigabit Ethernet, 1/3" (103870)) at a rate of 200 frames/sec was mounted on the microscope for recording and in-house developed software was used for sampling the recording. Specimens were examined using an oil immersion 100× objective lens.

Measurement of tracheal mucus transport velocity

Scintigraphy is a previously validated method to measure tracheal mucus transport velocity [19, 20]. All Dutch patients underwent scintigraphy in the Netherlands Cancer

Institute (Department of Nuclear Medicine). Patients with clinical signs of recent tracheal infection, i.e. less than 6 weeks prior to the planned scintigraphy, were excluded or postponed. Patients were instructed not to use short-acting bronchodilators for at least six hours prior to the measurement or long-acting bronchodilators at least twenty-four hours prior to the measurement.

Technetium-labeled Nanocolloid (99mTc-Nanocoll, GE Healthcare) was used as radioactive tracer in combination with methylene blue (Bleu Patenté, Guerbet) as a marker to visualize the droplet deposition. The particle size distribution of the Nanocolloid is such that 95 % are ≤80 nm in diameter. The radioactivity of the bolus is less than 5 Mbq, which is about 5 % of the standard dosage in diagnostic nuclear imaging [21].

During droplet bolus deposition, the patient was in supine position on the table of the gamma camera. A 20 µl bolus was placed about 6 cm distal from the stoma on the dorsal tracheal mucosa, using a sterile flexible tube connected to a 100 µl Gilson pipette without using local anesthetics. Inspection of the droplet deposition before and after the measurement was performed using a flexible fiberscope (Olympus, ENF Type GP, Olympus optical LTD, Japan).

After deposition of the radioactive bolus in the trachea, dynamic images were acquired with a single head camera (Argus, ADAC, Philips, The Netherlands). The camera setup was standard for 99mTc, with a 20 % window centered at 140 keV and a LEHR collimator. The camera was placed at approximately 10 cm above the thorax of the patient. Displacement of the radioactivity of 60 images of 30 s was recorded and all were acquired into a 128 × 128 matrix. Images were acquired for 30 min in the anterior projection on the gamma camera interfaced to a corresponding workstation. Four ⁵⁷Co markers were placed on reference points on the body and used for calculation of mucus transport velocity and to correct for body movements. The movement of the deposited bolus was followed in real time as well as being recorded to permit viewing later for analysis.

Data analysis

Ciliary brush biopsy

MiDas 2.0 software (free downloadable at www.xcitex.com) was used for analysis of the recordings. For each patient, the scores of the 10 recordings (per brush) by two observers (first and last author) were used to compute three endpoints: the total number of ciliated cells in the field of view of 10 recordings per brush, the fraction of attached cells with moving cilia and the fraction of attached cells that show coordinated cilia activity. This assessment/

evaluation is intended to map secondary-ciliated cell damage and determines loss of ciliated epithelium. Ciliary beat frequency (CBF) was not assessed as the *in vivo* situation in the trachea of these patients is different from the *in vitro* situation as CBF is temperature and humidity dependent [22, 23], and secondary-cilia damage leading to impaired mucociliary clearance is not always represented by a decreased CBF [24]. The field of view in the used setups allows the scoring of maximally approximately 80 ciliated cells per 10 recordings per one brush.

Tracheal mucus transport velocity

All scintigraphy scans were analyzed in consensus by the physician, who performed the scans (1st author) under supervision of the nuclear medicine physician (4th author), and the clinical physicist (2nd author). For each scintigraphy scan, the location of the leading and trailing edges of the bolus was determined in 14–20 frames using viewing software (Carestream Health, Inc 2011, VuePACS). These locations (distance towards the tracheostoma) were plotted as function of time (mm/min). The mucus transport velocity was determined in a time window of at least two minutes with a linear movement rate. Maximum droplet movement rate per scintigraphy was used as endpoint. Also the start- and end locations of the bolus movement (mean of leading and trailing edges) in the trachea towards the tracheostoma were assessed. Patients with mucus transport in the opposite direction of the tracheostoma (due to gravitational force) were scored as having 0 mm/min mucus transport velocity.

Statistical analysis

Ciliary brush biopsy

For ciliary brush analysis, the mean scores of the two observers were used. Inter- and intra-observer variability of the two observers was determined in terms of limits of agreement [25], i.e. by means of mean differences with corresponding standard deviations (SD). In case a patient missed one or more recordings, the total number of ciliated cells in the missing recordings was computed as the mean of the number of ciliated cells in the remaining recordings. For the subgroup of HME users, which underwent more than one biopsy, the mean score per brush per patient was used for analysis. The median number of cells per brush/per patient was used for multivariate and subgroup analysis because of the non-normal distribution of the number of cells. In a multivariate analysis the effects of country, location of brush biopsy (stoma versus trachea), and HME use on the number of ciliated cells were assessed using quasi-Poisson regression. Interaction terms were initially

included in the model and later discarded when they proved non-significant. The choice of quasi-Poisson regression above negative binomial regression was motivated by mean–variance plots, following Verhoef and Boveng 2007 [26]. Differences in number of ciliated cells, fractions of attached moving and attached coordinating ciliated cells between the tracheal and stoma brush within each patient were tested using a sign test for each patient group separately. In the Italian patients a paired *t* test was used for the prospective effect of an HME on ciliary score.

Tracheal mucus transport velocity

Based on previous measurements of tracheal mucus transport velocity by Morgan et al. [19] we expected the transport velocities to be log-normally distributed. This assumption was tested in our own data using qq-plots and seemed fairly accurate but a slight difference between HME users and non-HME users was noted. Hence, we used the log of the mucus transport velocities for analysis within the group of HME users and a non-parametric test to compare between the HME users and non-HME users.

The Wilcoxon test was used for the comparison between mucus transport velocity of the HME user group and the non-HME user group. The mean mucus transport velocity of the two performed scintigraphy scans with HME was used in the subgroup of the 14 HME users. Intra-patient variability in mucus transport velocity was calculated in the subgroup of 14 HME users that have had two scintigraphy scans with HME, using the Fisher's intraclass correlation coefficient. A one-sided paired *t* test was used in this subgroup to test whether 1 h non-HME breathing results in a lower log mucus transport velocity than HME breathing (where the log of the mean of the two velocities after HME breathing was used).

Results

Ciliary brush biopsy

Inter- and intra-observer variation for the number of ciliated cells in the Dutch patients was calculated for 948 analyzed recordings (10 recordings per patient). The limits of agreement between the two observers are [−1.57, 1.84] per observation (recording). The mean difference was 0.13 (SD = 0.87), which is considerably lower than the mean observed number of ciliated cells (2.78, SD = 1.56). The maximum total number of ciliated cells per brush per patient is 59 (Table 2) and this is within the range of maximum available ciliated cells that could be viewed and scored per patient due to the image size (c.a. 80 cells, method section). Examples of recorded tracheal ciliated

Table 2 Overview of all results: number of ciliated cells for stoma and tracheal location and fractions, median start- and end location of the bolus and the calculated tracheal mucus transport velocity from scintigraphy, and clinical complaints of mucus expectoration and coughing per 24-h

Patient group	Italy	Subgroup Italy		Netherlands	Netherlands	Subgroup Netherlands	
HME use	Non-HME	Non-HME	HME	Non-HME	HME	HME	1 h Non-HME
		<i>T</i> = 0	<i>T</i> = 4–9 months				
Total # patients	<i>N</i> = 16	<i>N</i> = 8	<i>N</i> = 8 ^c	<i>N</i> = 10	<i>N</i> = 21 ^a	<i>N</i> = 14 ^{a,d}	<i>N</i> = 6
Median (range) number ciliated cells							
Stoma	8 (0–33) <i>N</i> = 1 ^b	7 (0–18) <i>N</i> = 6 ^b	10 (0–27) <i>N</i> = 4 ^b	1 (0–51) <i>N</i> = 10	25 (0–49) <i>N</i> = 20	28 (6–49) <i>N</i> = 14	37 (28–50) <i>N</i> = 5
Trachea	11 (0–59) <i>N</i> = 16	5 (0–59) <i>N</i> = 8	16 (5–38) <i>N</i> = 8	22 (0–50) <i>N</i> = 10	32 (0–53) <i>N</i> = 20	38 (17–53) <i>N</i> = 14	35 (14–46) <i>N</i> = 6
Fraction moving attached ciliated cells							
Stoma	0.96 (0.29–1.00) <i>N</i> = 7	0.98 (0.29–1.00) <i>N</i> = 6	1.00 (0.97–1.00) <i>N</i> = 3	0.98 (0.91–1.00) <i>N</i> = 6	0.88 (0.50–1.00) <i>N</i> = 20	0.88 (0.50–1.00) <i>N</i> = 14	0.97 (0.78–1.00) <i>N</i> = 5
Trachea	0.99 (0.50–1.00) <i>N</i> = 13	0.91 (0.50–1.00) <i>N</i> = 8	0.95 (0.00–1.00) <i>N</i> = 8	0.95 (0.52–1.00) <i>N</i> = 9	0.91 (0.47–0.98) <i>N</i> = 20	0.89 (0.47–0.99) <i>N</i> = 14	0.95 (0.50–0.98) <i>N</i> = 6
Fraction coordinating attached ciliated cells							
Stoma	0.83 (0.67–1.00) <i>N</i> = 7	0.79 (0.67–1.00) <i>N</i> = 6	0.91 (0.90–1.00) <i>N</i> = 3	0.80 (0.42–1.00) <i>N</i> = 10	0.81 (0.25–1.00) <i>N</i> = 20	0.81 (0.42–1.00) <i>N</i> = 14	0.87 (0.79–0.95) <i>N</i> = 5
Trachea	0.96 (0.50–1.00) <i>N</i> = 13	1.00 (0.89–1.00) <i>N</i> = 8	0.93 (0.67–1.00) <i>N</i> = 8	0.73 (0.00–0.94) <i>N</i> = 10	0.87 (0.41–1.00) <i>N</i> = 20	0.89 (0.41–1.00) <i>N</i> = 14	0.98 (0.78–1.00) <i>N</i> = 6
Median start location bolus from stoma mm (range)	n.a.	n.a.	n.a.	68.7 (35–92) <i>N</i> = 10	47.5 (33–69) <i>N</i> = 21	47.5 (33–69) <i>N</i> = 14	50.4 (26–71) <i>N</i> = 14
Median end location bolus from stoma mm (range)	n.a.	n.a.	n.a.	59.5 (25.1–3.8) <i>N</i> = 10	38.6 (9.6–69) <i>N</i> = 21	28.5 (10–48) <i>N</i> = 14	30.4 (13–51) <i>N</i> = 14
Median mucus transport velocity mm/min (range)	n.a.	n.a.	n.a.	0.8 (0–12.3) <i>N</i> = 10	2 (0–7.9) <i>N</i> = 21	2.8 (0–7.9) <i>N</i> = 14	3.3 (0.7–0.6)
Median complaints per 24 h (range)							
Mucus	6 (0–15) <i>N</i> = 14	9 (0–15) <i>N</i> = 7	1 (0–16) <i>N</i> = 7	11 (3–26) <i>N</i> = 10	7 (1–42) <i>N</i> = 20	6 (1–20) <i>N</i> = 14	n.a.
Coughing	5 (3–39) <i>N</i> = 14	12 (5–39) <i>N</i> = 7	1 (2–11) <i>N</i> = 7	6 (0–16) <i>N</i> = 10	4 (1–16) <i>N</i> = 20	4 (1–9) <i>N</i> = 14	n.a.

n.a. not applicable

^a For the 14 patients of the subgroup the mucus transport velocities are the mean of the velocities at two measurements with HME^b In the Italian patient it was not always possible to collect the stoma brush due to logistic problems^c In three out of eight patients for the number of ciliated cells, the fractions of moving and coordinating attached cells of the trachea brush the means of the brush at 4 and 9 months were used^d In 8 out of 14 patients for the number of ciliated cells and the fractions of moving and coordinating attached cells the means of two brushes with HME were used

Fig. 1 Box plots of the number of ciliated cells in the different patient groups (Table 2) for both the stoma brush and tracheal brush. **a** Italian non-HME users and **b** Italian patients after 4–9 months HME use, **c** Dutch non-HME users and **d** Dutch HME users

cells are available as online supporting information (video showing: A: tracheal cells with loss of cilia, B: attached ciliated cells, with and without movement, C: attached ciliated cells with coordinated movement).

The median numbers of ciliated cells and fractions of moving and coordinating attached ciliated cells per patient/brush are shown in Table 2. Figure 1 graphically describes the number of ciliated cells in the different patient groups and between the stoma and tracheal brush using box plots. Multivariate analysis shows that the number of ciliated cells near the tracheostoma (stoma brush) was significantly lower than the number of cells deeper in the trachea (tracheal brush; $p = 0.01$), and that there are significantly more ciliated cells in Dutch HME users than in Italian non-HME users ($p = 0.02$). The analysis estimated that the number of ciliated cells in HME users is 1.39 times that of non-HME users ($p = 0.05$). The fractions of ciliated cells with moving and coordinating cilia did not differ statistically significantly between HME users and non-HME users, between brush locations or between the Dutch and the Italian groups.

Subgroup analysis

In the Dutch HME user group, a significantly higher number of ciliated cells were found than in the Dutch non-HME users ($p = 0.05$), adjusted for the effect of brush

location. In the Italian subgroup of eight patients, where pre- and post-HME treatment brushes were available, an increase in number of tracheal ciliated cells was seen after 4 months and an even higher increase after 9 months of HME use (Fig. 2a). However, the difference between the Italian non-HME and HME users is not significant (p value = 0.61). There are two Italian non-HME users (Fig. 2, orange and green), who already had a relatively high number of ciliated cells. One patient shows an extremely large increase in number of ciliated cells after 4 months with similar number of cells after 9 months (pink). Stoma brushes were available for four patients only after 4 months of using an HME (Fig. 2b). No correlation was found between patients with pre-existent chronic airway infection (Table 1) and the number of ciliated cells. The ratio of ciliated cells between HME users and non-HME users in The Netherlands (1.48) and in Italy (1.54) is similar.

Mucus transport velocity (scintigraphy)

Figure 3 shows an example of the leading and trailing edge of the bolus plotted as function of time (mm/min). The mean of leading and trailing edge yield the start- and end location of the bolus movement towards the tracheostoma. Figure 4 shows that the end location of the bolus corresponds with the mucus transport velocity, which means that

Fig. 2 Number of ciliated cells per patient in the Italian group without HME ($t = 0$) and after 4 and/or 9 months of HME breathing for the tracheal brushes (a) and the stoma brushes (b)

Fig. 3 Example of mucus transport analysis. Distance of the radiolabeled bolus (mm) from the tracheostoma stoma (y-axis = 0) as function of time (mins). The insertion of the bolus was at $t = 0$, the scintigraphy scan was divided into different time windows with linear movement rates to calculate the maximum mucus transport velocity. In this case the first window gives the maximum mucus transport velocity

the higher the maximum mucus transport velocity, the closer to the tracheostoma the bolus will end.

The median start- and end locations (in mm) of the intra-tracheal bolus movement towards the tracheostoma and the calculated tracheal mucus transport velocity (mm/min) are shown in Table 2. During the measurements, the ambient humidity and the temperature ranged from 34 to 53 % relative humidity and 20.5–24 °C, respectively.

Figure 5 shows the mucus transport velocity per patient for the HME users and non-HME users. The difference of

median mucus transport velocity between the HME users and non-HME users is 1.2 mm/min, however, this was not significant ($p = 0.37$).

In the subgroup of 14 patients, no significant difference in mucus transport velocity was found between HME breathing and 1-h non-HME breathing ($p = 0.13$). Also no difference in end location of the bolus movement was seen between HME users and 1 h breathing without HME ($p = 0.90$). The ICC (intraclass correlation coefficient) intra-patient variability is -0.39 .

Fig. 4 Relation of end location of the bolus and tracheal mucus transport velocity for all scintigraphy scans of HME users. *Red dots* with HME. *Black triangles* after 1-h exposure to open stoma (non-HME) breathing. The Pearson's product moment correlation coefficient for all observations together is -0.67 ($R^2 = 0.44$)

Overall, there was no significant correlation between the tracheal mucus transport velocity and cilia brush scores.

Clinical complaints

The median frequency of coughing and mucus expectoration per 24 h, scored by patients on tally sheets, is presented in Table 2. The overall median for the various clinical complaints in the Italian patients using an HME for 4 months are lower than those of the Dutch patients (both HME and non-HME groups). There are no large differences in clinical complaints between the Dutch HME users

and Dutch non-HME users. The median number of clinical complaints in the Italian non-HME users group after 4 months of HME use decreased from 9 to 1 and 12 to 1 per 24-h, respectively, for the mucus expectoration and coughing (Table 2; Fig. 6).

Discussion

In this study, mucociliary clearance was assessed in HME and non-HME users using both ciliary cell brush biopsies and scintigraphic measurements of mucus transport. The tracheal epithelium of HME users contains significantly more ciliated cells, both near the stoma and more distal in the trachea. In all studied TLE patient groups, the number of ciliated cells near the stoma is significantly lower than more distal in the trachea. The initial non-HME users, who were treated with HMEs for 4–9 months, overall showed an increase in the number of ciliated cells and a decrease in pulmonary complaints. Tracheal mucus transport velocity, though, was not significantly higher in HME users than in non-HME users. Moreover, no correlation was found between mucus transport velocity and number of ciliated cells. Finally, no significant short-term effect on mucus transport rate was measured after 1 h removal of the HME in HME users.

There are two possible explanations for the observation that the tracheal epithelium of HME users contains significantly more ciliated cells, both near the stoma and more distal in the trachea. Most of the Dutch patients started with HME use immediately post-TLE, which might suggest that the loss of ciliated cells is (partly) prevented. This is in line with earlier observations that early onset of HME use is significantly preventing the development of pulmonary complaints [27, 28]. However, the observation in the Italian

Fig. 5 Tracheal mucus transport velocity (mm/min) for the non-HME users ($n = 10$, in open blue diamonds, median value in closed blue diamond) and the HME users ($n = 21$, in open pink squares, median value in closed pink square)

Fig. 6 Frequency of coughing (a) and mucus expectoration (b) per 24-h reported by patients without using an HME ($t = 0$) and after 4 months of HME use ($t = 4$)

patients of a trend towards more ciliated cells after 4–9 months of HME use suggests that the mucosa can recover from the epithelial damage post-TLE. This latter explanation concurs well with the observed decrease in pulmonary complaints as reported by the Italian patients. The clear reduction in complaints in the Italian patient group (Fig. 6) is in line with earlier findings in the Netherlands and elsewhere in Europe [10, 11, 29–31].

The finding that there are less ciliated cells near the tracheostoma is in line with the literature [5]. This is most likely caused by the anatomical change resulting from total laryngectomy and is probably related to better temperature and humidity conditions lower in the trachea or a consequence of the radiotherapy which most patients have received, since the normal distribution of ciliated cell in the trachea is similar in the cranial and distal trachea [5]. The finding that no differences were found in the fraction of moving or coordinating ciliated cell strips between the various patient groups might be an artifact. The in vitro environment (optimal immersion in cell medium) is not similar to the in vivo intra-tracheal environment after TLE, where relatively dry and cold air is passing the epithelium. It is also possible that the fraction of moving or coordinating ciliated cells, which has mostly been used to map primary ciliary damage [24], is not well suited to map secondary ciliary damage.

An unexpected finding was that there were overall significantly more ciliated cells in the Dutch than in the Italian patients, both in the HME users as non-HME users (Table 2) even though the ratio between HME users and non-HME users in both countries was similar. The explanation for this might be that more Italian patients reported

pre-existent chronic lung disease (COPD/lung emphysema, Table 1) compared to the Dutch patient groups and hence significantly less ciliated cells. A further difference between the two countries is the median postlaryngectomy follow-up, which is much longer in the 10 Dutch non-HME users (20 years) than that of the 16 Italian non-HME users (3 years). Differences in smoking history are probably not the explanation for this difference, because these are most likely comparable. It cannot be excluded that environmental differences between the two countries, such as climate or air pollutants (dust) might play a role.

The finding that the difference in median mucus transport velocity between Dutch HME users (2 mm/min) and the Dutch non-HME users (0.8 mm/min) was not significant, is probably (partly) due to the low number of available non-HME users and the wide range of velocities measured. However, similar results were found in a comparison study of bronchial mucus transport velocities in mechanically ventilated patients in a semi-closed system between 11 HME users and 11 non-HME users [32]. Also, an animal study by Eckerbom et al. [33] that assessed the tracheal mucus transport velocity in 10 ventilated tracheotomized pigs has shown no statistic difference between the group of five with HME and the group of five without HME. Overall, the mucus transport velocity rates in laryngectomized patients are lower than in healthy individuals but quite similar to patients with a chronic airway disease such as COPD, asthma or chronic bronchitis [7, 13, 19]. However, only 2 of the 31 (Dutch) TLE patients have a pre-existent chronic airway disease (sarcoidosis/COPD, Table 1). It remains, therefore, intriguing that despite the significant improvement of intra-tracheal

ciliated cells with HME use, the mucus transport velocity is not significantly improved. The reason might be that the mucus quality in patients still is decreased even after long-term HME use, which coincides with other observations in the pig study by Eckerbom et al. [33]. In that study also the changes in mucus quality in the 10 tracheotomized pigs (5 with HME and 5 without HME) were assessed by bronchoscopy, and after 6 h of ventilation mucus quality with HME use was better than without, but still there was no significant difference in mucus transport velocity [33].

This study also has measured the effect of short-term (one hour) breathing without HME on tracheal mucus transport velocity in regular HME users. Patients served as their own control, eliminating bias factors such as age, radiotherapy, and smoking history. We found that breathing without HME for a short time (for example during stoma cleaning) does not negatively influence the tracheal mucociliary clearance. This finding seems in contrast with the previously described (short term) significant tracheal epithelial damage after 60 min of breathing dry air in 15 tracheotomized-guinea pigs [9]. However, the air used in the guinea pigs was probably much dryer (“dry air”, no exact humidity value was given) than the humidity differences between HME and non-HME breathing [9, 34]. As no impact of one-hour breathing without HME could be shown, it is probably safe if patients occasionally do not wear their HME for a short period, although we cannot yet recommend how long this period can safely be.

Typical for our study is the large inter-individual variance in mucociliary clearance rates (Table 2), which corresponds with findings in previous studies [7, 19, 33], and which limits the statistical power. This variance and the limited number of patients might be the explanation that we did not find a correlation between the number of ciliated cells and mucus transport velocity. Moreover, possible differences due to seasonal changes during the measurements (brush biopsies were taken in the spring, summer and winter and the scintigraphy measurements were performed both in the winter and spring) could not be taken into account.

Overall, this study has given more insight in the effects of an HME on mucociliary clearance in TLE patients. Although long-term HME users have higher number of ciliated cells in the trachea, they still have an impaired tracheal mucus transport velocity. As mucociliary clearance depends on the quality of mucus [13] and on ciliary function [14], this indicates that mucus quality, despite the improvements in humidification caused by HMEs, still is not optimal. Short-term (one hour) removal of an HME from the stoma does not affect the mucus transport velocity. The well-known improvements in pulmonary functioning achieved with the continued use of HMEs thus might not only be resulting from the improvements in

humidification, but also from the restored/prevented loss of tracheal ciliated epithelium.

Conclusions

The long-term use of an HME restores/prevents the loss of tracheal ciliated cells. Significant improvement in mucus transport velocity was not found. Short-term (one hour) detachment of an HME has no measurable effect on mucus transport velocity.

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