The Influence of Purulence on Ciliary and Cough Transport in Bronchiectasis

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BACKGROUND: Individuals with bronchiectasis exhibit colonization and infection of the respiratory system, with a consequent alteration of the macroscopic appearance of secretions, which ranges from mucoid to purulent. Purulence is related to the structural conformation, rheological profile, and transport indexes of mucus. We analyzed and compared the transport properties of respiratory secretions with mucoid appearance versus those with purulent appearance in patients with bronchiectasis and in subjects without lung disease. METHODS: In a simulated cough machine we assessed the mucociliary transport and contact angle of 32 mucoid and 19 purulent samples from subjects with bronchiectasis, and 21 samples from subjects without lung disease. RESULTS: Mucociliary transport was lower in the mucoid samples (0.78 ± 0.22) and in the purulent samples (0.73 ± 0.22) than in the samples from subjects without lung disease (1 ± 0.19). The purulent samples had less displacement in the simulated cough machine (7.57 ± 3 cm) than did the mucoid samples (23 ± 15 cm) or the samples from subjects without lung disease (34 ± 8.4 cm), as did the mucoid samples compared to the samples from subjects without lung disease. The purulent samples had a higher contact angle (25 ± 6.1°) than the mucoid samples (17 ± 7.8°) or the samples from subjects without lung disease (10 ± 2.5°), as did the mucoid samples compared to the samples from subjects without lung disease. CONCLUSIONS: Respiratory secretions in individuals with bronchiectasis have poor transport properties, which manifest as reduced mucociliary transport, reduced mucus transport by cough, and higher contact angle. These features were more accentuated in the purulent samples. This simple classification can be used by therapists to plan treatments, and by researchers to obtain more homogeneity between groups of subjects. *Key words: respiratory system; mucus; sputum; color; bronchiectasis; mucociliary clearance.* [Respir Care 2013; 58(12):2101–2106. © 2013 Daedalus Enterprises]

Introduction

Bronchiectasis is characterized by abnormal and permanent dilatation of the bronchi,[1,2] which is mainly caused by inadequate clearance of microorganisms and recurrent or chronic infection.[2] Damage to the ciliated epithelium decreases mucociliary clearance, and these patients exhibit stimulation of mucus-secreting cells, which produce stiffer mucus,[1] which induces secretion retention in the respiratory system.

Because individuals with bronchiectasis have bacterial colonization,[3,4] the macroscopic appearance of their respiratory secretions ranges from mucoid to purulent. The purulence may be related to the structural conformation, rheo-

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logical profile, and transport indexes of the mucus,[5] which are associated with a greater secretion retention and infections. We assessed the transport properties of mucoid and purulent respiratory secretions from subjects with bronchiectasis, and secretions from subjects without lung disease.

QUICK LOOK

Current knowledge

Bronchiectasis is characterized by abnormal and permanent dilation of the bronchi, resulting in recurrent or chronic infection. Alteration of the ciliated epithelium reduces the effectiveness of the mucociliary escalator, stimulates mucus-secreting cells, and results in the production of thick mucus, often inducing secretion retention.

What this paper contributes to our knowledge

Respiratory secretions from patients with bronchiectasis exhibited poor transport properties, as demonstrated by decreased mucociliary and cough transport, and increased contact angle. These abnormalities were accentuated in the samples with a purulent appearance.

Methods

This was a prospective study that included clinically stable subjects, 22–81 years old, diagnosed with non-cystic-fibrosis-related bronchiectasis based on clinical history, complete examination, and computed tomography. This study also included volunteers without lung disease, who were undergoing surgery under general anesthesia and orotracheal intubation. We excluded patients who did not exhibit respiratory secretions in sufficient amounts for the analysis, and those who had developed respiratory infections within the 4 weeks prior to the study. All subjects gave informed consent, and the study was approved by our institution's ethics committee.

The mucus samples were collected with voluntary cough and expectoration into a glass. The mucus was separated from the saliva and assessed for purulence, then placed into a plastic tube, covered with fluid petroleum jelly to prevent desiccation, and frozen until analysis.[6,7]

The mucus was stored at –20°C for no more than 90 days. According to a study in which frog mucus was stored at −20°C or −80°C, periods up to 90 days did not lead to any significant differences in mucus transportability.[6]

In the without-lung-disease subjects the samples were collected immediately after removal of the endotracheal tube. The secretion sample that was deposited inside the tube was removed with a swab, according to the criteria and procedures described by Rubin et al.[8]

Respiratory secretions were assessed as follows:

Purulence

The mucus was assessed for purulence with a score based on a previously described numerical visual scale,[9] which ranges from 1 (mucoid) to 5 (yellow/green). Samples with a purulence index between 1 and 3 were classified as mucoid, and samples with scores of 4 or 5 were classified as purulent. The purulence evaluation was performed by 2 researchers.

Mucociliary Transport

A convenient system for studying mucociliary transport is the frog palate, since frog palate epithelium is similar to that of the airways of other vertebrates.[10-12] Anesthetized frogs were decapitated, their jaws disarticulated, and the upper portion of the head was removed. The frog palate was kept in a refrigerator at 4°C for 2 days, covered with plastic wrap, in a humidified chamber to deplete the frog mucus. The frog mucus was then collected, covered with Vaseline, and used as a control in relation to the sputum sample tested.

During the examination of the samples the palate was kept at approximately 25°C in an acrylic chamber, coupled to an ultrasonic nebulizer (Max Inhales, Nsam, Brazil), at 100% humidity, maintained by a saline solution (0.45% NaCl).

Ciliary transport time was established by measuring the time between the placement of a small sample of mucus on the ciliated epithelium of the frog palate and 6 mm of displacement, viewed with a stereoscope (Stemi 1000, Carl Zeiss, Germany) equipped with 10× ocular (eyepiece) and a 0.8× magnification lens.

Each sample was tested 5 times, and the transport speed was expressed in terms of relative velocity, which is equal to the velocity of sputum displacement, divided by the speed of the frog mucus samples.[7,11] The average of the 5 measurements was used for statistical analysis.

Simulated Cough Machine Transport

Analysis of the cough machine mucus transport was completed according to the model described by King et al.[13] The model was composed of a pressure source (oxygen cylinder), a solenoid valve, and a simple scheme of airways, represented by a dry acrylic cylinder measuring 30 cm in length and 4 mm in internal diameter, with the 3 elements connected in a series.[14] When the cough simulator machine was activated, the timing device controlled the opening of the solenoid for one second, allowing the exit of air at 4.2 kgf/cm^2 of pressure, thus moving the secretion sample.[7,11] Each mucus sample was tested 5 times, and the

Table. Subjects

	Subjects With Bronchiectasis		Subjects Without Pulmonary Disease
	Mucoid $n = 32$	Purulent $n = 19$	Mucoid $n = 21$
Male/female	9/23	7/12	9/12
Age, mean ± SD y	54.5 ± 14.1	52.1 ± 15.5	23.2 ± 9.3
Duration of intubation, mean ± SD d	NA	NA	112.4 ± 50.1

NA = not applicable

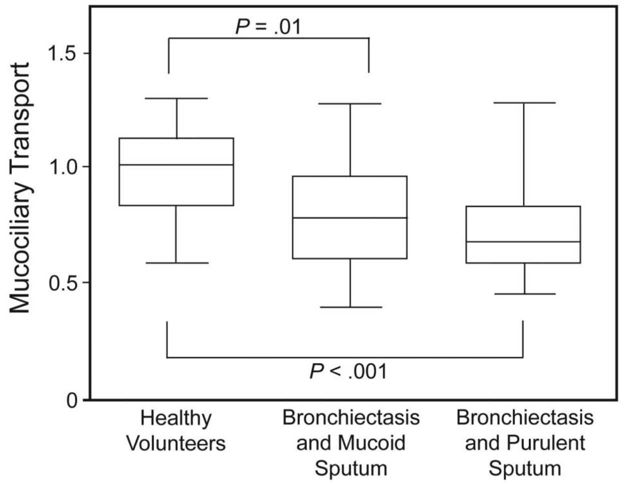

Fig. 1. Mucociliary transport, over frog palate, of mucus samples from subjects with bronchiectasis and from volunteers without lung disease. In the data bars, the horizontal lines represent the medians, the tops and bottoms of the bars represent the 75th and 25th percentiles, respectively, and the whisker bars represent the maximum values.

average of these 5 measurements was used for statistical analysis.

Contact Angle

The angle, in degrees, formed between the mucus and the glass surface was considered the contact angle, and was measured using a goniometer with a 20× magnification lens. The glass surface used for these analyses was treated with sulfochromic acid to remove any electrical charge.[7,11] The sample was evaluated 5 times, and the average of these measurements was used for statistical analysis.

Statistical Analysis

Initially, the Shapiro-Wilk test was used to check normality. Because the data presented normal distribution, the comparisons were carried out using analysis of variance, followed by the Tukey-Kramer test. Multiple comparisons were performed with statistics software (Statistica 7.0, StatSoft, Tulsa, Oklahoma). $P < .05$ was considered statistically significant.

The sample size was calculated for mucociliary transport as the main outcome, based on data from Tambascio et al (2009),[7] and considering an average difference of 2.41, standard deviation of 1.88, power of 90%, and α of 5%. The calculated sample size was 10 subjects.

Results

We assessed samples from 51 subjects with bronchiectasis: 17 had idiopathic bronchiectasis, 22 had bronchiectasis related to recurrent infections, and 12 had tuberculosis lesions. By macroscopic appearance, 32 samples were classified as mucoid and 19 as purulent (Table).

We assessed samples from 21 without-lung-disease volunteers who were undergoing surgery under general anesthesia and orotracheal intubation. All these samples were macroscopically classified as mucoid (see the Table).

Relative Transport Speed on Frog Palate

The relative transport speed was lower with the mucoid (0.78 ± 0.22) and purulent samples (0.73 ± 0.22) from the bronchiectasis subjects than with the samples from the without-lung-disease subjects (1 ± 0.19) ($P = .01$ and $P < .001$, respectively) (Fig. 1).

Displacement in the Simulated Cough Machine

The purulent samples had less displacement in the simulated cough machine (7.57 ± 3 cm) than did the mucoid samples (23 ± 15 cm, $P < .001$) or the samples from the without-lung-disease subjects (34 ± 8.4 cm, $P < .001$). The mucoid samples had less displacement in the simulated cough machine than did the samples from the without-lung-disease subjects ($P = .01$) (Fig. 2).

Contact Angle

The purulent samples had a higher contact angle (25 ± 6.1°) than the mucoid samples (17 ± 7.8°, $P < .001$) or the samples from the without-lung-disease subjects (10 ± 2.5°, $P < .001$). The mucoid samples had a higher contact angle than the samples from the without-lung-disease subjects (.01) (Fig. 3).

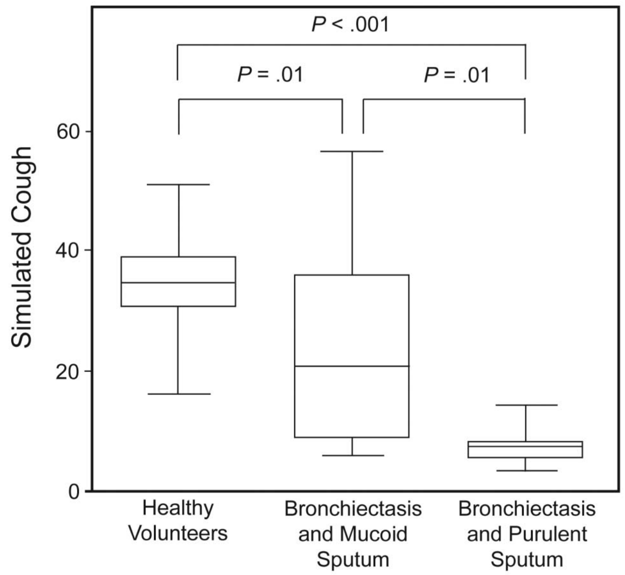

Fig. 2. Mucus transport, in a simulated cough machine, of mucus samples from subjects with bronchiectasis and from volunteers without lung disease. In the data bars, the horizontal lines represent the medians, the tops and bottoms of the bars represent the 75th and 25th percentiles, respectively, and the whisker bars represent the maximum values.

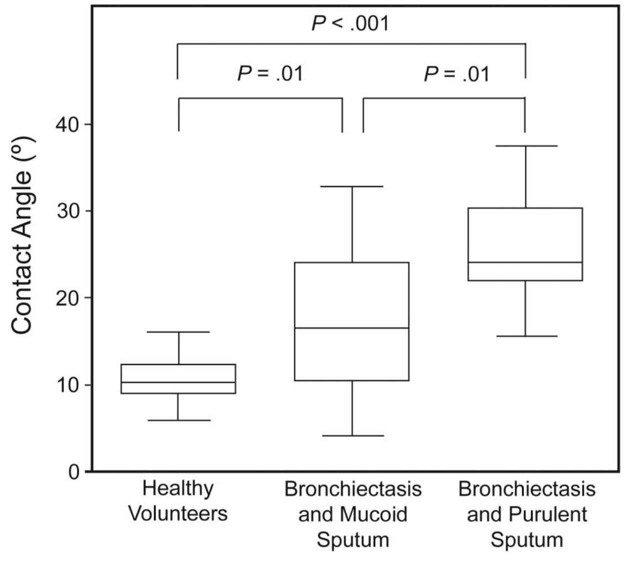

Fig. 3. Contact angle of mucus samples from subjects with bronchiectasis and from volunteers without lung disease. In the data bars, the horizontal lines represent the medians, the tops and bottoms of the bars represent the 75th and 25th percentiles, respectively, and the whisker bars represent the maximum values.

Discussion

Mucociliary transport was lower in the mucoid and purulent samples from the subjects with bronchiectasis than the samples from the without-lung-disease subjects. The purulent samples had less displacement in the simulated cough machine and a greater contact angle than the mucoid samples. The samples from the without-lung-disease subjects had more displacement in the simulated cough machine and a lower contact angle than did the samples from the subjects with bronchiectasis.

Our findings suggest a simple method that would allow greater homogeneity in secretion samples, by classifying the samples as mucoid or purulent, thus facilitating the identification of alterations induced by interventions. This finding may reduce the confounding factors that increase the odds of variability of the results, which could hinder our understanding of the progression of disease and the mechanisms and/or benefits of therapies.

In addition, our findings confirm the adequacy of the respiratory mucus from without-lung-disease subjects in ciliary transport, and suggest that the mucus alterations in subjects with bronchiectasis, who are exposed to frequent aggressions, exhibit impairment in ciliary and cough mechanisms. This impairment was particularly evident in the non-purulent samples.

Similar results were previously published by our group. Tambascio et al[7] assessed the respiratory secretions of subjects with bronchiectasis and found that adherent and purulent samples exhibited less displacement in a simulated cough machine, and a greater contact angle, than non-adherent and mucoid samples. However, no results were obtained from without-lung-disease subjects in that study.

Several studies have correlated respiratory secretions with a purulent appearance, which is characterized by yellow/green color, inflammatory markers,[15] and bacteria[12,16,17] in patients with bronchiectasis,[12,15] COPD,[16,17] and chronic bronchitis.[15]

Stockley et al[15] suggested that, even with categorization systems that have more than 2 alternatives (purulent and mucoid) and are used to assess the macroscopic appearance of secretions, there is no difference between raters in classifying the purulent or mucoid appearance of the samples.[15] In our study the evaluation of macroscopic appearance of the secretions was always performed by 2 investigators. To reduce the subjectivity of those evaluations we used the scale described by Deneuville et al.[9]

Regarding the samples from the without-lung-disease subjects, the mucus analysis was difficult, because small amounts were collected, so for this group we applied the method described by Rubin et al,[8] in which mucus is collected from the endotracheal tube used during a short surgical procedure in without-lung-disease subjects. The characteristics of the mucus deposited inside and outside the tube was compared. The viscoelastic properties of the mucus from inside versus outside the tube were similar, but the mucus from inside the tube was less hydrated.

Therefore, to increase the homogeneity we collected only samples from inside the tubes.

Although the mean duration of orotracheal intubation of the without-lung-disease subjects was 112.4 ± 50.1 min, these mucus samples presented normal transport values. A review by Houtmeyers et al[18] suggests that the relative transport velocity is within the normal range and the rheological properties are unchanged after anesthesia.

A study has yielded evidence of an inverse relationship between mucociliary clearance and age, but this influence needs to be better investigated. According to Houtmeyers et al,[18] it is not known whether or not age affects the transport properties of respiratory secretions. Our results from our without-lung-disease group are comparable to those presented by Jeanneret-Grosjean, with ages ranging from 19 to 50 years.[19]

Dulfano and Adler[20] found that increased mucus purulence was associated with greater viscosity and reduced elasticity. The rheological properties of the respiratory secretions were not assessed in our study; however, the rates of mucociliary transport or transport by cough are known to be influenced by the rheological characteristics, including viscosity and elasticity of the respiratory mucus.[9,21,22]

Zanchet et al[12] found a lower relative transport speed on frog palate in samples with a purulent appearance (0.8 ± 0.06), compared to mucoid samples (0.9 ± 0.1) from subjects with bronchiectasis. In the present study, the differences between the transport of the purulent samples (0.73 ± 0.22) and the mucoid samples (0.78 ± 0.22) were lower than those of Zanchet et al.[12] Our results also presented a higher variability, which can be compared to other authors.[7,11,23] However, we found significant differences only in the comparison between the secretions of subjects with bronchiectasis and those of the without-lung-disease subjects (1 ± 0.19), thus confirming that the without-lung-disease subjects exhibited the ideal relative transport speed to ensure the best transport, which was approximately 1.0–1.1.[11,23]

High contact angle may impair the ciliary and cough transportability of bronchial mucus.[21] According to Girord et al,[24] mucus samples with a contact angle lower than 20° provide better protection and lubrication. In our study the purulent samples had an average contact angle of 25°, demonstrating a poor rheological profile.

The main limitation of our study is the assessment of purulence, considering that we evaluated only the macroscopic appearance of the secretions and did not measure inflammatory markers. However, the assessment was based on color, which is a simple and quick method that reflects the alterations of the transport indexes that were exhibited by the investigated samples.

The impaired mucociliary and cough transport of secretions from the subjects with bronchiectasis, even the secretions with mucoid appearance, reinforces the indication of airway clearance techniques to help these patients. This method can be easily introduced into clinical practice[25] and can help clinicians and researchers to plan appropriate therapies.

Conclusions

The respiratory secretions of subjects with bronchiectasis exhibited poor transport properties, as demonstrated by decreased mucociliary and cough transport, and increased contact angle, to a greater degree in those with a purulent appearance. The method we used to distinguish mucoid from purulent secretions allows the separation of samples into more homogeneous groups, facilitating the identification and interpretation of changes caused by interventions in future studies.

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