Adhesiveness and purulence of respiratory secretions: implications for mucociliary transport in patients with bronchiectasis*

Adesividade e purulência de secreções respiratórias: implicações no transporte mucociliar em pacientes com bronquiectasias

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Abstract

Objective: To analyze and compare the transport properties of respiratory secretions, classified by selected parameters, in individuals with bronchiectasis unrelated to cystic fibrosis. **Methods:** We collected mucus samples from 35 individuals with bronchiectasis unrelated to cystic fibrosis. The samples were first classified by their surface properties (adhesive or nonadhesive), as well as by their aspect (mucoid or purulent). We then tested the samples regarding relative transport velocity (RTV), displacement in a simulated cough machine (SCM), and contact angle (CA). For the proposed comparisons, we used ANOVA models, with a level of significance set at 5%. **Results:** In comparison with nonadhesive samples, adhesive samples showed significantly less displacement in the SCM, as well as a significantly higher CA (6.52 ± 1.88 cm vs. 8.93 ± 2.81 cm and 27.08 ± 6.13° vs. 22.53 ± 5.92°, respectively; $p < 0.05$ for both). The same was true in the comparison between purulent and mucoid samples (7.57 ± 0.22 cm vs. 9.04 ± 2.48 cm and 25.61 ± 6.12° vs. 21.71 ± 5.89°; $p < 0.05$ for both). There were no significant differences in RTV among the groups of samples, although the values were low regardless of the surface properties (adhesive: 0.81 ± 0.20; nonadhesive: 0.68 ± 0.24) or the aspect (purulent: 0.74 ± 0.22; mucoid: 0.82 ± 0.22). **Conclusions:** The respiratory secretions of patients with bronchiectasis showed decreased mucociliary transport. Increased adhesiveness and purulence cause the worsening of transport properties, as demonstrated by the lesser displacement in the SCM and the higher CA.

Keywords: Bronchiectasis; Mucus; Mucociliary clearance; Adhesiveness.

Resumo

Objetivo: Analisar e comparar as propriedades de transporte de secreções respiratórias, classificadas através de parâmetros selecionados, de indivíduos com bronquiectasias não secundárias à fibrose cística. **Métodos:** Foram avaliadas amostras de muco respiratório, classificadas como com propriedades de superfície adesivas ou não adesivas, assim como com aspecto mucoide ou purulento, de 35 participantes com bronquiectasias não secundárias à fibrose cística, quanto a velocidade relativa de transporte (VRT), deslocamento em máquina simuladora de tosse (MST) e ângulo de contato (AC). Para as comparações propostas, foram utilizados modelos de ANOVA, com nível de significância estabelecido em 5%. **Resultados:** Houve uma diminuição significativa no deslocamento em MST, assim como um aumento significativo no AC, das amostras adesivas quando comparadas às não adesivas (6,52 ± 1,88 cm vs. 8,93 ± 2,81 cm e 27,08 ± 6,13° vs. 22,53 ± 5,92°, respectivamente; $p < 0,05$ para ambos). O mesmo ocorreu na comparação entre as amostras purulentas e mucoides (7,57 ± 0,22 cm vs. 9,04 ± 2,48 cm e 25,61 ± 6,12° vs. 21,71 ± 5,89°; $p < 0,05$ para ambos). Não houve diferença na VRT entre os grupos, embora os valores estivessem diminuídos, independentemente da adesividade (adesivas: 0,81 ± 0,20; não adesivas: 0,68 ± 0,24) ou do aspecto (purulentas: 0,74 ± 0,22; mucoides: 0,82 ± 0,22) das amostras. **Conclusões:** A secreção respiratória de pacientes com bronquiectasia apresentou uma diminuição do transporte ciliar. Maior adesividade e purulência favorecem a piora das propriedades de transporte, demonstradas pela diminuição do deslocamento em MST e pelo aumento do AC.

Descritores: Bronquiectasia; Muco; Depuração mucociliar; Adesividade.

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Introduction

Bronchiectasis is an airway disease of various etiologies, pathologically characterized by abnormal, permanent bronchodilation,[1,2] and is principally caused by the perpetuation of inflammatory processes, induced by frequent episodes of bacterial infection and inflammation. Consequently, the ciliated epithelium of the airways is altered, which reduces the effectiveness of mucociliary clearance.[3]

In addition to the impairment of the ciliated epithelium, patients with bronchiectasis show stimulation of mucus-secreting cells and production of mucus that is more tenacious,[1] which together induce secretion accumulation in the respiratory tract. This respiratory mucus accumulation can be reduced by the cough mechanism, which compensates for impaired mucociliary clearance.[4]

Changes in respiratory mucus are principally related to changes in its rheological and surface properties, which, depending on the existing characteristics, interfere with mucociliary transport and transport by cough in a distinct manner.[5,6] Therefore, certain rheological and surface properties of bronchial mucus (e.g., viscosity, elasticity, spinnability, adhesiveness, and wettability) are determinants of effective bronchial mucus transport.[7]

Individuals with bronchiectasis commonly present with bacterial colonization and infection in the respiratory tract,[8,9] which result in changes in the macroscopic appearance of secretions, which can range from mucoid to purulent. Purulence is intimately related to the structural conformation of mucus and, therefore, to its rheological profile, both of which affect the transport velocity.[10]

The objective of the present study was to compare the transport properties of respiratory secretions, classified by their surface properties (adhesive or nonadhesive) and by their appearance (mucoid or purulent), in individuals with bronchiectasis unrelated to cystic fibrosis.

Methods

In this study, we evaluated 35 clinically stable patients treated at the Pulmonology Outpatient Clinic of the University of São Paulo at Ribeirão Preto School of Medicine *Hospital das Clínicas*, located in the city of Ribeirão Preto, Brazil. Patient ages ranged from 22 to 81 years, and all of the patients had been diagnosed with bronchiectasis unrelated to cystic fibrosis. The diagnoses had been made on the basis of history-taking and complete clinical examination, having been confirmed by CT.

Patients who presented with an amount of respiratory secretion that was insufficient for the analyses were excluded from the study, as were those who had developed any type of respiratory infection in the last four weeks.

The study was approved by the local research ethics committee, and all participants gave written informed consent.

At the time of their inclusion in the study, some of the participants were using medication: 19 were using bronchodilators; 11 were using corticosteroids; and 2 were using expectorants. In addition, 2 participants were using nocturnal home oxygen therapy.

On the day of collection, the patients who had been using expectorants were instructed to discontinue the use of the drugs. Mucus samples collected after voluntary cough were placed into glass containers. The mucus was separated from the saliva and then evaluated in terms of its adhesiveness and purulence. After this initial evaluation, the respiratory secretions were placed into plastic tubes and covered with mineral oil to prevent dehydration of the samples, which were then frozen for subsequent analysis.[11]

We evaluated the following parameters:

- Surface properties—Immediately after the secretion had been separated from the saliva, the mucus was evaluated in terms of its "pourability" (adhesiveness), being graded on a scale ranging from 1 (adheres closely to the container) to 4 (pours easily).[12] The samples that received adhesiveness grades of 1 or 2 were classified as adhesive, whereas those that received adhesiveness grades of 3 or 4 were classified as nonadhesive.
- Appearance—Immediately after the analysis of adhesiveness, the mucus was evaluated in terms of its appearance, being graded on a scale ranging from 1 (mucoid) to 5 (yellow or green).[13] The samples that received grades from 1 to 3 were classified as mucoid, whereas those that received grades of 4 or 5 were classified as purulent.

- Measurement of relative transport velocity (RTV) on frog palate—Frogs (*Rana catesbeiana*) were decapitated, and their palates were removed. Frog palates were stored at 4°C for 48 h, after which the endogenous (control) mucus was collected. Small quantities of mucus previously collected from the patients were removed from the plastic tubes, submerged in ether to remove excess mineral oil, and placed in a region of the frog palate. Sample displacement was observed with the aid of a stereomicroscope (Stemi1000; Carl Zeiss, Oberkochen, Germany), at a magnification of ×8, and transport time was registered with the aid of a stopwatch (8904; Herweg, Timbó, Brazil).[14,15] The results are expressed as RTV, which was defined as the ratio between the transport velocity of the experimental mucus (mean of five measurements) and that of the frog mucus (mean of two measurements—initial and final).
- Measurement of transport in a simulated cough machine (SCM)—The analyses of mucus displacement in the SCM were performed in accordance with the model initially described by King et al.[4] The SCM was manufactured in the precision mechanics workshop at the Heart Institute of the University of São Paulo School of Medicine *Hospital das Clínicas*, located in São Paulo, Brazil. The SCM comprised a pressure source, a solenoid valve, and a simple facsimile of the airways, composed of a dry acrylic cylinder (length, 30 cm; internal diameter, 4 mm). The three components of the SCM were connected in series. The pressure source was composed of an oxygen tank with a pressure-reducing valve for maintaining a constant pressure of 4.2 kgf/cm^2 throughout the experiment.[14] When the SCM was activated, the timing mechanism controlled the opening of the solenoid valve, which remained opened for 1 s, releasing a burst of air at the aforementioned pressure, thus dislodging the secretion. All mucus samples were tested five times, and the mean of these displacement measurements was used.
- Measurement of the contact angle (CA)—The CA represents the surface tension of the mucus on a solid, flat glass surface. In order to measure the CA, the samples were prepared as previously described, and the angle formed between the mucus and the glass surface was measured in a goniometer equipped with a 20× eyepiece. The glass surface used for these analyses was treated with sulfochromic acid to eliminate electrical currents. The samples were evaluated five times, and the mean of these measurements (in degrees) was used.[14,15]

For the proposed comparisons, we used ANOVA models, with a level of significance set at 5%. These models are used in order to compare two or more independent groups and is based on the assumption that the residual obtained by subtracting the values predicted by the model from the values observed, or vice versa, presents normal distribution, with a mean of zero and constant variance.

Results

Of the 35 participants of the present study, 15 were female and 20 were male. The mean age of the participants was 54.14 years (range, 22-81 years). The etiologies of bronchiectasis were as follows: idiopathic, in 8 (23%) of the patients; recurrent pneumonia with a history of infections, principally in childhood, in 18 (51%) of the patients; tuberculosis sequelae, in 8 (23%) of the patients; and immunoglobulin deficiency, in 1 (3%) of the patients.

Table 1 shows the distribution of the secretion samples from the 35 participants of the present study, by macroscopic appearance and adhesiveness.

Of the 19 samples of purulent appearance, sputum culture was performed in 12, of which 7 tested negative and 5 tested positive

Table 1 - Distribution of the secretion samples collected from the 35 participants, by macroscopic appearance (mucoid or purulent) and adhesiveness (adhesive or nonadhesive).

Characteristics	n
Macroscopic appearance	
Mucoid	16
Purulent	19
Adhesiveness	
Adhesive	10
Nonadhesive	25

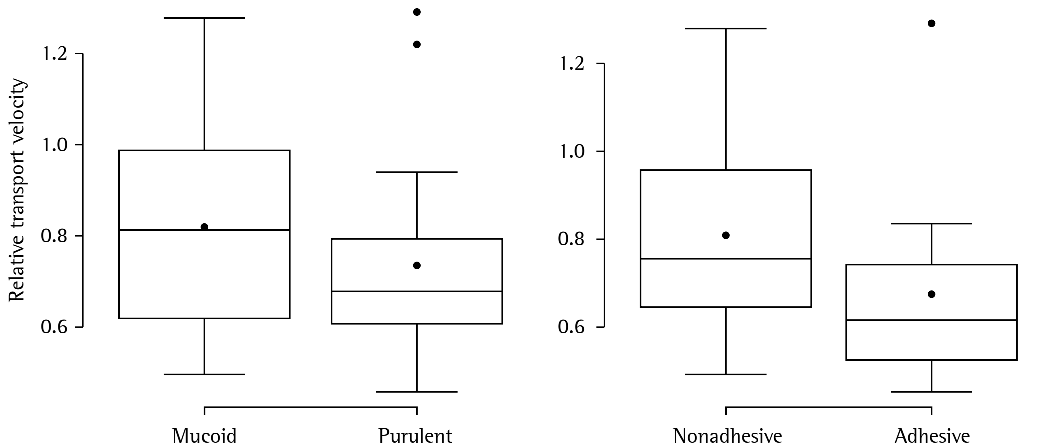

Figure 1 - Median, lower quartile, upper quartile, and interquartile range of the relative transport velocity values for the samples (mucoid vs. purulent and nonadhesive vs. adhesive).

(3 for *Pseudomonas aeruginosa*, 1 for *Serratia marcescens*, and 1 for *Acinetobacter baumannii*).

Regarding smoking status, 3 of the patients were smokers (mean smoking history, 43 pack-years), 12 were former smokers (mean time since smoking cessation, 23 years), and 10 were never smokers.

The spirometric data revealed that 8 of the participants presented normal pulmonary function test results, 2 presented a restrictive pattern, and 25 presented airflow obstruction. Among the 25 participants who presented airflow obstruction, the obstruction was extremely severe in 5, severe in 11, moderate in 7, and mild in 2. For the sample as a whole, the means and standard deviations of the values for FVC, FEV_1, and FEV_1/FVC (all expressed as percentages of the predicted values) were, respectively, 74.00 ± 21.05%, 56 ± 27%, and 62.00 ± 22.86%.

In terms of the RTV on frog palate, there were no significant differences between mucoid and purulent samples (0.82 ± 0.22 and 0.74 ± 0.22, respectively) or between nonadhesive and adhesive samples (0.81 ± 0.20 and 0.68 ± 0.24, respectively).

Figure 1 compares the mucoid and purulent samples, as well as the nonadhesive and adhesive samples, in terms of the RTV.

In terms of displacement in the SCM, there were significant differences between nonadhesive and adhesive samples (8.93 ± 2.81 cm vs. 6.52 ± 1.88 cm; $p < 0.05$), as well as

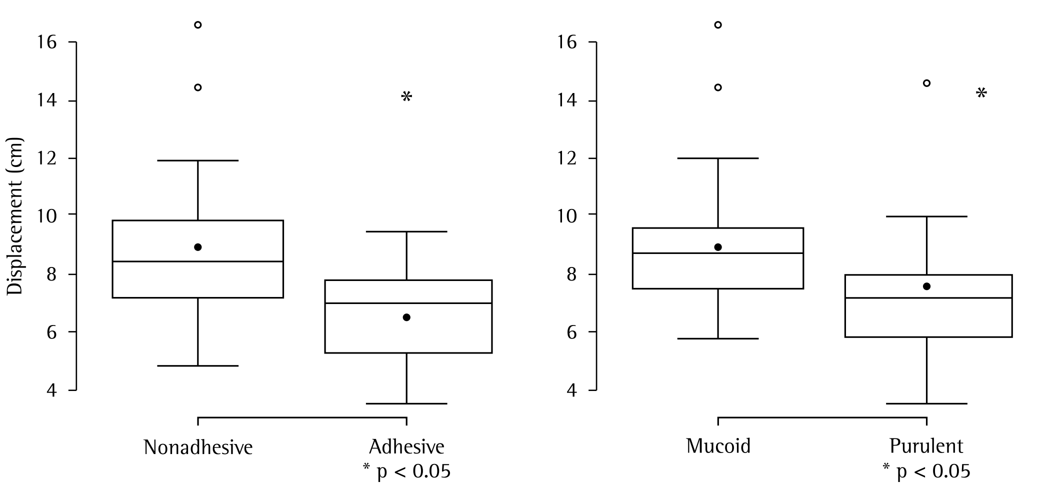

Figure 2 - Median, lower quartile, upper quartile, and interquartile range of the simulated cough machine displacement values for the samples (nonadhesive vs. adhesive and mucoid vs. purulent).

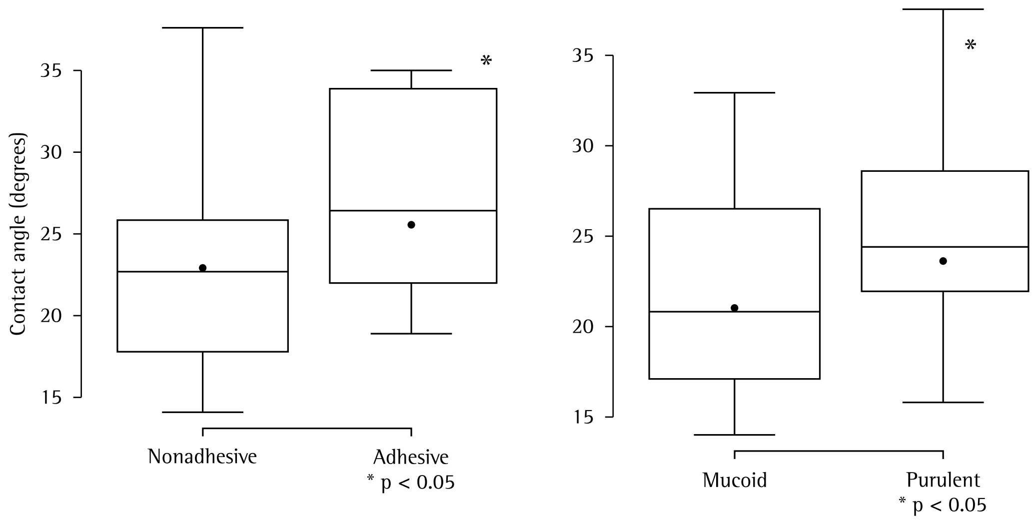

Figure 3 - Median, lower quartile, upper quartile, and interquartile range of the contact angle values for the samples (nonadhesive vs. adhesive and mucoid vs. purulent).

between mucoid and purulent samples (9.04 ± 2.48 cm vs. 7.57 ± 2.91 cm; $p < 0.05$).

Figure 2 compares the nonadhesive and adhesive samples, as well as the mucoid and purulent samples, in terms of the displacement in the SCM.

In terms of the CA values, there were significant differences between nonadhesive and adhesive samples (22.53 ± 5.92° vs. 27.08 ± 6.13°; $p < 0.05$), as well as between mucoid and purulent samples (21.71 ± 5.89° vs. 25.61 ± 6.12°; $p < 0.05$).

Figure 3 compares the nonadhesive and adhesive samples, as well as the mucoid and purulent samples, in terms of the CA values.

Discussion

In the present study, we evaluated respiratory secretion samples collected from 35 patients with bronchiectasis unrelated to cystic fibrosis. After classifying the samples by purulence and adhesiveness, we determined the RTV on frog palate, displacement in an SCM, and CA.

The adhesive and purulent samples showed less displacement in the SCM and a higher CA than did the nonadhesive and mucoid samples.

Our results contribute to a better understanding of the changes in mucus transport that are caused by the underlying disease and associated factors. The results of the present study, in combination with those of other studies, will allow us to identify reference parameters to evaluate respiratory system impairment, isolating factors such as the underlying disease, the severity of the disease, and the degree of contamination. If these reference parameters are not established in the literature, findings will not be reproducible, which will limit the understanding of the evolution of the disease and of the mechanisms or benefits of potential therapeutic intervention strategies.

In individuals with bronchiectasis, the secreted expectoration can range from mucoid to purulent, a fact that is intimately related to the general status of patients, since these individuals are commonly infected with germs, such as pneumococci, *Haemophilus influenzae*,[8,9] *Streptococcus pneumoniae*, *Moraxella catarrhalis*, and *P. aeruginosa*.[8]

Although the classification based on the macroscopic appearance of sputum is controversial among certain authors due to the lack of homogeneity of the color and the subjectivity of the choice,[16] other authors have stated that, in patients with bronchiectasis,[17,18] COPD,[19] or chronic bronchitis,[20] respiratory secretion purulence (an increase in color) correlates with the presence of inflammatory mediators and bacteria,[18,20] as well as with greater secretion volume,[17] worsening of the rheological profile,[10,21] lower ciliary beat frequency, and greater transport by cough[20] whereas a transparent to white (mucoid) appearance does not. Therefore, specific scales have been developed in order to make the evaluation of the macroscopic appearance more definitive and subjective.[16]

One group of authors[17] suggested that, even when the macroscopic appearance of sputum is evaluated with scales that have more than two alternatives (purulent and mucoid), there is no difference between raters in terms of the choice between these two alternatives (i.e., which samples are classified as purulent and which are classified as mucoid).[17]

In the present study, the secretion samples were collected by means of voluntary expectoration, and the macroscopic appearance was evaluated with the purulence index described by Deneuville et al.[13] At the time of the evaluation of the variables, we used the purulence index to classify the samples, according to their appearance, as mucoid (index of 1 to 3) or purulent (index of 4 or 5).

One group of authors[20] studied respiratory secretion samples collected from patients with bronchiectasis and found that the RTV on frog palate ($p < 0.05$) was lower in those of purulent appearance (0.80 ± 0.06) than in those of mucoid appearance (0.9 ± 0.1). Although the difference, in terms of the mucociliary transport velocity, between the purulent and mucoid samples evaluated in the present study (0.74 ± 0.22 vs. 0.82 ± 0.22) was slightly lower than was that found in the aforementioned study,[20] the difference between the two studies was not significant.

Another group of authors[19] used a bovine trachea model to evaluate mucociliary transport in patients with bronchiectasis and reported a decrease in the transport of purulent and mucoid secretions. However, no correlation was found between purulence and the presence of *P. aeruginosa*.

The authors of another study[21] stated that a more purulent mucus translated to a greater viscosity and a lower elasticity; although the rheological properties of the respiratory secretions were not evaluated in the present study, it is known that mucociliary transport rates and cough transport rates are influenced by the rheological characteristics (viscosity and elasticity) of respiratory mucus.[5,6,13]

Although previous studies have demonstrated a decrease in the ciliary transport of purulent secretions,[19,20] the results of the present study revealed that the impairment of the ciliary transport of purulent samples was not greater than was that of the ciliary transport of mucoid samples, which is probably due to the fact that transport was already decreased in all samples (including in those of mucoid appearance), since the ideal relative velocity for optimal transport ranges from 1.0 to 1.1.[14,22]

Another variable that can interfere with the transport of respiratory secretions, principally with the mechanism of displacement by airflow, is adhesiveness,[7] which characterizes the attraction between an adherent (mucous) surface and an adhesive system[23] and is related to the surface tension on surfaces and to the CA formed between the solid and liquid surfaces.[24]

Since the methods for determining the surface tension of gelatinous substances (such as mucus) are difficult to perform,[25] we evaluated the CA and adhesiveness, described in some studies as "pourability".

Since we are not familiar with the classification of adhesiveness by CA, we adopted the adhesiveness grading system, which also evaluates factors such as viscosity and surface tension,[24] as a reference for the evaluation of secretion samples and their categorization as adhesive or nonadhesive.

In the present study, adhesiveness was graded as previously described,[12] and, at the time of the evaluation of the variables, the samples were classified as adhesive (grade 1 or 2) or nonadhesive (grade 3 or 4). In order to avoid the problems related to the evaluation of small subgroups, including the possibility that a large number of subdivisions might not express markedly distinct types, we decided to classify the adhesiveness of the samples as one of only two types, following the example of systems used in order to classify samples by color.[17]

In the present study, the evaluation of the samples by adhesiveness revealed no significant differences in mucociliary transport, which is in agreement with the findings of another study[24] and is due to the fact that truly effective mucociliary transport is more complex and is dependent on the viscoelastic properties of the secretion.

In the present study, adhesive samples showed less displacement in the SCM than did nonadhesive samples (6.52 ± 1.88 cm vs. 8.93 ± 2.81 cm). This result can be explained by the very mechanism of secretion transport by airflow, which corresponds to the passage of gas, which can separate the mucus from the respiratory epithelium,[24] and depends principally on the rheological properties and adhesiveness of the respiratory secretion.

Purulence and adhesiveness are major aspects of respiratory secretion, as are the rheological properties,[10,13,23] hydration,[3,23] and surface tension.[13] Although we did not evaluate aspects related to the hydration status of the existing secretion, we found that adhesive and purulent samples showed higher CAs and less displacement in the SCM than did nonadhesive and mucoid samples, a fact that might reflect an increase in the forces of attraction and cohesion between molecules,[26] changing the structural conformation of the secretion and, consequently, its rheological profile.[10]

With regard to displacement in the SCM, we found mean values ranging from 6.52 to 9.04 cm. This small variation, explained by the division into groups according to the macroscopic appearance and adhesiveness grade—which created groups that were more homogeneous—does not correspond to the variation found in studies that used the same model of SCM, studies in which the displacement measurements varied greatly, the mean values ranging from 2.2 to 19.6 cm.[14,22,27]

High CA values can impair mucociliary transport and bronchial mucus transport by cough.[5] According to one group of authors,[28] mucus samples with a CA lower than 20° provide better protection and lubrication. In the present study, the groups of samples evaluated showed mean CA values ranging from 21° to 27°.

The possible interference of certain drugs with secretion transport is an issue that remains controversial. In the present study, 19 patients were using bronchodilators, and 11 were using corticosteroids. However, the effect of these medications on bronchial secretion transport has yet to be clearly defined.[29] Regarding the use of expectorants, only 2 patients used this medication, and they were instructed to discontinue the use of the drugs on the day of secretion collection. In addition, a recent systematic review failed to clearly establish any effects of these drugs on secretion transport.[30]

Studies of bronchiectasis, principally those conducted in developing countries, represent attempts to gain a better understanding of respiratory system changes and impairment in individuals with the disease. However, as we gain a better understanding of the issue, the formulation of increasingly homogeneous groups becomes necessary, a task that is extremely difficult, since bronchiectasis is related to various underlying etiologies, age brackets, and degrees of contamination, among other confounding factors. The obstacles encountered in the present study include the difficulty in monitoring hydration and the limited number of tools for the evaluation of the rheological properties of respiratory secretion. However, our results show that easily assessable, macroscopic characteristics can provide important information regarding the transport properties of respiratory secretions collected from patients with bronchiectasis.

We conclude that, regardless of adhesiveness or purulence, the respiratory secretions of patients with bronchiectasis have properties that impair mucociliary transport. In addition, secretions that are more adhesive or purulent have worse transport properties, as demonstrated by the lesser degree of displacement in the SCM and the higher CA values.

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