Original Article

Physicochemical properties of human tracheobronchial sputum maintained at room temperature*

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Abstract

Objective: To evaluate the effect that maintaining tracheobronchial sputum at room temperature has on the analysis of ciliary activity and cough transportability, as well as on the contact angle. **Methods:** Mucoid sputum was collected from 30 individuals without pulmonary diseases, and purulent sputum was collected from patients with bronchiectasis. The samples were analyzed immediately after collection and again after 24 h. **Results:** After 24 h at room temperature, the purulent sputum presented an increase in cough-induced displacement (96 ± 50 *vs.* 118 ± 61 mm) and a decrease in the contact angle (32 ± 6 *vs.* 27 ± 6 degrees) ($p < 0.05$). For the mucoid sputum, there were no alterations in the parameters analyzed. **Conclusion:** Mucoid tracheobronchial sputum can be stored at room temperature for 24 h without presenting alterations in ciliary transport or contact angle. However, purulent sputum should not be stored at room temperature for many hours, since cough transportability and contact angle might be altered as a result.

Keywords: Temperature; Sputum; Cough; Mucociliary Clearance.

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Introduction

Human tracheobronchial sputum is commonly studied in vitro[1] with the objective of evaluating the physiology and physiopathology of the respiratory tract[2] as well as the response to different therapeutic measures.[3]

The in vitro study of human sputum consists of evaluating the physicochemical properties of the sputum, such as its capacity to be transported through ciliary activity and cough, which is also known as transportability. The chemical properties, which express, in detail, the composition of the sputum, as well as the physical properties, such as viscoelasticity, adhesiveness, spinnability and wetability, influence in vitro and in vivo sputum transport.[4]

The methods of studying in vitro transport have the advantage of determining the transportability of the sputum, irrespective of existing alterations in the ciliated epithelium of the airways or in the capacity to generate adequate flow for efficacious coughing.[4] Those methods are very important, principally in terms of the scientific validation of bronchial hygiene techniques or the use of mucolytic drugs, both of which have the ultimate objective of eliciting the expectoration of sputum.

With the increasing interest in studies on tracheobronchial sputum, the detailing of technical factors that may interfere with the analyses of the sample properties is essential. It is known that sputum should be analyzed immediately after collection[5] or frozen for later analysis at temperatures of -20 °C,[6] -70 °C[1] or -80 °C.[6] However, until the sputum sample is frozen or analyzed, it remains at room temperature. That period should be short in duration but, when that is not possible, what are the consequences of sputum remaining at room temperature for prolonged periods? Do samples of different macroscopic aspects present different behaviors in relation to their physical properties?

There are reports of sputum analysis carried out 60 min,[5] 120 min,[7,8] 180 min[9] and 240 min[10] after the collection. Some authors have observed that storing sputum at 37 °C for 60 min,[9] or at room temperature for 24 h,[7] decreases its viscosity. However, these authors utilized the sputum samples of only one patient and did not clarify the degree of purulence of the samples.

The objective of the present study was to determine the effect that maintaining sputum at room temperature for 24 h has on the contact angle and transportability of tracheobronchial mucoid and purulent sputum.

Methods

The sputum of individuals without pulmonary diseases, considered mucoid, was collected from 21 men and 9 women with a mean age of 37 ± 21 years who had been submitted to general anesthesia for extrathoracic surgery. Those individuals had no history of pulmonary diseases or any signs of viral or bacterial respiratory tract infection within the preceding six weeks.

Purulent sputum was collected from 20 patients (12 men and 8 women) with bronchiectasis not resulting from cystic fibrosis. The mean age was 52 ± 10 years. The bronchiectasis was diagnosed through clinical testing, chest X-rays or computed tomography scans of the chest. Yellowish or greenish sputum samples were considered purulent, with the number of leukocytes per field being ≥ 24 and the number of epithelial cells per field being ≤ 10.[11] The number of cells in ten microscopic fields at a magnification of ×1000 was observed.

This study was approved by the research ethics committees at the Triangle University Center and the Federal University of Sao Paulo. All of the patients gave written informed consent.

The mucoid sputum was obtained by scraping the inner surface of the endotracheal tube, after extubation, of patients submitted to extrathoracic surgery under general anesthesia.[12] The purulent sputum was obtained from phlegm, thereby avoiding salivary contamination. The sputum was collected in, at most, three coughing sessions without the prior use of any muco-active medication or physical therapy technique.

Immediately after collection, the samples were stored in plastic containers submersed in mineral oil, which was utilized to avoid the dehydration of the sputum.[13] Prior to the analysis of the sputum samples, the mineral oil was removed by immersing the samples in petroleum ether.

The temperature of the laboratory where the samples were stored was maintained at approximately 20 °C, although the relative humidity was not regulated.

One sputum sample from each individual was analyzed at 1–2 h after collection and again after 24 h at room temperature. The mucoid sputum, due to the small quantity collected, was evaluated only in terms of the ciliary transportability and contact angle.

The purulent sputum was studied in terms of the contact angle, as well as in terms of the ciliary and cough transportability.

The contact angle of the sputum was measured in accordance with the method described by Girod *et al.*,[4] immediately after the sample had been deposited on a slide. Five measurements of the contact angle were taken, and the mean value was calculated.

Ciliary transportability was evaluated using the frog palate method.[6,14] The isolated palate of *Rana catesbeiana* frogs was used. The time required for the sample to move 6 mm in the frog palate was measured five times per sample. The velocity of the sputum was expressed as relative velocity, which consists of the mean velocity of the sputum test divided by the mean velocity of frog mucus, which was used as a control.

The cough transportability was studied with a simulated cough machine developed by King *et al.*,[15] adapted by Gastaldi *et al.*[6] and having the following characteristics: oxygen pressure of 4.2 kgf/cm^2; solenoid valve opening time of one second; cylindrical acrylic tube of 4 mm in internal diameter and 30 cm in length. For each sputum sample, five measurements of cough-induced displacement were taken, and the mean value was calculated.

The results were analyzed using the Wilcoxon test, and a level of 5% was considered statistically significant. Despite the fact that nonparametric statistics were used, we opted to describe the data using means and standard deviations in order to facilitate the comprehension of the results.

Results

In this study, it was noted that there was no statistically significant difference between the samples of mucoid sputum analyzed at 1–2 h after the collection and those analyzed after 24 h at room temperature, both with respect to ciliary transportability (0.87 ± 0.19 *vs.* 0.9 ± 0.2, respectively) and contact angle (26 ± 3 *vs.* 23 ± 3 degrees, respectively) (Figure 1).

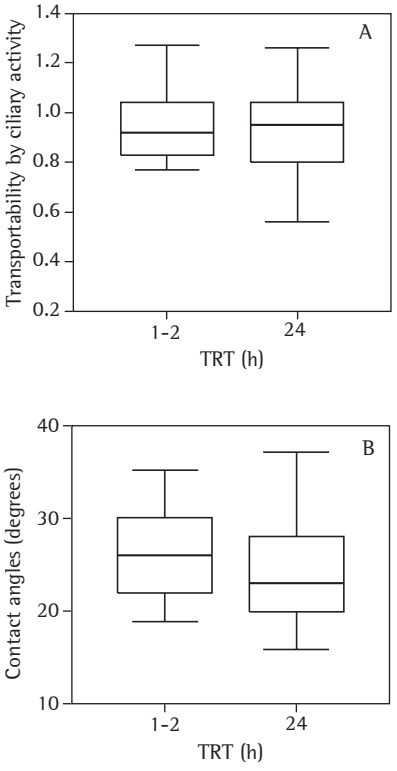

Figure 1 - Transportability by ciliary activity and contact angle (degrees) of mucoid tracheobronchial sputum analyzed at 1–2 h and again at 24 h after collection. TRT = time at room temperature (prior to analysis).

In relation to the purulent sputum, there was no statistically significant difference with respect to ciliary transportability (0.72 ± 0.24 *vs.* 0.69 ± 0.19, respectively), although there was an increase in the displacement in the simulated cough machine (96 ± 50 *vs.* 118 ± 61 mm, respectively; $p < 0.05$) and a decrease in the contact angle values (32 ± 6 *vs.* 27 ± 6 degrees, respectively) (Figure 2).

The statistical calculations were conducted for only 19 of the 20 samples studied, since one of them became liquefied 24 h after collection, precluding its analysis. In addition, all of the samples of purulent sputum presented a decrease in their consistency, i.e. they became more fluid, thereby making their handling difficult.

Discussion

In the present study, there were no statistically significant differences in the ciliary transportability

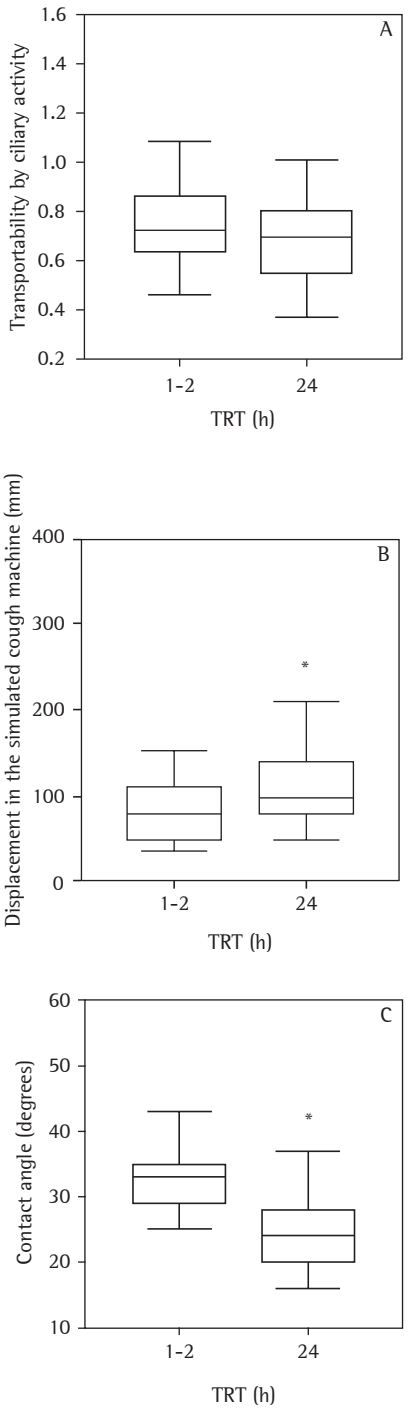

Figure 2 - Transportability by ciliary activity, displacement (in mm) in the simulated cough machine and angle of contact (degrees) of purulent tracheobronchial sputum analyzed at 1–2 h and again at 24 h after collection. $p < 0.05$. TRT = time at room temperature (prior to analysis).

or contact angle of the mucoid sputum after 24 h at room temperature. We believe that the lack of alteration of the sputum properties was due to the small number of inflammatory cells, bacteria and enzymes present in the sputum of individuals without pulmonary diseases,[16] in addition to the fact that the method of collection results in little contamination by saliva.

However, the purulent sputum presented alterations not only in ciliary transportability; there was a statistically significant decrease in the contact angle values and an increase in displacement in the simulated cough machine at 24 h after collection ($p < 0.005$). This indicates a degenerative effect on the structure of the sputum, which was confirmed by the decrease in its consistency. Some authors[17] have associated the liquefaction of sputum stored at room temperature with enzymatic activity. Confirming this precept, other authors[18] observed that alpha-naphthyl acetate esterase, present in the alveolar macrophages of sputum, bronchial secretions and alveolar lavage, still presented some function after 72 h, despite having presented a progressive loss of activity after 24 h at room temperature.

It is known that the sputum of bronchiectasis patients contains a large number of enzymes, principally neutrophilic proteases.[19] Therefore, although the enzymatic activity of the samples of the present study was not evaluated, it is believed that the alterations of the purulent sputum may have occurred due to the action of enzymes released by the lysis of leukocytes and bacteria in the mucin secretory pathway. One example of an enzyme released in sputum by bacteria is the metalloproteinase elastase derived from *Pseudomonas aeruginosa*.[20]

In conclusion, tracheobronchial mucoid sputum can be stored at room temperature for 24 h without any alteration in its ciliary transportability or contact angle. Purulent tracheobronchial sputum, however, should not remain at room temperature for many hours, since it undergoes alterations in its physical properties and can become liquefied. However, since no intermediate analyses between 2 h and 24 h after collection were carried out, future studies are warranted in order to determine the maximum time that purulent sputum can remain at room temperature, thereby allowing alterations in its structure and, consequently, transportability to be avoided.

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