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The many ways sputum flows – Dealing with high within-subject variability in cystic fibrosis sputum rheology

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ABSTRACT

We evaluated test-retest reliability of sputum viscoelastic properties in clinically stable patients with cystic fibrosis (CF). Data from a prospective, randomized crossover study was used to determine within-subject variability of sputum viscoelasticity (G', storage modulus and G", loss modulus at 1 and 10 rad s^{-1}) and solids content over three consecutive visits. Precision of sputum properties was quantified by within-subject standard deviation (SD_{ws}), coefficient of variation (CV) and intraclass correlation coefficients (ICC). Fifteen clinically stable adults with CF (FEV_1 range 24–94% predicted) were included. No differences between study visits (mean ± SD 8 ± 2 days) were observed for any sputum rheology measure. CV's for G', G" and solids content ranged between 40.3–45.3% and ICC's between 0.21–0.42 indicating poor to fair test-retest reliability. Short-term within-subject variability of sputum properties is high in clinically stable adults with CF. Investigators applying shear rheology experiments in future prospective studies should consider using multiple measurements aiming to increase precision of sputum rheological outcomes.

1. Introduction

Increased production of mucus with abnormal biophysical properties is a typical feature of cystic fibrosis (CF) lung disease (Wine et al., 2018). One of the goals of CF therapy is to improve mucociliary clearance by changing aberrant rheological properties of airway mucus that trigger mucus plugging, advancing chronic infection and inflammation (Ehre et al., 2014). Rheological measurements have been applied in different research contexts in CF, for example, in the study of the biophysical properties of mucus (Hill et al., 2014; Horsley et al., 2014), its association with clinical status (Tomaiuolo et al., 2014), acute exercise (Dwyer et al., 2011, 2017), and in clinical trials investigating effects of intravenous antibiotics (Serisier et al., 2009) or mucolytic therapy alone (Bucki et al., 2015; Daviskas et al., 2010; Shah et al., 1996) or in combination with airway clearance therapy (Dasgupta et al., 1998). Surprisingly, there is a clear lack of shear rheology studies investigating test-retest reliability characteristics of sputum viscoelastic properties in CF. Importantly, knowledge on variability characteristics of these markers is a prerequisite for the correct interpretation of intervention effects and the basis for sample size calculations. To our knowledge, only a conference communication reported on within-subject variability of sputum viscoelasticity in adults with CF (Dwyer et al., 2008) indicating overall poor reliability for G' (storage modulus) and G" (loss modulus).

The aim of this study was to evaluate the short-term within-subject variability (test-retest reliability) of sputum properties in clinically stable adults with CF.

2. Methods

2.1. Study design

We analyzed sputum rheological data from a randomized, controlled, crossover trial (Clinicaltrials.gov, NCT02750722) conducted at the Adult CF Center of the University Hospital Zurich. Inclusion criteria were: i) a confirmed diagnosis of CF based on either two CF-causing mutations and/or a sweat chloride concentration during two tests of > 60 mmol/l, ii) age ≥18 years and iii) ability to provide sputum samples. Exclusion criteria were: i) listing for lung transplantation or status post lung transplantation, ii) infection with *Burkholderia* cepacia

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complex, iii) unstable clinical condition (i.e., major hemoptysis or pneumothorax within the last 3 months, acute pulmonary exacerbation (Fuchs et al., 1994), intravenous antibiotic treatment during the last 4 weeks, change in pulmonary medication during the study period).

2.2. Study participants

Patients visited our laboratory facility on three different occasions, scheduled at the same time of the day (+ −1 h deviation). The time period between study visit 1 and 3 was 8 ± 2 days, respectively. Prior to each study visit, patients were told to abstain from fatty meals (for 3 h), caffeine-containing substances (for 4 h) and to avoid vigorous physical activity during the last 24-h. Moreover, patients were told not to perform airway clearance therapy before the visits. All assessments were done pre-bronchodilation (i.e., withheld of short-acting bronchodilators and anticholinergic drugs for at least 4 h, long-acting bronchodilators for at least 12 h, and once-daily, long-acting bronchodilators for at least 24 h). Ethical approval was obtained from the Cantonal Ethics Committee of Zurich (2015–00153), Switzerland. All patients provided written informed consent.

2.3. Sputum samples

At the beginning of each study visit, one sputum sample was collected. Sputum was gently expectorated and collected into sterile and coded containers (cryotubes 5 mL, VWR). The containers were immediately stored at −4 °C and transferred on ice into a deep freezer (−80 °C) after each study visit.

2.4. Sputum rheology

Rheological measurements were performed on a MCR 702 rheometer (Anton Paar, Austria) in parallel plate mode, using sandblasted 25 mm diameter stainless steel plates (PP25 S, Anton Paar, Austria) and a gap size of 0.5 mm. First, a frequency sweep was performed (0.1–100 rad s^{-1}, $\gamma = 1\%$) followed by an amplitude sweep (0.1–1000%, $\omega = 1$ rad s^{-1}). The snap frozen sputum samples (−80 °C) were transferred to the fridge (4 °C) at least 6 h before the measurement. The slowly thawed samples were then gently transferred from the cryotubes to the lower measuring plate using a 1 mL micropipette. The micropipette tips were cut in the front with a scalpel to have a larger die, thus minimizing shear on the sample. The upper plate was slowly lowered onto the sputum, and a solvent trap containing moist sponges was placed over the sample. Prior to measurements, the sputum was let to rest for five minutes. All measurements were performed temperature controlled at 20 °C (Serisier et al., 2009).

2.5. Solids content

To estimate sputum solids content, 0.25 mL aliquots of sputum were filled in 1.5 mL HPLC vials (VWR, Switzerland) and weighed with a high precision scale (Mettler AE 163, Mettler Toledo, Switzerland). The samples were then dried for 24 h at 50 °C and a pressure of 100 mbar using a vacuum drying oven (SalvisLab, Switzerland) and subsequently weighed again.

2.6. Spirometry

Spirometry was performed (MasterScreen™ PFT Pro, Jaeger, PanGas AG Healthcare, Switzerland) according to American Thoracic Society/European Respiratory Society standards (Miller et al., 2005). Percent-predicted values for forced expiratory volume in 1 s (FEV_1) were calculated based on equations published by Quanjer et al. (Quanjer et al., 2012).

2.7. Patient-reported health status

We assessed patient-reported health status with the Feeling Thermometer. The Feeling Thermometer is part of the EQ-5D, a common instrument used for healthy economic analyses and established by the EuroQol group (EuroQol, 1990). The Feeling Thermometer is a modified visual analogue scale in form of a thermometer. The instrument has marked intervals from 0 (worst health state = dead) to 100 (perfect health).

2.8. Statistical analysis

All statistical analyses were performed with the statistical software package SPSS version 23 (IBM Corp. Armont, NY, USA). Data are presented as median (interquartile range, IQR) and means (95% confidence intervals, CI). Differences in sputum properties between the three different study visits were analyzed with the non-parametric Friedman Test. Precision of sputum properties was quantified by the within-subject standard deviations (SD_{ws} = root mean square error) calculated by the root-mean-square (RMS) method and the coefficient of variation (CV, SD_{ws}/overall mean) (Gluer et al., 1995). Reproducibility was calculated as 1.96 * √2 * SD_{ws} (95% level of confidence) (Bland and Altman, 1996). In addition, we calculated the least significant change (LSC = 1.96 * √2 * CV), that denotes the smallest difference between measurements that can be considered to represent a true change. Intraclass correlation coefficients (ICC's) and their 95% CI's were calculated using a two-way mixed model [consistency, single measurement, (ICC, 3,1)] (Shrout and Fleiss, 1979). Interpretation of ICC's is based on (Cicchetti, 1994).

3. Results

Sixteen patients participated in the study and all patients were able to provide sputum samples. One patient was excluded from the analysis due to oral antibiotic treatment for acute pulmonary exacerbation between visit 1 and 2. Fifteen patients with a median (IQR) age of 23 (22, 25) years and a forced expiratory volume in 1 s (FEV_1) of 57 (45, 74) % predicted were included. Of those, 53% had CF-related diabetes, 87% were pancreatic insufficient and 40% were chronically colonized with *Pseudomonas* aeruginosa.

3.1. Test-retest reliability

There were no differences in sputum properties between the three study visits (Table 1). No trial effects (i.e., systematic error) were observed for any viscoelasticity variable. Table 2 displays test-retest reliability characteristics for sputum properties. Overall CV's for G' and G" at 1 and 10 rad s^{-1}, and sputum solids content were high (40–45%) and ICC's ranged from 0.22 to 0.42 with wide 95% CI's indicating poor to fair reliability.

4. Discussion

This study demonstrates mainly poor test-retest reliability of sputum properties in adults with CF. This is in line with data from a previous conference communication on 15 CF adults (mean age 29 ± 10 years; FEV_1 52 ± 25% predicted) reporting poor test-retest reliability for G' and G" with ICC's ranging from 0.25 to 0.43 (Dwyer et al., 2008). Despite highly standardized testing conditions and measurement procedures, we found high visit-to-visit variability for shear rheology outcomes. Reasons are likely to be multifactorial, but differences in airway surface liquid composition and/or variations in the contamination of samples with saliva (which we controlled rigorously) could contribute to our findings (Antus, 2016). The low ICC's for G' and G" indicate the challenge of identifying treatment effects and maybe one reason (amongst others) why previous studies failed to show