CHANGE OF MUCUS RHEOLOGY IN PATIENTS WITH CYSTIC FIBROSIS, COPD AND ASTHMA DIAGNOSIS

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Aims

Abnormal level of sputum viscosity is related to an inefficient mucociliary clearance for patients with COPD[1] or Cystic Fibrosis[1,2]. Rheology provides biophysical markers based on elasticity and viscosity measurements of sputum, which promise to identify exacerbations crisis[2,3,4]. In patients with COPD[1], Cystic Fibrosis[1,2] (CF) and Asthma (AST), the present study aims to check the differences in sputum rheology and the reproducibility of rheological measurements over 48h.

Methods

The clinical protocol NCT02682290 was led by and in the Grenoble University Hospital (France) with 4 groups: 10 CF, 10 COPD, 10 AST and 10 healthy volunteers (HV). The eligibility criteria are CF, COPD (any class) and AST patients with bronchial disorder, confirmed by Grenoble University Hospital. The exclusion criteria are: FEV1 ≤ 40%, PaO2 < 60 mmHg at rest, acute exacerbation during the last month, and/or contraindications for spirometry.

Two visits V1 and V2 were performed 48 hours apart. Patients with COPD have a spontaneous expectoration. Patients with AST and HVs need an standard induced expectoration, using a saline solution (4.5%) during 10 minutes to generate an induced sputum. The first sputum out is taken for analysis. Saliva is extracted from the sputum and the mucus plug are homogenized before measurements using a vortex during 30 s. Rheology tests are made at 37°C.

Rheology is the study of the flow of matter that exhibits a combination of elastic, viscous and plastic behaviors. Elasticity, viscosity and plasticity are measured with a rheometer Rheomuco (Rheonova, France; fig. 1). An oscillatory (1 Hz) shear sequence is exerted with a strain sweep (0.1–3000%), from which the sputum rheological properties are extracted (fig. 2). Classically, two rheological properties are extracted: the elastic (G'_p) and viscous (G''_p) moduli at the plateau (in the low-strain region, typically below 10%). New properties were also explored in this study: the damping ratio (tan δ), yield strain (γ_c), yield stress (τ_c) and Elastic force (EF). Rough plates in contact with the sample ensure the sample does not slide during the strain sweep so that the yield point can be reached.

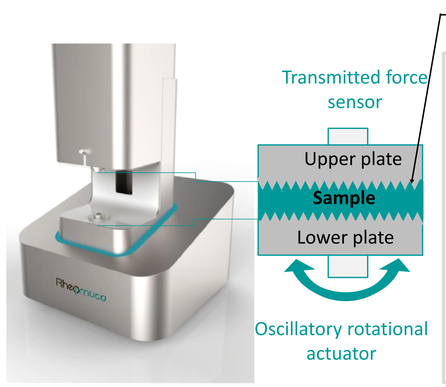


Figure 1: Rheomuco, plate-plate oscillatory rheometer with rough surfaces

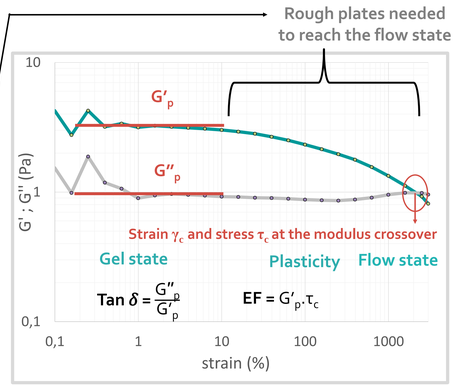


Figure 2: Rheological data extracted from strain sweep curves

Results

Relative variations of rheological properties between the two visits are showed on fig. 3. Linear properties G'_p G''_p, and tan δ are not significantly different ($p >> 0.05$) between both visits, as already reported in the literature[5]. Non linear properties Elastic Force (EF), yield strain (γ_c) and yield stress (τ_c) are all stable ($p >> 0.05$) for CF and COPD. Moreover, variations are at least 3 times lower for non linear properties compared to linear properties. In contrary, γ_c and τ_c of HV and AST show variations over 200%: the induced sputum protocol may be the cause of this variation.

For linear properties on fig. 4, G'_p and G''_p of CF patients are significantly different ($p < 0.05$) from the other groups (fig. 4), because of bacteria DNA in suspension[2]. Indeed G'_p and G''_p characterize the sputum microstructure (molecular network). Both moduli are also significantly higher ($p < 0.05$) in COPD patients compared to healthy volunteers, but not significantly different from the AST group.

For non linear properties on fig. 4, γ_c is not significantly different between the 4 groups. This parameter is related to the common stretchability of sputa, which elongate in strings under stretch. But the level of force needed to stretch sputa, represented by τ_c, is significantly different ($p < 0.05$) between AST/HV groups and CF/COPD groups. The yield stress τ_c represents the gel force of the sputum, and this level of force ($\tau_c > 10$ Pa, fig. 4) may block the cilia, causing with time the bronchial obstruction known in CF and COPD. Elastic Force (EF) is the only parameter significantly different ($p < 0.05$) between each group. EF represents the amount of elastic energy needed to make the sputum flow.

Figure 3: 48 h evolution of the rheological parameters (base = 100%)

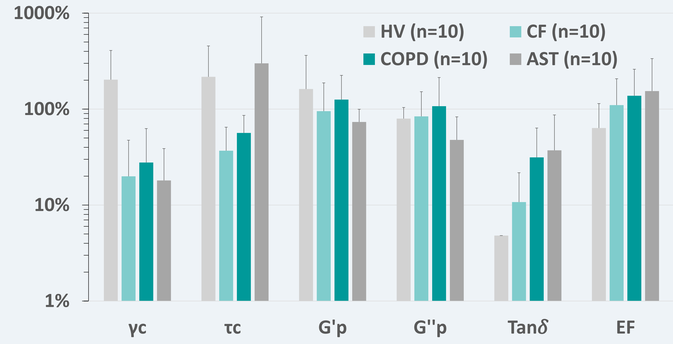
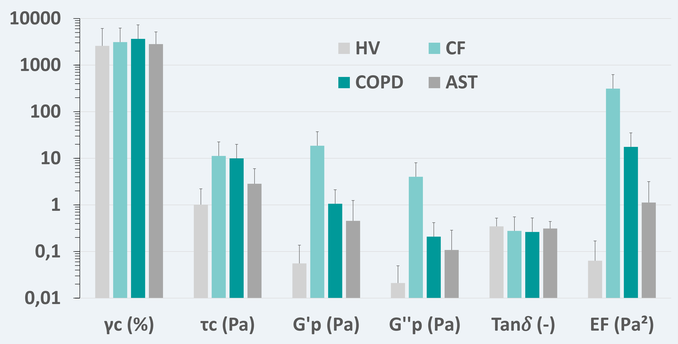


Figure 4 : Change of rheological properties for each group



Conclusions

- The rheology of sputum is different for airways diseases, limited in this study to CF, COPD and AST. Results regarding AST and HV are debatable, since the sputa are induced with a saline solution which may modify sputum properties by dilution. Moreover AST type (mild/severe) has not been differentiated.
- For CF and COPD, non linear properties γ_c and τ_c are more stable than the linear viscoelasticity moduli G'_p and G''_p, because G'_p and G''_p are related to the molecular network and may be sensitive to hydration level of the patient. This effect should be investigated in more details, but in the meantime γ_c and τ_c seem better suited as endpoints. Still EF seems the most relevant single biophysical marker for patient classification and to evaluate the bronchial congestion.
- The high level of elastic behavior (G'_p and EF) in CF is a marker of macromolecules like DNA[2]. G'_p has actually been related to the type of bacteria colonies[2] for CF patients.

The next step is to evaluate the potential of rheology (i) to monitor treatments in CF and COPD patients as a companion test, and (ii) to establish the prognosis of exacerbations. For COPD patients, rheology would give an early-diagnosis and classification criteria.

References

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