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 COPD1 or Cystic Fibrosis1,2. Rheology provides biophysical markers based on elasticity and viscosity measurements of sputum, which promise to identify exacerbations crisis2,3,4. In patients with COPD, Cystic Fibrosis1 (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 HV 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 Rheonuco (Rheonova, France; fig. 1). An oscillatory (1 Hz) shear excitation 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') and viscous (G'') 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 (γy), yield stress (τy) and Elastic force (EF). Rough plates in contact with the sample ensure the same model does not slide during the strain sweep so that the yield point can be reached.

Results
Relative variations of rheological properties between the two visits are shown on fig. 3. Linear properties G', G'', and tan δ are not significantly different (p > 0.05) between both visits, as already reported in the literature4. Non linear properties Elastic Force (EF), yield strain (γy) and yield stress (τy) 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, γy and τy 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' and G'' of CF patients are significantly different (p < 0.05) from the other groups (fig. 4), because of bacteria DNA in suspension5. Indeed G' and G'' 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, γy is not significantly different between the 4 groups. This parameter is related to the common stretchability of sputa, which elongate in strings under stress. But the level of force needed to stretch sputa, represented by τy, is significantly different (p < 0.05) between AST/HV groups and CF/COPD groups. The yield stress τy represents the gel force of the sputum, and this level of force (τy > 10 Pa) 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.

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 γy and τy are more stable than the linear viscoelasticity moduli G' and G''5, because G' and G'' 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 γy and τy 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 modulus G' and EF in CF is a marker of macromolecules like DNA2. G' has actually been related to the type of bacteria colonies1 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
4 Nettle C.J. et al. 2018 “Linear rheology as a potential monitoring tool for sputum in patients with Chronic Obstructive Pulmonary Disease.” Biotechnology 54, 67

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

Figure 2: Rheological data extracted from strain sweep curves

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

Figure 4: Change of rheological properties for each group

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