# Sputum Rheology: a Robust Biophysical Marker for the Monitoring of Cystic Fibrosis Patients

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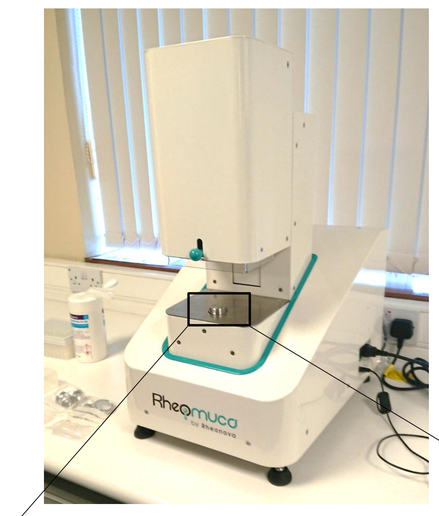
## Aims

In cystic fibrosis (CF), the difficulty to expectorate is associated with abnormal mucus viscoelasticity [1,2]. Sputum rheology has thus been proposed as a physical biomarker to quantitatively assess the patients' condition [3,4] and the treatments' efficacy [5,6]. However, checking the reproducibility of rheological measurements is a prerequisite to validate the potential of sputum rheology as biomarker. In this study, we investigated the within-measurement and within-subject variabilities of sputum rheological measurements [7] in healthy volunteers (HV) and stable CF patients.

## Methods

Rheology is the science of flowing matter: human sputum are fluids that exhibit a combination of elastic, viscous and plastic behaviors. Rheomuco (Rheonova, France; fig. 1) is a medical rheometer that measures viscoelastic properties. Rheomuco was tested at the Grenoble University Hospital (France) in the clinical protocol NCT02682290. 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') and viscous (G") moduli at the plateau (in the low-strain region, typically below 10% strain). New properties were also explored in this study: the properties at the moduli crossover that characterize the mucus flow point, using the crossover modulus $G'_c$ and the yield stress $\tau_c$. 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.

11 CF patients and 11 HV were recruited with the exclusion criteria: 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: during a single visit, rheology measurements were carried out immediately or at t = 10 min (fig. 3). CF patients have a spontaneous expectoration, followed also by 20 minutes rhDNAse nebulisation and again a spontaneous expectoration 1 hour later. HVs undergo an standard sputum induction, using a saline solution (4.5%) during 10 minutes. 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.



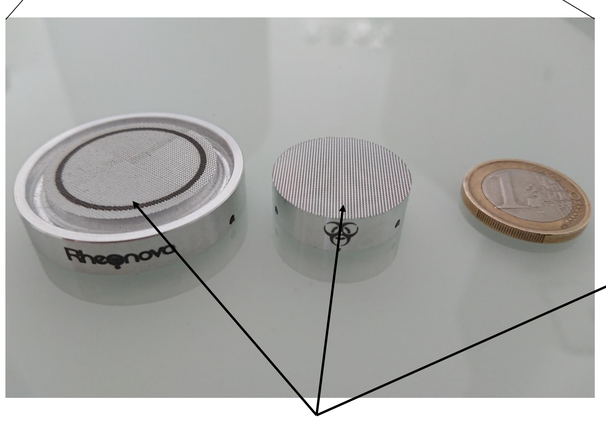




**Figure 1: Rheomuco, plate-plate oscillatory rheometer with rough surfaces**

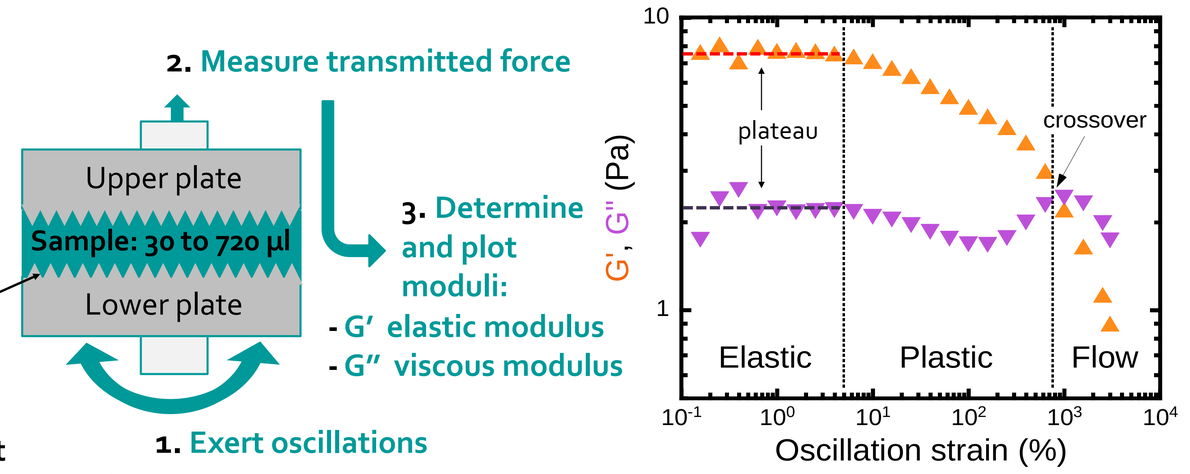



**Figure 2: Sputum properties measurments and data extraction from strain sweep curves**

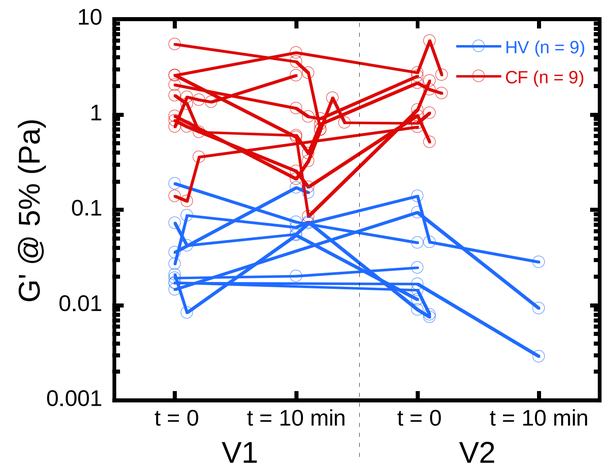
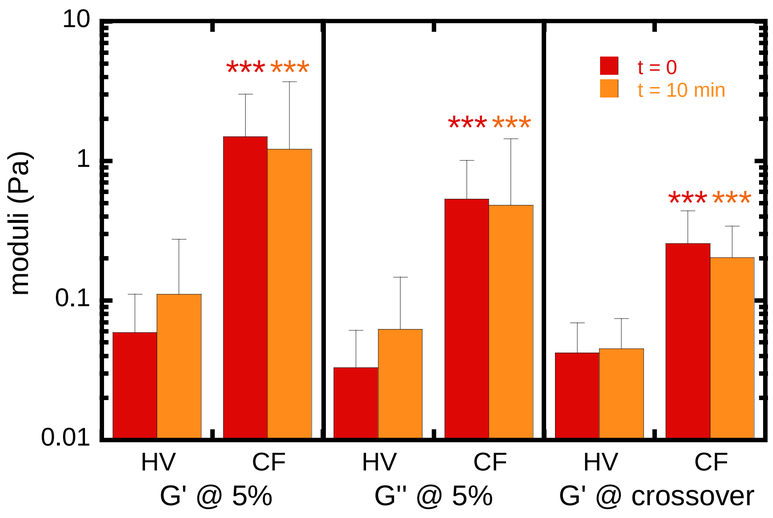



**Figure 3: Aggregated evolutions of the linear elastic modulus G' for 9 HV and 9 CF patients between the 2 visits V1 and V2**

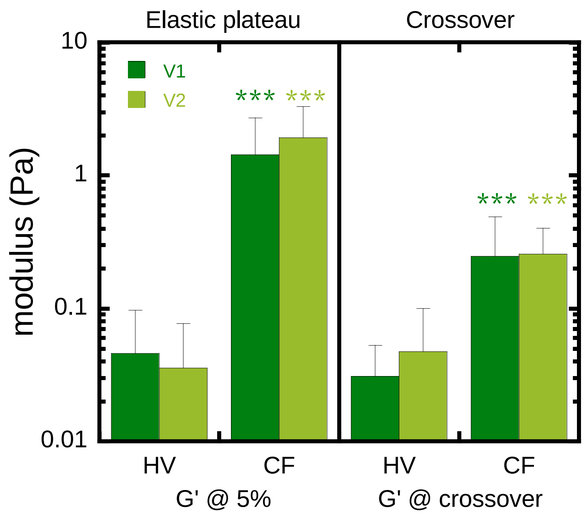
## Results

### I. Repeatability at 10min



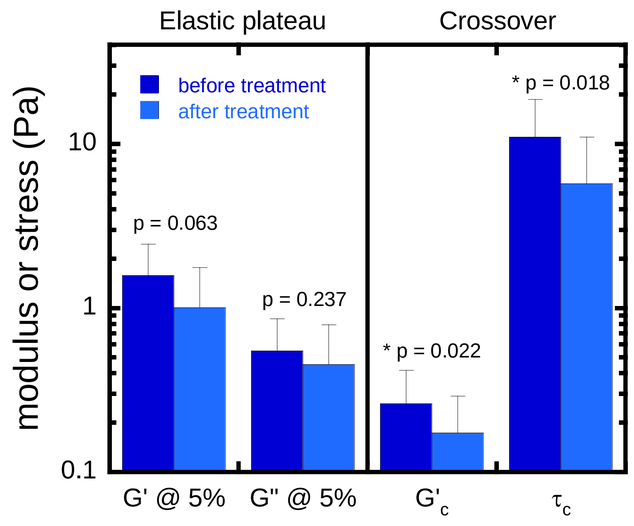

Both linear and crossover moduli strictly distinguish healthy and CF subjects (*** p < 0.001). They vary slightly in consecutive measurements, more markedly in healthy subjects even if only 4 healthy subjects were able to produce enough sputum to make a second test after 10 minutes. Nevertheless, these variations remain within the inter-subject deviation, and the populations remain strictly distinct.

### II. Long-term variability over 48h




Linear and crossover moduli strictly distinguish healthy and CF subjects for 9 HV and 9 CF patients between the 2 visits V1 and V2 (*** p < 0.001). The observed two-days variations of G' can be very strong (increase or decrease by a factor of up to 10), but again the populations remain strictly distinct even on the long term.

### III. Effect of rhDNase




RhDNase is expected to reduce viscoelastic parameters as it favours mucus mobilisation. We observe a small decrease of the linear moduli (statistically non significant), and a more pronounced ($p < 0.05$) decrease of the crossover parameters (modulus $G'_c$ and yield stress $\tau_c = G'_c\gamma_c$ with $\gamma_c$ the crossover strain).

## Conclusions

1. The rheology of sputum varies with time, and these variations can be noticeable in day-to-day basis. However, the temporal variability in a given subject remains smaller than the variability between subjects of the considered population. More importantly, the healthy and CF subjects remain always clearly distinct which confirms that **sputum rheology is a robust biomarker of CF patients condition**.

2. RhDNase has a significant rheological signature at the crossover, i.e. when the sputum starts to flow. This result suggests that **the large-deformation rheology can thus be exploited to assess the physiological efficacy of mucolytics**.

## Perspectives

Sputum rheology is robust to assess the condition of CF patients. However, the within-subject variability of linear viscoelastic moduli remains too high for reliable individual monitoring. The non-linear domain seems promising, as mucus transport processes (cough, tidal breath, mucociliary) involve large deformations. More research is needed to unveil these mechanisms and find biomarkers featuring high levels of both specificity and stability.

## References

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