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Detection of pepsin in sputum: a rapid and objective measure of airways reflux

To the Editor:

The diagnostic test, Peptest (RD Biomed Ltd, Cottingham, UK), detects pepsin in expectorated saliva and is established as a simple, noninvasive measure of reflux of gastric contents. It has been used to detect pepsin A in patients with both gastro-oesophageal reflux disease (GORD) and extra-oesophageal reflux into the laryngopharynx and airways [1–5].

Reflux has a substantial, but as yet undefined, component in the aetiology of cough hypersensitivity syndrome [6]. Currently evaluation is by the subjective Hull Airways Reflux Questionnaire (HARQ) [7] or objective, but costly and invasive, measures such as 24-h pH-metry, impedance [8], and high resolution manometry [8, 9]. These current diagnostic pathways have their limitations in detecting low levels of airways reflux that may be sufficient to cause chronic cough. We hypothesised that Peptest could provide simple objective confirmation of airways reflux in unselected patients with chronic cough.

Peptest was used in routine clinical practice in out-patients attending the Hull Cough Clinic at Castle Hill Hospital (Hull, UK), a secondary and tertiary referral centre. Verbal consent was obtained at the time of attendance. Chronic cough was defined as cough lasting >8 weeks.

Patients were instructed to provide three expectorated saliva/sputum samples into sample collection tubes (containing 0.01M citric acid) during daily activities and immediately after three spontaneous coughing episodes. Sample collection was optimised by providing patient leaflets and a video. The presence and concentration of pepsin was measured using Peptest by trained analysts and with a lateral flow-test reader calibrated with known concentrations of pepsin A standard. The lower limit of detection is 16 $ng{\cdot}mL^{-1}$ and upper limit of quantification was 500 $ng{\cdot}mL^{-1}$. Peptest is specific for pepsin A (isoforms 1, 3a, 3b and 3c) and does not detect pepsin C/Gastricsin (isoform 5) putatively suggested to be expressed in the lungs [10, 11]. As pepsin concentrations do not follow a normal distribution a non-parametric statistical analysis was performed.

We have tested 93 (55 female) chronic cough patients mean±SD age 58.4±13.8 years between August 2014 and December 2014. Smoking status was: smoker n=5, nonsmoker n=55, ex-smoker n=24 and unknown n=9. The mean±SD HARQ score (upper limit of normal 13) was 31.9±13.1 and cough duration was 5.6±7.0 years. Over a period of 4 months the 93 patients provided 262 evaluable samples for testing. 80 patients had at least one pepsin positive sample (86.0%). Pepsin concentrations ranged from 0 to 500 $ng{\cdot}mL^{-1}$ with a median (interquartile range (IQR)) of 31 $ng{\cdot}mL^{-1}$ (0–113.5) $ng{\cdot}mL^{-1}$.

We previously [1] used a similar triple sampling strategy in a thoroughly investigated normal asymptomatic healthy volunteer population. The absence of gastro-oesophageal reflux was confirmed by pH-impedance testing. In contrast to this chronic cough study these control samples were provided first thing in the morning, 1 h after lunch and 1 h after the evening meal. Of the 87 control subjects only 33 were found to have at least one positive sample (37.9%) but the pepsin concentration in those that were Peptest positive was very low, median (IQR) of 0 (0–0) $ng{\cdot}mL^{-1}$ pepsin, which represents physiological reflux.

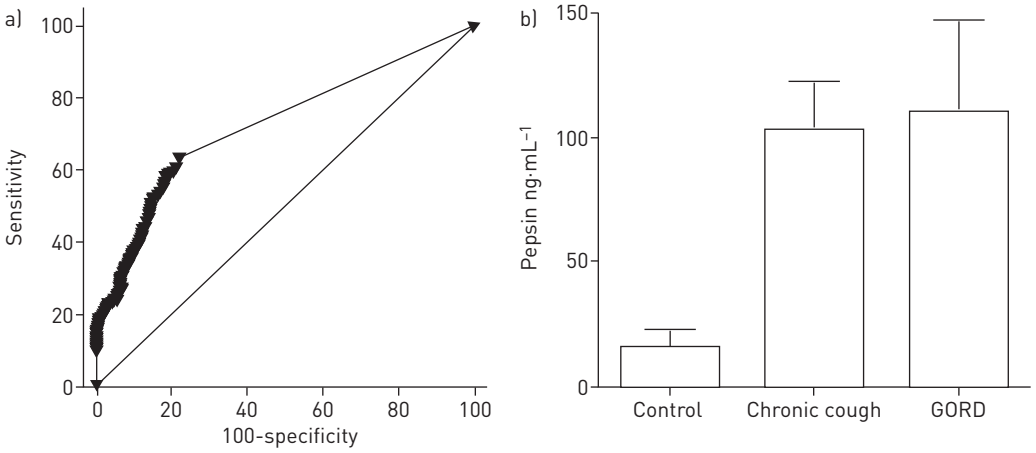

FIGURE 1 a) Receiver operating characteristics curve for pepsin concentration measured in 93 chronic cough patients (262 samples) measured using Peptest (RD Biomed Ltd, Cottingham, UK). b) The mean (95% CI) pepsin concentration (measured using Peptest) of 93 chronic cough patients and 59 gastro-oesophageal reflux disease (GORD) patients providing samples within 15 min of a symptomatic episode [12] compared to 87 healthy controls [1].

Patients with chronic cough in our study had significantly increased prevalence of pepsin detection ($p<0.0001$ Fisher's exact test; 86% *versus* 38%) and increased pepsin concentration ($p<0.0001$ Mann–Whitney U-test, 31 ng·mL^{-1} *versus* 0 ng·mL^{-1}) compared to the control group. There was a significant receiver operating characteristic (ROC) curve (0.7244, $p<0.0001$ area under the ROC curve) for chronic cough patients against the control population (figure 1a).

Other primary lung pathology, such as COPD or IPF, was present in only 6% of the patients and did not influence the rate of pepsin detection (prevalence 83% with lung pathology *versus* 86% without lung pathology).

Interestingly, the mean pepsin concentration in this chronic cough population is comparable to the mean pepsin concentration seen in 59 symptomatic GORD patients [12] at the time of symptomatic episodes, as was the prevalence of pepsin detection (80%). Thus suggesting that the level of pepsin associated reflux seen in chronic cough patients may be pathologically relevant (figure 1b).

Acid suppression medication, usually proton pump inhibitors (PPI), had been prescribed to 30% of the patients at presentation. Acid suppression did not impact on the detection or concentration of refluxed pepsin (81%, 37 ng·mL^{-1} on PPI *versus* 89%, 28.5 ng·mL^{-1} off PPI; $p>0.05$ Fisher's exact test). We have previously shown in a randomised double-blind placebo-controlled study of esomeprazole [13] that acid suppression is of no significant benefit in the treatment of airways reflux associated chronic cough. For a patient with airway hypersensitivity a small amount of reflux (usually non-acid) may be sufficient to induce cough [14] and a pro-motility medical strategy (*e.g.* baclofen, metaclopramide or azithromycin) should be considered.

This prospective cohort study in a real-life setting has demonstrated Peptest has a high sensitivity to detect pepsin in the overwhelming majority of chronic cough patients during symptomatic episodes. Since some reflux is physiological a lack of specificity must be considered. In conjunction with subjective assessment this assay may aid in diagnosis, leading to more appropriate management strategies in chronic cough.

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Detection of salivary pepsin (Peptest) may be a useful adjunct in the diagnosis of airway reflux
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