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Clinical Trial Paper

Detecting laryngopharyngeal reflux in patients with upper airways symptoms: Symptoms, signs or salivary pepsin?

Alexander Spyridoulias [a], Siobhan Lillie [a], Aashish Vyas [a], Stephen J. Fowler [a, b, *]

[a] Respiratory Medicine, Lancashire Teaching Hospitals NHS Foundation Trust, Preston, UK
[b] The University of Manchester, Manchester Academic Health Science Centre, NIHR South Manchester Respiratory and Allergy Clinical Research Facility, University Hospital of South Manchester, Manchester, UK

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ABSTRACT

Background: Laryngopharyngeal reflux (LPR) can induce laryngeal hyper-responsiveness, a unifying feature underlying chronic cough and vocal cord dysfunction. The diagnosis of LPR currently relies on invasive oesophageal pH impedance testing. We compared symptoms, laryngeal signs and salivary pepsin as potential diagnostic methods for identifying LPR in patients with upper airway symptoms.
Methods: Symptoms were assessed using the Reflux Symptom Index (RSI) and signs of laryngeal inflammation quantified using the Reflux Finding Score (RFS) during laryngoscopy. Saliva samples were analysed for the presence of pepsin. A sub-group of patients with severe symptoms and signs of LPR were investigated with oesophageal pH monitoring and impedance study.
Results: Seventy eight patients with chronic cough and/or suspected vocal cord dysfunction were recruited, mean (SD) age, 54.6 (15.6) years. The majority (87%) had significant symptoms of reflux (RSI > 13). There were clinical signs of LPR (RFS > 7) in 51% of cases. Pepsin was detected in the saliva of 63% of subjects and 78% of those with a high RFS. Salivary pepsin had a sensitivity of 78% and specificity of 53% for predicting a high RFS. There was a correlation between the RSI and RFS ($r = 0.51$, $p < 0.001$) and between the severity of laryngeal inflammation and the concentration of pepsin ($r = 0.28$, $p = 0.01$). All cases investigated with pH-impedance study had objective evidence of proximal reflux.
Conclusion: Salivary pepsin may be used as a screening adjunct to supplement the RFS in the clinical workup of patients with extra-oesophageal symptoms and upper respiratory tract presentations of reflux.

1. Introduction

Reflux is a commonly reported finding in patients with respiratory disease, with a prevalence as high as 50% in patients with chronic cough, severe COPD and difficult to control asthma [1–3]. Despite increasing evidence for an association between reflux and respiratory symptoms [4–6] the pathological mechanism for this has not yet been established [7]. Laryngeal hyper-responsiveness is one feature proposed to underlie conditions such as chronic cough and vocal cord dysfunction [7] that may be induced by laryngopharyngeal reflux (LPR).

Laryngopharyngeal reflux describes the retrograde flow of gastric refluxate into the laryngopharynx, an area which is highly susceptible to both the acidic and non-acidic components of reflux, where as few as three episodes a week may induce laryngeal inflammation [8]. The symptoms associated with LPR include persistent throat irritation and tightness, dysphonia, globus, excessive mucus production as well as dyspnoea and stridor, with the typical symptoms of dyspepsia or heartburn being less common [9]. Current methods employed in the diagnosis of LPR have inherent limitations. The Reflux Symptom Index (RSI) questionnaire is useful in quantifying severity of symptoms and variance with treatment [10] but may not differentiate LPR amongst other causes of upper respiratory tract symptoms [11]. The Reflux Finding Score (RFS) quantifies the severity of laryngoscopic findings of inflammation [12] but may be exaggerated in the presence of chronic laryngeal irritation from other sources. Twenty-four hour oesophageal pH monitoring detects changes in pH associated with reflux however it is invasive and labour intensive, and simultaneous intraluminal impedance monitoring is required to detect episodes of weakly- or non-acidic reflux [13,14].

* Corresponding author. Education and Research Centre, University Hospital of South Manchester, Southmoor Road, Manchester M23 9LT, UK.
E-mail address: stephen.fowler@manchester.ac.uk (S.J. Fowler).

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Studies in patients with persistent symptoms despite maximal acid suppressive therapy have established a positive symptom and temporal association between non-acid reflux episodes and symptoms such as chronic cough [15,16]. The presence of pepsin in the laryngopharynx has also been shown to correlate with reflux events [17] and has been found in the laryngeal epithelial mucosa of patients with reflux-attributed laryngeal disease [18,19]. Pepsin has been identified in the sputum and bronchoalveolar lavage fluid of patients with chronic cough and LPR [6,20], and it has been used to indicate aspiration in patients with lung allografts [21] and the effectiveness of anti-reflux surgery [22]. Salivary pepsin has a moderate sensitivity and specificity for the diagnosis of gastro-oesophageal reflux disease in patients with heartburn [23], and its association with scores obtained by clinical diagnostic tools such as the RSI and RFS has been investigated only in smaller studies to date [24,25].

The presence of pepsin in the upper airway is therefore indicative of reflux suggesting that it may be used as a biomarker for the objective assessment of LPR. The aim of this study was to evaluate the correlation and performance of the RSI, the RFS and the presence of pepsin in saliva and examine their role as diagnostic adjuncts in the investigation of patients with predominantly upper respiratory tract symptoms.

2. Methods

2.1. Subjects

Participants were recruited from the Airways Clinic of the Royal Preston Hospital, UK. Patients who had at least eight weeks' history of upper respiratory tract symptoms suggestive of laryngeal hyperresponsiveness, (for example persistent throat irritation or clearing, dysphonia, globus, episodic choking or breathlessness, chronic cough or vocal cord dysfunction) due to be investigated with fibre-optic laryngoscopy, were invited to participate in the study. Patients on treatment for obstructive airways disease or on anti-reflux treatment were included in the cohort. The protocol was approved by the Greater Manchester Research Ethics Committee, (12/NW/0016) and all patients provided written, informed consent.

2.2. Measurements

A flow diagram outlining the study procedures is shown in Fig. 1. Participants completed the self-administered RSI questionnaire, a validated tool which quantifies the nature and severity of symptoms over the previous month. The RSI is made up of nine components each of which is rated on a scale of 0–5, with a score of more than 13/45 considered suggestive of LPR [10]. They were then asked to produce a sample of saliva from their throat. Following this, subjects underwent flexible fibre-optic laryngoscopy to assess for any evidence of laryngeal mucosal inflammation or structural changes, which were quantified using the RFS. The RFS is an assessment tool that quantifies the severity of inflammation and other structural changes seen during laryngoscopy and is based on eight components, with a score of more than 7/26 being suggestive of LPR [12]. Where clinically indicated, patients with symptoms and/or signs of laryngeal inflammation, usually despite previous anti-reflux treatment, were also referred for oesophageal pH studies.

Salivary sample collection: Saliva samples were all collected by one researcher explaining the same technique; subjects were encouraged to produce a deep salivary sample through a throat clearing manoeuvre into a collecting tube containing 0.5 ml of 0.01 M citric acid preserving medium. Samples were collected within four hours of participants' last meal. Samples were anonymised and analysed for the presence of pepsin by an independent investigator blinded to subjects' symptom scores and laryngoscopy findings. All samples were analysed within 36 h of collection.

Pepsin assay: The pepsin assay used in the study was a lateral flow device, the Peptest (RDBiomed, Hull, UK) [26]. Analysis involved extraction of 0.5 mls of each clinical sample, micro-centrifugation and separation of a clear supernatant layer which was then extracted and mixed with a migration buffer and added to the test well of the lateral flow device. The detection monoclonal antibody (specific to pepsin-3) labelled with blue latex beads was solubilised by the clinical sample and carried across a nitrocellulose membrane, sandwiched to the capture monoclonal antibody. A positive result resented as a blue test line showing on the device after an analysis time of 15 min. A control line formed by a third antibody in the presence of pepsin confirmed the validity of the result. Pepsin concentration was quantitated in ng/ml by measuring the intensity of the test line against the standard curve of human pepsin-3, with a lower limit of detection of 25 ng/ml [26].

Flexible fibre optic laryngoscopy: This was performed using the Pentax FNL-10RP3 flexible fibre-optic laryngoscope (Pentax Medical, Hamburg, Germany) and laryngeal inflammation and structural changes quantified using the RFS by a specialist respiratory consultant blinded to the results of the pepsin assay.

Oesophageal pH study: 24 h oesophageal pH-monitoring was performed using a dual channel antimony catheter. The distal pH probe was positioned 5 cm above the lower oesophageal sphincter (LOS) and the upper probe was placed in the proximal oesophagus 2 cm below the upper oesophageal sphincter using manometry. Multichannel intraluminal impedance pH study (Impedance catheter and Bioview analysis; Sleuth system, Sandhill Scientific Inc, Oxon, UK) was performed using an antimony catheter with six paired impedance rings positioned 3, 5, 7, 9, 15 and 17 cm above the LOS and pH electrodes positioned at 10 cm below and 5 cm above the LOS as determined by manometry. Patients were asked to record medication and meal times, symptoms and periods they were supine and upright but otherwise to maintain normal activities. Reflux events were identified as acidic when pH < 4 and weakly acidic when pH > 4 with an associated rapid drop in intraluminal impedance progressing proximally; gas events were identified when impedance increased in all channels, and gas/liquid events when there was a combination of both. Proximal reflux events were identified when the signal reached the proximal two electrode rings. Patients were maintained on anti-reflux treatment during the study.

2.3. Statistical analysis

Performance characteristics were calculated for the pepsin assay versus a clinical diagnosis of LPR based on an RSI > 13, RFS > 7 and impedance–pH study results. Mann–Whitney U test was used to test for significant between group differences in symptoms, signs of inflammation and pepsin concentrations for anti-reflux treatment and quantified pepsin levels; chi-squared test was used for categorical parameter. Correlations between symptoms, signs, pepsin concentrations and reflux events were assessed using Spearman's rank correlation in StatsDirect (version 2.7.9, StatsDirect Ltd, Cheshire, UK).

3. Results

Seventy eight subjects were recruited into this study (mean [+/−SD] age, 54.6 [+/− 15.6 years]), 24% male. In 68 patients the indication was for assessment of possible vocal cord dysfunction, which was confirmed in 45 subjects, and the remaining 10 for chronic cough. Thirty patients had concomitant asthma, and 42 were taking at least one form of anti-reflux therapy.

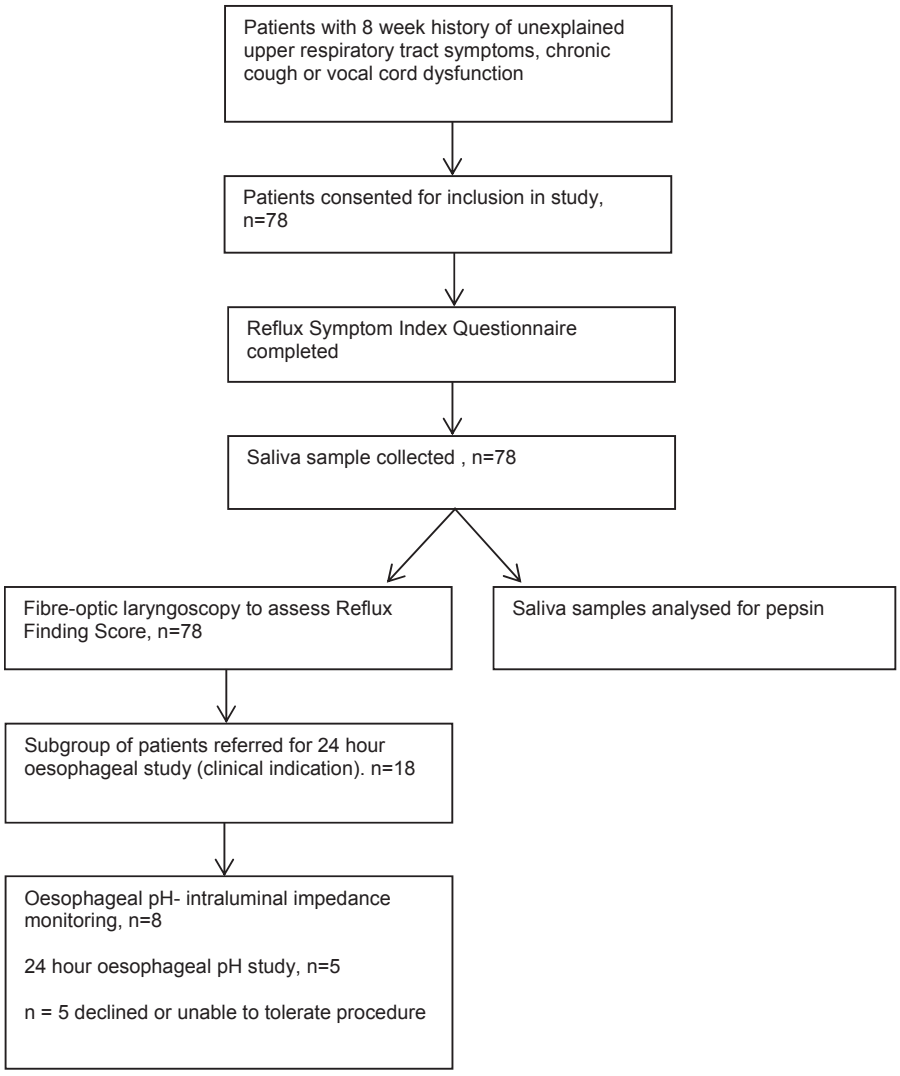

Fig. 1. Flow diagram of the study procedures.

3.1. Reflux Symptom Index

The median (IQR) RSI score in this cohort was 27 (18.8–32.3) with 87% of subjects indicating significant symptoms (RSI > 13/45) on their questionnaire. The frequency of symptom occurrence is shown in Table 1.

3.2. Reflux Finding Score

The median (IQR) RFS score was 7 (5–10) and 51% showed significant signs of laryngeal inflammation with an RFS > 7/26 at laryngoscopy. Average RFS scores for each component of the RFS are shown in Fig. 2.

3.3. Pepsin

Pepsin was detected in the saliva of 63% of patients. The median (IQR) concentration of pepsin was 49 (17–92) ng/ml. Demographic details for patients with and without a positive salivary pepsin test are summarized in Table 2. Pepsin was detected in 63% who had a score of >13 on the RSI questionnaire, but also in 60% who did not have significant symptoms. It was found to be present in the saliva

Table 1
The frequency n (%) of reflux symptoms (n = 50) and frequency of most severe form (a score of 5/5) according to the Reflux Symptom Index.

Symptom	Frequency n (%)	Frequency n (%) with severe symptoms
Hoarseness or problem with your voice	46 (92)	10 (20)
Clearing your throat	44 (88)	14 (28)
Excess throat mucus or post nasal drip	40 (80)	7 (14)
Difficulty in swallowing	32 (64)	5 (10)
Coughing after eating or lying down	41 (82)	10 (20)
Breathing difficulties or choking episodes	42 (84)	13 (26)
Troublesome or annoying cough	46 (92)	18 (36)
Sensation of something sticking in the throat or lump in the throat	43 (86)	19 (38)
Heartburn, chest pain, indigestion or stomach acid coming up	38 (76)	11 (22)

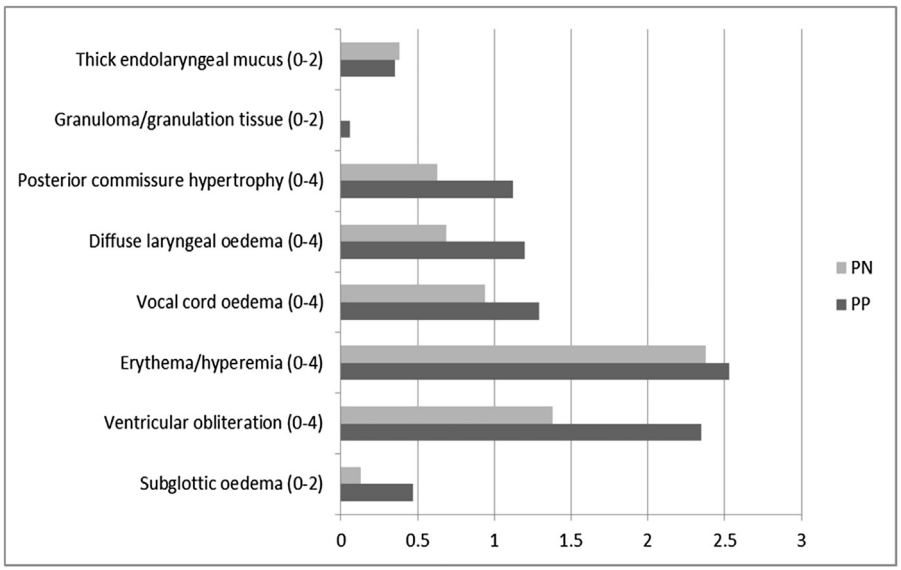

Fig. 2. Average score for each component of the RFS for subjects who were pepsin positive (PP) and pepsin negative (PN).

of 78% with significant signs of laryngeal inflammation on laryngoscopy (RFS > 7) and in 47% without such signs ($p < 0.05$). The degree of ventricular obliteration in subjects who had pepsin present in their saliva was greater than for those who were pepsin negative, ($p = 0.006$) but there was no significant difference in the other components of the RFS between pepsin groups. Salivary pepsin was detected in 7 of the 13 subjects (58%) who were investigated further with oesophageal impedance or pH study.

3.4. Oesophageal intraluminal impedance and pH monitoring studies

Eighteen patients with severe symptoms or signs of LPR were referred for further investigation, although five of these either could not tolerate or declined the test. As can be seen from Fig. 3 these did have suggestive symptoms but not all had clear signs of reflux, at least as determined by the RFS.

Eight patients were investigated with multichannel intraluminal impedance and pH monitoring, six of whom were on anti-reflux therapy at that time. All eight were found to have objective evidence of non-acid reflux reaching the proximal probe and six of these had acid reflux occurring during the study. There were median 6 (IQR 0.5–9.5) acidic and 18 (14.5–29.5) non-acidic reflux episodes recorded reaching the proximal probe. Three of the five patients investigated with 24 h dual probe oesophageal pH monitoring had a positive test.

Table 2
Demographic details for pepsin positive and pepsin negative patients.

Characteristic	Pepsin negative, n = 29	Pepsin positive, n = 49
Age, mean (SD) yrs	52 (16.1)	56 (15.1)
Gender, n (%) female	24 (83)	35 (71)
Vocal cord dysfunction, n (%)	14 (48)	31 (63)
Chronic cough, n (%)	4 (13)	6 (12)
Asthma, n (%)	10 (34)	20 (41)
Bronchodilator treatment, n	6	7
LABA/Steroid combination, n	4	13
Anti-reflux treatment, n (%)	15 (52)	27 (55)
Alginate only, n	1	2
PPI only, n	7	5
Alginate and PPI, n	5	5
Alginate, PPI and prokinetic, n	2	5
Reflux Symptom Index, median (IQR)	24 (18–31)	27 (20–33)
Reflux Finding Score, median (IQR)	5 (4–9)	8 (6–11)[a]
Pepsin conc, median (IQR) ng/ml	NA	49 (17–92)

[a] Indicates significant between-group difference ($p < 0.05$).

3.5. Effect of anti-reflux therapy

Of the 42 patients on anti-reflux therapy at the time of the study, 22 were on a proton pump inhibitor (PPI), three on an alginate alone, 10 on a PPI and prokinetic or alginate, and seven on a combination of these three agents. When compared to those not on anti-reflux therapy there were no differences in the number of patients with significant symptoms (high RSI: 86% versus 81%), signs (high RFS: 50% vs 54%) or presence of salivary pepsin (64% vs 63%).

3.6. Comparison of diagnostic methods

Fig. 3 shows the correlation between symptoms and signs for patients that were pepsin positive and negative and according to pH study results. Although there was correlation between the severity of symptoms and signs of inflammation seen on laryngoscopy, ($r = 0.51$, $p < 0.001$), there were many patients with symptoms who did not have objective evidence of reflux. We did not find a correlation between the RSI and pepsin concentration ($p = 0.4$) however there was a significant correlation between the RFS scores and pepsin concentration, ($r = 0.28$, $p = 0.01$). The components of the RFS which showed significant correlation with the concentration of pepsin were ventricular obliteration ($p = 0.001$), vocal fold oedema ($p = 0.02$) and diffuse laryngeal oedema ($p = 0.04$). There was a significant difference in the concentration of pepsin found in subjects who had an RFS >7 compared to those with an RFS of 7 or less ($p = 0.009$). Salivary pepsin had a sensitivity of 0.78 and a specificity of 0.53 for predicting an RFS >7. We did not see a significant correlation between the number of acidic ($p = 0.53$) or non-acidic reflux ($p = 0.2$) episodes with the RFS score or the concentration of pepsin ($p = 0.44$ and $p = 0.66$ respectively).

4. Discussion

To our knowledge this is the first study to investigate and report the prevalence of LPR in patients with upper respiratory symptoms using multiple methods. Our patients were typically referred with

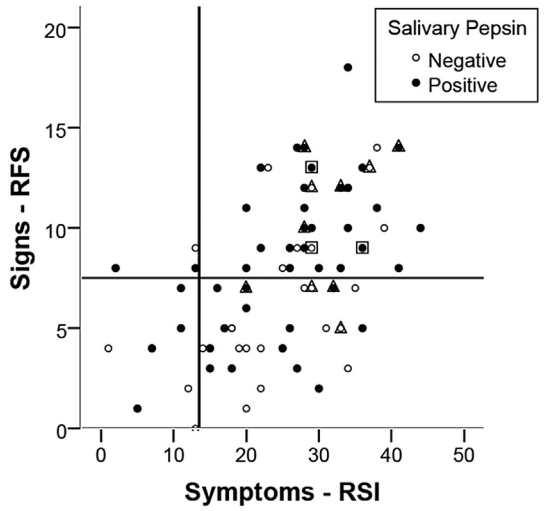

Fig. 3. Scatter plot demonstrating relationship between symptoms and signs of reflux for pepsin positive and pepsin negative subjects. RSI = Reflux Symptom Index; RFS = Reflux Finding Score. Cut-off lines added to indicate a high RSI of >13 and RFS of >7. Superimposed shapes are shown for 13 patients who also had oesophageal studies: squares = negative study; triangles = positive study.

symptoms such as persistent dyspnoea and/or chronic cough which on clinical assessment were suspected to be associated with laryngeal hypersensitivity, with many having coexistent asthma. We found evidence of reflux in the majority of these patients by the presence of diagnostic signs and salivary pepsin, confirmed by oesophageal pH and impedance monitoring in a subgroup. Signs of laryngeal inflammation correlated with reflux symptoms and salivary pepsin, but there were a large number of patients with discordant findings between tests.

The majority of subjects in this cohort who were undergoing investigation for upper airways symptoms scored highly on the RSI questionnaire. Although the severity of patient symptoms correlated reasonably well with signs of laryngeal inflammation, there was a high number of presumed false positive results, with a quarter of the patients with a high RSI having no objective evidence of reflux by either RFS or salivary pepsin. There are a number of possible explanations for this. Many of the symptoms on the RSI may not be specific enough for reflux in respiratory patients, particularly those questions relating to breathlessness, mucus production and voice problems [11]. Secondly patients may be having episodes of laryngopharyngeal reflux but not of sufficient severity or frequency to cause signs. This latter hypothesis is supported by the finding that almost half of the patients with symptoms but few signs nevertheless had pepsin in their saliva at low concentrations.

A study undertaken in patients with heartburn has shown that both the prevalence and concentration of salivary pepsin was significantly higher in patients with reflux disease and oesophageal hypersensitivity compared to patients with functional heartburn not related to reflux, with higher concentrations predicting reflux with a greater probability; (>100 ng/ml had a PPV 54.5%, >150 ng/ml had a PPV 75% and >210 ng/ml had a PPV 95.7%) [23]. Pepsin has previously been found in the saliva of asymptomatic control subjects that is occurring as a result of physiological reflux [23,27] but not in the lower airways [28]. Such reflux becomes pathological when it starts to cause inflammation and injury producing symptoms. A higher concentration of pepsin and frequency of exposure through an increased number of reflux events is more likely to result in injury of the susceptible laryngopharynx [29,30]. The threshold concentration of pepsin at which this occurs is however unknown.

Objective signs of laryngeal inflammation were frequently present in these subjects, with just over half having an RFS > 7, i.e. suggestive of LPR [12]. Furthermore, the severity of reflux as measured by the RFS related to salivary pepsin, with higher RFS scores in subjects who had pepsin present compared to those who did not and a weak but significant correlation between the RFS and salivary pepsin concentration.

A previous study of patients with symptoms of reflux laryngitis comparing clinical findings of LPR using the RSI and RFS with the presence of pepsin showed that patients with pepsin in their sputum had more significant symptoms and laryngoscopic signs of reflux than those who were pepsin negative [25], and found associations between its presence in the upper respiratory tract and episodes of proximal reflux detected on oesophageal studies [17,31] finding it to be sensitive for detecting LPR [32].

We found approximately a quarter of patients with signs of LPR but negative salivary pepsin. This may be due to taking a single clinical sample and the sampling time not coinciding with recent reflux events. This is supported by the finding that four of the five patients with a negative salivary pepsin who were referred on for oesophageal investigations nevertheless had evidence of significant reflux reaching the proximal probe. It is likely that the sensitivity of salivary pepsin testing could be improved by increasing the frequency of sampling and sampling when symptomatic. Previous studies have shown that patients with proximal reflux as defined by oesophageal pH or impedance testing have higher levels of pepsin in their saliva [24,33].

Our findings reinforce previous evidence that reflux is also a common finding in patients with chronic cough. Reflux could stimulate the cough reflex by microaspiration, directly irritating the upper respiratory tract or by a vagally mediated oesophagobronchial reflex. Coughing causes a transient increase in intra-abdominal pressure and a functional decrease of the lower oesophageal sphincter pressure that could precipitate reflux. Chronic cough was one of the most frequently occurring symptoms in our patient cohort although the majority were suspected to have vocal cord dysfunction, which typically causes inspiratory airflow limitation and would hence be associated with a decrease in intra-abdominal pressure, as air is drawn in through partially adducted cords. A recent study in chronic cough patients showed that lower airway pepsin concentration (in sputum or bronchoalveolar lavage) was only weakly related to the number of proximal reflux episodes detected by pH-impedance study but was inversely related to symptoms of coughing, suggesting that a hyper-reactive cough reflex may in fact be protective [6]. Another proposed explanation is that medication such as adrenoreceptor agonists may decrease lower oesophageal sphincter tone, promoting reflux [34] however this has not been proven in a larger review [35] and in our study there was no difference in the proportion of patients with and without asthma who had detectable salivary pepsin.

Whilst more than half of our patients were taking some form of anti-reflux therapy at the time of the study measurements, we did not find any significant difference in the severity of symptoms, signs, or pepsin concentrations compared to those not on any treatment. It has been postulated that non-acid reflux, a significant contributor to respiratory symptoms such as chronic cough, is not suppressed by commonly used treatments such as PPIs [13] and whilst pepsin activity declines with decreasing acidity, there is still clinically relevant activity even at a higher pH [36].

Patients in our study underwent a thorough clinical workup to exclude other causes for their symptoms, although all patients did not undergo a protocolised workup to rigorously exclude asthma or other comorbidities. There was variability in the timing of spot saliva sample collection which may have been between one to four hours in relation to the subjects' last meal, affecting the reflux

volume and pepsin concentration found. This may have been improved by collecting samples at specific times after meals throughout the day or when symptomatic. The pepsin concentration was extrapolated for those clinical samples which tested positive but which were below the lower limit of detection of the pepsin assay which may have affected the accuracy of the concentration that was actually present. Only a small subgroup of our patients with severe symptoms or signs underwent oesophageal studies and it would have been informative to have performed it in patients with mild symptoms and laryngeal signs for comparison. A proportion of our patients were on different forms of anti-reflux treatment according to the severity of their clinical findings which may have affected our results although we did not find a significant difference between patient groups on and off treatment. Laryngoscopic appearance was scored using the RFS by an experienced respiratory airways specialist consultant however this is still a subjective assessment. We also did not recruit healthy control subjects in which to compare the diagnostic methods, although the RFS has previously been shown to lack specificity for reflux as asymptomatic volunteers may also display features of laryngeal irritation [37–39].

We showed that the detection of salivary pepsin, even at low concentrations had a baseline sensitivity of 78% and a specificity of 53% for predicting patients with laryngeal signs and symptoms of LPR, which is similar to that of Hayat et al. for predicting reflux in patients with heartburn. We found lower concentrations of salivary pepsin in our study which may be explained by the fact that our patients had extra-oesophageal symptoms which may be occurring with a lower severity of reflux in the susceptible larynx compared to their patients who primarily had heartburn related to hypersensitive oesophagus and reflux disease.

In summary we have shown that a higher concentration of salivary pepsin is present in patients with severe LPR symptoms, and that the presence and concentration of salivary pepsin is associated with greater severity of laryngeal signs as determined by the RFS. On the other hand there were many patients in whom the findings of LPR were discordant between the three methods used. This adds to the evidence base on the use of salivary pepsin in patients with extra-oesophageal symptoms and upper respiratory tract presentations of reflux in a clinical setting. We believe that salivary pepsin may be a simple to use test to supplement the findings of the RFS when assessing patients with such heterogenous symptoms. Further study should however be performed to establish a sensitive concentration at which pepsin could predict LPR in patients with atypical symptoms, and its ability to direct the use of anti-reflux treatment in such patients.

Competing interests

None.

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References

[1] A.B. Chang, T.J. Lasserson, T.O. Kiljander, et al., Systematic review and meta-analysis of randomised controlled trials of gastro-oesophageal reflux interventions for chronic cough associated with gastro-oesophageal reflux, Br. Med. J. 332 (2006) 11–17.
[2] J.J. Leggett, B.T. Johnston, M. Mills, et al., Prevalence of gastroesophageal reflux in difficult asthma: relationship to asthma outcome, Chest 127 (2005) 1227–1231.
[3] R.R. Kempainen, K. Savik, T.P. Whelan, et al., High prevalence of proximal and distal gastroesophageal reflux disease in advanced COPD, Chest 131 (2007) 1666–1671.
[4] B. Avidan, A. Sonnenberg, T.G. Schnell, et al., Temporal associations between coughing or wheezing and acid reflux in asthmatics, Gut 49 (2001) 767–772.
[5] J.A. Smith, S. Decalmer, A. Kelsall, et al., Acoustic cough-reflux associations in chronic cough: potential triggers and mechanisms, Gastroenterology 139 (2010) 754–762.
[6] S. Decalmer, R. Stovold, L.A. Houghton, et al., Chronic cough: relationship between microaspiration, gastroesophageal reflux, and cough frequency, Chest 142 (2012) 958–964.
[7] K. Kenn, R. Balkissoon, Vocal cord dysfunction: what do we know? Eur Respir J 37 (2011) 194–200.
[8] J.A. Koufman, The otolaryngologic manifestations of gastroesophageal reflux disease (GERD): a clinical investigation of 225 patients using ambulatory 24-hour pH monitoring and an experimental investigation of the role of acid and pepsin in the development of laryngeal injury, Laryngoscope 101 (1991) 1–78.
[9] J.A. Koufman, Larynogopharyngeal reflux is different from classic gastroesophageal reflux disease, Ear Nose Throat J. 81 (2002) 7–9.
[10] P.C. Belafsky, G.N. Postma, J.A. Koufman, Validity and reliability of the Reflux Symptom Index (RSI), J. Voice 16 (2002) 274–277.
[11] C.N. Ford, Evaluation and management of laryngopharyngeal reflux, JAMA 294 (2005) 1534–1540.
[12] P.C. Belafsky, G.N. Postma, J.A. Koufman, The validity and reliability of the reflux finding score, Laryngoscope 111 (2001) 1313–1317.
[13] I. Mainie, R. Tutuian, S. Shay, et al., Acid and non-acid reflux in patients with persistent symptoms despite acid suppressive therapy: a multicentre study using combined ambulatory impedance-pH monitoring, Gut 55 (2006) 1398–1402.
[14] J.A. Kastelik, A.E. Redington, I. Aziz, et al., Abnormal oesophageal motility in patients with chronic cough, Thorax 58 (2003) 699–702.
[15] R.S. Irwin, J.K. Zawacki, M.M. Wilson, et al., Chronic cough due to gastroesophageal reflux disease: failure to resolve despite total/near total elimination of esophageal acid, Chest 121 (2002) 1132–1140.
[16] R. Tutuian, I. Mainie, A. Agrawal, et al., Nonacid reflux in patients with chronic cough on acid-suppressive therapy, Chest 130 (2006) 386–391.
[17] J. Knight, M.O. Lively, N. Johnston, et al., Sensitive pepsin immunoassay for detection of laryngopharyngeal reflux, Laryngoscope 115 (2005) 1473–1478.
[18] G.A. Gill, N. Johnston, A. Buda, et al., Laryngeal epithelial defenses against laryngopharyngeal reflux: investigations of E-cadherin, carbonic anhydrase isoenzyme III, and pepsin, Ann. Otol. Rhinol. Laryngol. 114 (2005) 913–921.
[19] A. Jiang, M. Liang, Z. Su, et al., Immunohistochemical detection of pepsin in laryngeal mucosa for diagnosing laryngopharyngeal reflux, Laryngoscope 121 (2011) 1426–1430.
[20] M. Grabowski, A. Kasran, S. Seys, et al., Pepsin and bile acids in induced sputum of chronic cough patients, Respir. Med. 105 (2011) 1257–1261.
[21] C. Ward, I.A. Forrest, I.A. Brownlee, et al., Pepsin like activity in bronchoalveolar lavage fluid is suggestive of gastric aspiration in lung allografts, Thorax 60 (2005) 872–874.
[22] E. Wassenaar, N. Johnston, A. Merati, et al., Pepsin detection in patients with laryngopharyngeal reflux before and after fundoplication, Surg. Endosc. 25 (2011) 3870–3876.
[23] J.O. Hayat, S. Gabieta-Somnez, E. Yazaki, et al., Pepsin in saliva for the diagnosis of gastro-oesophageal reflux disease, Gut 64 (2015) 373–380.
[24] M. Sereg-Bahar, A. Jerin, R. Jansa, et al., Pepsin and bile acids in patients with laryngopharyngeal reflux – a prospective comparative study, Clin. Otolaryngol. (2014), http://dx.doi.org/10.1111/coa.12358.
[25] L. Wang, X. Liu, Y.L. Liu, et al., Correlation of pepsin-measured laryngopharyngeal reflux disease with symptoms and signs, Otolaryngol. Head Neck Surg. 143 (2010) 765–771.
[26] http://www.rdbiomed.com/peptest/resources/ (accessed 01.08.14).
[27] E. Saritas Yuksel, S.K. Hong, V. Strugala, et al., Rapid salivary pepsin test: blinded assessment of test performance in gastroesophageal reflux disease, Laryngoscope 122 (2012) 1312–1316.
[28] J.K. Bohman, D.J. Kor, R. Kashyap, et al., Airway pepsin levels in otherwise healthy surgical patients receiving general anesthesia with endotracheal intubation, Chest 143 (2013) 1407–1413.
[29] J.P. Pearson, S. Parikh, R.C. Orlando, et al., Review article: reflux and its consequences—the laryngeal, pulmonary and oesophageal manifestations, Aliment. Pharmacol. Ther. 33 (2011) 1–71.
[30] K.D. Bardhan, V. Strugala, P.W. Dettmar, Reflux revisited: advancing the role of pepsin, Int. J. Otolaryngol. 2012 (2012), 646901.
[31] S. Potluri, F. Friedenberg, H.P. Parkman, et al., Comparison of a salivary/sputum pepsin assay with 24-hour esophageal pH monitoring for detection of

gastric reflux into the proximal esophagus, oropharynx, and lung, Dig. Dis. Sci. 48 (2003) 1813–1817.
[32] T.H. Kim, K.J. Lee, M. Yeo, et al., Pepsin detection in the sputum/saliva for the diagnosis of gastroesophageal reflux disease in patients with clinically suspected atypical gastroesophageal reflux disease symptoms, Digestion 77 (2008) 201–206.
[33] J.O. Hayat, E. Yazaki, A.T. Moore, et al., Objective detection of esophagopharyngeal reflux in patients with hoarseness and endoscopic signs of laryngeal inflammation, J. Clin. Gastroenterol. 48 (2014) 318–327.
[34] M.D. Crowell, E.N. Zayat, B.E. Lacy, et al., The effects of an inhaled beta(2)-adrenergic agonist on lower esophageal function: a dose-response study, Chest 120 (2001) 1184–1189.
[35] B.D. Havemann, C.A. Henderson, H.B. El-Serag, The association between gastro-oesophageal reflux disease and asthma: a systematic review, Gut 56 (2007) 1654–1664.
[36] N. Johnston, P.W. Dettmar, B. Bishwokarma, et al., Activity/stability of human pepsin: implications for reflux attributed laryngeal disease, Laryngoscope 117 (2007) 1036–1039.
[37] C.F. Milstein, S. Charbel, D.M. Hicks, et al., Prevalence of laryngeal irritation signs associated with reflux in asymptomatic volunteers: impact of endoscopic technique (rigid vs. flexible laryngoscope), Laryngoscope 115 (12) (2005) 2256–2261.
[38] D.M. Hicks, T.M. Ours, T.I. Abelson, et al., The prevalence of hypopharynx findings associated with gastroesophageal reflux in normal volunteers, J. Voice 16 (2002) 564–579.
[39] M.F. Vaezi, D.M. Hicks, T.I. Abelson, et al., Laryngeal signs and symptoms and gastroesophageal reflux disease (GERD): a critical assessment of cause and effect association, Clin. Gastroenterol. Hepatol. 1 (2003) 333–344.