Diagnostic value of the pepsin concentration in saliva and induced sputum for gastroesophageal reflux-induced chronic cough: a prospective clinical study

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A salivary pepsin concentration >76.10 ng·mL^{-1} is of good diagnostic value for gastroesophageal reflux-induced chronic cough (GERC), especially in non-acidic GERC. The induced sputum pepsin concentration has a low diagnostic value. https://bit.ly/3Jh9keQ

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Abstract

Background Finding a simple, effective and rapid diagnostic method to improve the diagnosis of gastroesophageal reflux-induced chronic cough (GERC) is indicated. Our objective was to determine the diagnostic value of the pepsin concentration in saliva and induced sputum for GERC.

Methods 171 patients with chronic cough were enrolled. The diagnosis and treatment followed the chronic cough diagnosis and treatment protocol. Saliva and induced sputum were collected, and the pepsin concentration was determined using Peptest. A Gastroesophageal Reflux Diagnostic Questionnaire (GerdQ) was completed. The diagnostic value of the pepsin concentration in saliva and induced sputum for GERC was analysed and compared.

Results The salivary pepsin concentration predicted GERC with an area under the receiver operating characteristic curve (AUC) of 0.845. The optimal cut-off value was 76.10 ng·mL^{-1}, the sensitivity was 83.58% and the specificity was 82.69%. The pepsin concentration in the induced sputum supernatant for GERC had an AUC of 0.523. When GerdQ was used for GERC diagnosis, the AUC was 0.670 and the diagnostic value of salivary pepsin was better compared to GerdQ (DeLong test, p=0.0008). Salivary pepsin had a comparable diagnostic value to GerdQ (AUC 0.779 *versus* 0.826; p=0.4199) in acidic GERC. Salivary pepsin had superior diagnostic value compared to GerdQ (AUC 0.830 *versus* 0.533; p<0.0001) in non-acidic GERC.

Conclusions A salivary pepsin concentration >76.10 ng·mL^{-1} is of good diagnostic value for GERC, especially in non-acidic GERC. The pepsin concentration in induced sputum has a low diagnostic value.

Introduction

Gastroesophageal reflux-induced chronic cough (GERC) is a clinical syndrome characterised by the reflux of gastric acid and other gastric contents into the oesophagus, resulting in cough as a prominent manifestation [1, 2]. Current diagnostic methods for GERC include multichannel intraluminal impedance pH monitoring (MII-pH), endoscopy, barium meal, empirical anti-reflux therapy and related questionnaires [3]. The MII-pH test is the most essential supplementary test for the diagnosis of GERC. Although MII-pH is sensitive and reliable, it is invasive, poorly tolerated by patients, expensive and difficult to perform in primary care settings. Endoscopic detection of oesophagitis and barium meal examination demonstrating barium reflux are two of the foundation methods for GERC diagnosis, but the sensitivity is low and the

diagnosis is easily missed. Anti-reflux treatment consisting of omeprazole (20 mg, twice daily) and domperidone (10 mg, three times per day) does not completely establish the diagnosis. The Gastroesophageal Reflux Diagnostic Questionnaire (GerdQ) is a diagnostic tool for GERC. GERC should be considered with a GerdQ score ⩾8. The GerdQ has high sensitivity in diagnosing acid GERC but poor diagnostic value for non-acid GERC [4]. It is necessary to find a simple, effective and rapid diagnostic method to improve the diagnosis of GERC.

Pepsin can be detected in saliva, sputum, the trachea, lungs and sinuses, making pepsin suitable as a biomarker for the detection of reflux [5]. Peptest (RD Biomed, Cottingham, UK) is a clinically certified tool for detecting the presence of pepsin in samples. The diagnosis of gastroesophageal reflux disease (GERD) and gastroesophageal reflux-associated disease by Peptest is currently a major focus of research, but the diagnostic value for GERC has rarely been reported [6–10]. Therefore, we conducted a prospective clinical trial to assess the diagnostic value of the pepsin concentration in patients with GERC using the Peptest method to measure the pepsin concentration in saliva and induced sputum of patients with chronic cough.

Materials and methods

Patients

Chronic cough patients attending the Outpatient Clinic in the Department of Respiratory and Critical Care Medicine at Tongji Hospital of Tongji University (Shanghai, China) were consecutively enrolled in this study from February 2021 to October 2022. The inclusion criteria were: 1) age 16–80 years; 2) cough course >8 weeks; 3) forced expiratory volume in 1 s (FEV_1)/forced vital capacity >70% and FEV_1 >80% predicted; 4) no other symptoms, such as wheezing, haemoptysis or fever; 5) no rales on lung auscultation; 6) no abnormal findings on chest radiography or computed tomography (CT); 7) ability to correctly complete the GerdQ; and 8) no proton pump inhibitor (PPI) use within 1 week or H2 receptor inhibitor, gastric motility drugs or antacid use within 3 days. The exclusion criteria were: 1) history of gastroesophageal or pharyngeal surgery, severe benign gastrointestinal or respiratory disease (*e.g.* severe ulcers and pharyngeal polyps), pregnancy or breastfeeding; 2) smokers or those who quit smoking within 2 years; 3) difficulty reading or writing; and 4) a history of cardiac and other organ disease that precluded the patient from undergoing our study. Dropout criteria were: 1) patients who were lost to follow-up and 2) patients whose information was incomplete. The study was approved by the Ethics Committee of Tongji Hospital (K-2015-007) and registered in the Chinese Clinical Trials Register (ChiCTR1800020221). Informed consent was obtained from all patients.

Diagnostic criteria for GERC

GERC was diagnosed in patients who met the following criteria [11, 12]. 1) A persistent cough that was prevalent throughout the day and, in a small percentage of cases, nocturnal. 2) MII-pH meeting at least one of three requirements (oesophageal acid exposure time (AET) >6%, reflux episodes >80 times/24 h and symptom association probability (SAP) ⩾95%). Reflux was classified as acid GERC (AET >6% and/or with acid reflux >80 times/24 h; acid reflux SAP ⩾95%) and non-acid GERC (non-acid reflux >80 times/24 h; non-acid reflux SAP ⩾95%). 3) Stepwise anti-reflux therapy was effective. Patients with suspected GERC were first treated with standard anti-reflux therapy. If there was no improvement in the cough or if the cough worsened after 8 weeks, intensive anti-reflux therapy (doubling the dose of a PPI or combining a neuromodulator) was given to resolve or significantly relieve the symptoms of cough. Cough completely resolved or significantly improved (cough symptom score decreased by >50%) indicated that the treatment worked. 4) Other common aetiologies of chronic cough, such as upper airway cough syndrome (UACS), cough variant asthma (CVA), atopic cough (AC) and eosinophilic bronchitis (EB), were ruled out.

Study design

This was a prospective clinical trial. The diagnostic process for the aetiology of chronic cough was based on the guidelines for the diagnosis and treatment of cough [11, 13]. All patients had the following assessments to clarify the aetiology of chronic cough after a detailed history was obtained and a physical examination was performed: chest radiograph and/or CT scan, pulmonary function tests, induced sputum cytology, bronchial provocation test and MII-PH. Saliva and induced sputum specimens were obtained, the GerdQ was completed, and the pepsin concentration in saliva and induced sputum supernatants was measured by Peptest. When a diagnosis of UACS, CVA, EB, GERC or AC was suspected after the tests were completed, aetiological treatment was performed to confirm the diagnosis. We analysed the diagnostic value of the salivary and induced sputum pepsin concentration for GERC and compared the findings with the GerdQ. Figure 1 depicts the study flowchart.

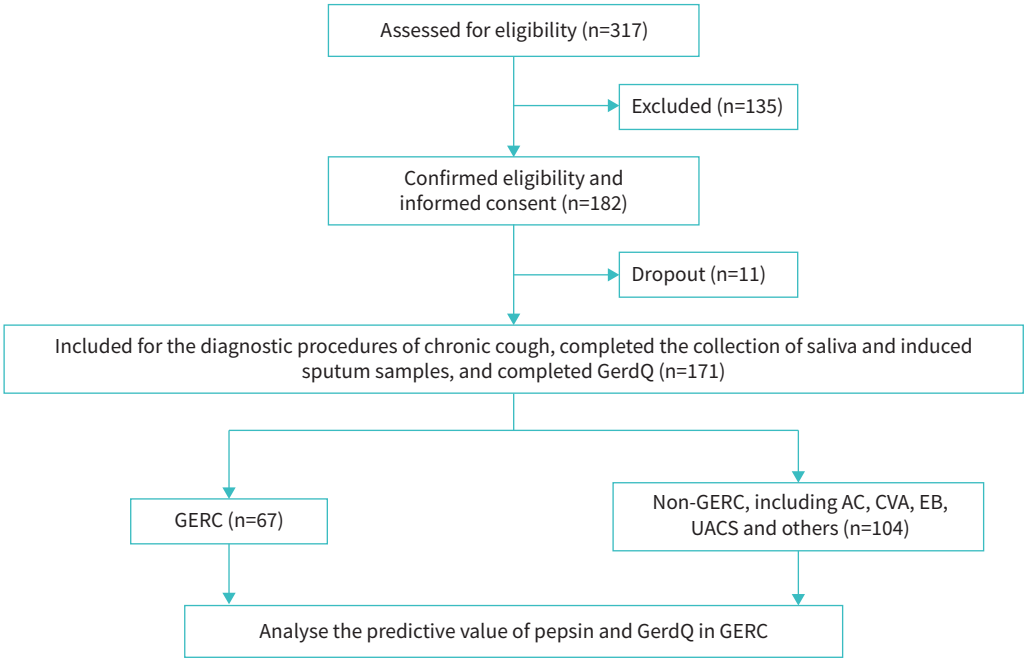

FIGURE 1 Study flowchart. GerdQ: Gastroesophageal Reflux Disease Questionnaire; GERC: gastroesophageal reflux-induced chronic cough; AC: atopic cough; CVA: cough variant asthma; EB: eosinophilic bronchitis; UACS: upper airway cough syndrome.

Laboratory investigations

Peptest

Saliva samples were obtained early in the morning while the patient was fasting. Patients were instructed to gently generate at least 1 mL of saliva sample from the larynx into a 15-mL sterile plastic tube containing 0.5 mL of 0.01 mol·L^{-1} citric acid. Samples were quickly transferred to a 4°C refrigerator. After the sputum was treated with dithiothreitol, the supernatant was stored in a −80°C refrigerator. Samples were processed 48 h after collection. Peptest Migration Buffer (240 µL) was pipetted into a screw-cap microfuge tube with 80 µL of the supernatant from the surface layer. The sample was combined on a vortex shaker for 10 s. The test strip was removed from the foil pouch and placed in the viewing window facing up on a horizontal table. Approximately 80 µL of the prepared sample was transferred into the test strip injection well. The test findings were read by the colloidal gold immunochromatographic strip smart detector 15 min later. After three tests for each sample, the average value was determined to be the final result and noted.

MII-pH

MII-pH was performed according to an established procedure [14, 15]. A combined MII-pH probe has six impedance channels (consisting of seven impedance sensors) and one pH sensor. The combined MII-pH catheter with six impedance channels (K6011-E10632; Unisensor, Zurich, Switzerland) was inserted transnasally into the oesophagus 3, 5, 7, 9, 15 and 17 cm above the lower oesophageal sphincter at locations determined by oesophageal manometry. An antimony pH electrode (819100; Medical Measurement Systems, Enschede, The Netherlands) was placed 5 cm above the proximal border of the lower oesophageal sphincter. A connected portable data logger (Ohmega; Medical Measurement Systems) stored data from all seven channels over 24 h. Reflux events recorded in the MII-pH tracings were manually characterised as liquid, gas or mixed liquid/gas reflux based on the impedance values or as acidic (pH <4.0), weakly acidic (pH 4.0–<7.0) or weakly alkaline (pH ⩾7.0) reflux based on the pH measurements.

GerdQ

The GerdQ is a six-item symptom questionnaire containing four reflux-positive-related symptom questions and two reflux-negative-related symptom questions [16]. The totals are summed to obtain the total GerdQ score (0–18). GERC should be considered with a GerdQ score ⩾8.

Other laboratory tests

Induced sputum cytology was performed according to a previously described protocol [17]. The total number of sputum inflammatory cells and cell classification results were determined to clarify the type and degree of airway inflammation in the patients. The cough symptom score was based on a scale developed by Hsu *et al.* [18]. The scale was split into two sections (the scores for daytime and nocturnal cough symptoms). The levels ranged from 0 (no coughing) to 5 (severe coughing most of the day). Pulmonary ventilation function tests and histamine bronchial provocation tests were performed according to guidelines established by the American Thoracic Society [19]. Capsaicin cough sensitivity was measured by a modified capsaicin challenge test, as reported by Fujimura *et al.* [20].

Sample size

Based on the pre-experiment results, the sensitivity (S_N) was calculated to be 0.79 and the specificity (S_P) was calculated to be 0.87. The prevalence of GERC in chronic cough was 40%. According to the formulae: $Z^2_{1-\alpha/2}\times S_N\times(1-S_N)/L^{-2}\times$prevalence and $Z^2_{1-\alpha/2}\times S_P\times(1-S_P)/L^{-2}\times(1-$prevalence), a total of 160 patients with chronic cough needed to be included. $Z_{1-\alpha/2}$ is the Z-value in a normal distribution when the cumulative probability is equal to $\alpha/2$ and when α is 0.05, $Z_{1-\alpha/2}$ is 1.96. L, which is the width of the 95% interval of the allowable sensitivity or specificity, was set to 0.1 in this study. Our final analysis was conducted on 171 included patients [21, 22].

Statistical analysis

Statistical analysis was performed using SPSS version 25.0 (IBM, Armonk, NY, USA). Data with a normal distribution are presented as mean with standard deviation. Data with an abnormal distribution are presented as median (range interquartile), while C2 and C5 (capsaicin solution concentration for $\geqslant$2 and $\geqslant$5 coughs, respectively) were log-transformed to normalise the data and presented as geometric mean with standard deviation. ANOVA or non-parametric tests were used to compare the data for differences between groups. A Chi-squared test was performed using Pearson and continuity modified Chi-squared tests. The receiver operating characteristic (ROC) curve was used to evaluate the diagnostic efficacy. Different areas under the ROC curve (AUCs) were compared using the DeLong test. A $p<0.05$ was considered statistically significant.

Results

Basic information

From February 2021 to October 2022, a total of 317 patients with chronic cough were seen in our Outpatient Clinic. A total of 182 patients met the inclusion criteria; five withdrew from the study due to incomplete data and six were lost to follow-up. A total of 171 patients with chronic cough were ultimately included in the study, including 74 males and 97 females with a mean±SD age of 48.07±15.09 years. The distribution of aetiologies is shown in table 1.

Comparison of general clinical data between patients in GERC and non-GERC groups

This study included 67 patients with GERC and 104 patients with non-GERC. There were no significant differences in gender, age, height, weight, body mass index and pulmonary ventilation function between

TABLE 1 Aetiologies of chronic cough patients (n=171)

Single aetiology	146 (85.38)
GERC	56 (32.75)
CVA	20 (11.70)
EB	20 (11.70)
AC	16 (9.36)
UACS	17 (9.94)
PIC	9 (5.26)
ACEI-related chronic cough	3 (1.75)
Others	5 (2.92)
Dual aetiologies	11 (6.43)
GERC+CVA	5 (2.92)
GERC+AC	3 (1.75)
GERC+EB	3 (1.75)
Refractory cough	14 (8.19)

Data are presented as n (%). GERC: gastroesophageal reflux-induced chronic cough; CVA: cough variant asthma; AC: atopic cough; EB: eosinophilic bronchitis; UACS: upper airway cough syndrome; PIC: post-infectious cough; ACEI: angiotensin-converting enzyme inhibitor.

TABLE 2 Comparison of general clinical data between patients in the gastroesophageal reflux-induced chronic cough (GERC) and non-GERC groups

	GERC (n=67)	Non-GERC (n=104)	Statistical value
Gender (male/female)	27/40	47/57	χ^2=0.398, p=0.528
Age (years)	49.58±15.78	47.06±14.60	t=1.062, p=0.290
Height (cm)	163.87±8.86	165.05±7.88	t= −0.909, p=0.365
Weight (kg)	64.86±13.52	66.20±12.33	t= −0.664, p=0.508
BMI (kg·m^{-2})	24.03±3.93	24.21±3.70	t= −0.304, p=0.761
FEV_1 (% pred)	105.71±15.01	99.68±17.15	t=2.006, p=0.862
FVC (% pred)	101.87±13.87	99.58±14.63	t=0.868, p=0.956
FEV_1/FVC (%)	83.85±8.02	81.67±8.74	t=1.403, p=0.393
Cough symptom score			
Day	3.00 (1.00)	3.00 (1.00)	*Z*= −1.470, p=0.142
Night	1.00 (1.00)	1.00 (1.00)	*Z*= −1.844, p=0.065
C2 (μmol·L^{-1})	0.86±0.07$^{\#}$	0.84±0.07$^{\#}$	t=1.176, p=0.229
C5 (μmol·L^{-1})	0.95±0.13$^{\#}$	0.93±0.14$^{\#}$	t=0.312, p=0.802
GerdQ	7.94±2.23	6.50±1.61	t=4.905, p<0.001*

Data are presented as n, mean±SD or median (interquartile range), unless otherwise stated. GERC: gastroesophageal reflux-induced chronic cough; BMI: body mass index; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; GerdQ: Gastroesophageal Reflux Disease Questionnaire; C2: capsaicin solution concentration for ⩾2 coughs; C5: capsaicin solution concentration for ⩾5 coughs. $^{\#}$: geometric mean±SD. *: p<0.05.

the two groups. There were no statistically significant differences in cough symptom scores and capsaicin cough thresholds between the two groups. The GerdQ scores were significantly higher in the GERC group than the non-GERC group, as shown in table 2.

Comparison of pepsin concentrations in saliva and induced sputum in patients with different chronic cough aetiologies

The salivary pepsin concentration was significantly higher in patients with GERC than non-GERC (132.50 ±103.90 *versus* 30.05±64.33 ng·mL^{-1}; *Z*= −7.651, p<0.001). The salivary pepsin concentration in patients with GERC was also significantly increased compared with AC, CVA, EB and UACS (*F*=61.613, p<0.001). The pepsin concentration in induced sputum supernatant was low in patients in the GERC and non-GERC groups (0.00±22.98 *versus* 0.00±16.00 ng·mL^{-1}; p=0.642). There was no significant difference in the pepsin concentration in induced sputum supernatant between chronic cough aetiologies (p>0.05).

Predictive diagnostic value of salivary and induced sputum pepsin concentrations in patients with GERC

The diagnostic value of salivary and induced sputum pepsin concentrations in patients with GERC is shown in table 3 and figure 2. The diagnostic value of salivary pepsin was better than the induced sputum pepsin

TABLE 3 Prediction of gastroesophageal reflux-induced chronic cough based on the salivary or induced sputum pepsin concentration

	Salivary pepsin	Induced sputum pepsin
AUC	0.845	0.523
Cut-off value (ng·mL^{-1})	76.10	53.90
Youden index	0.663	0.095
Sensitivity (%)	83.58	20.45
Specificity (%)	82.69	89.06
Positive predictive value (%)	74.67	60.00
Negative predictive value (%)	88.54	62.11
κ-value	0.640	0.120
p-value in κ-test	<0.001	0.106

AUC: area under the receiver operating characteristic curve.

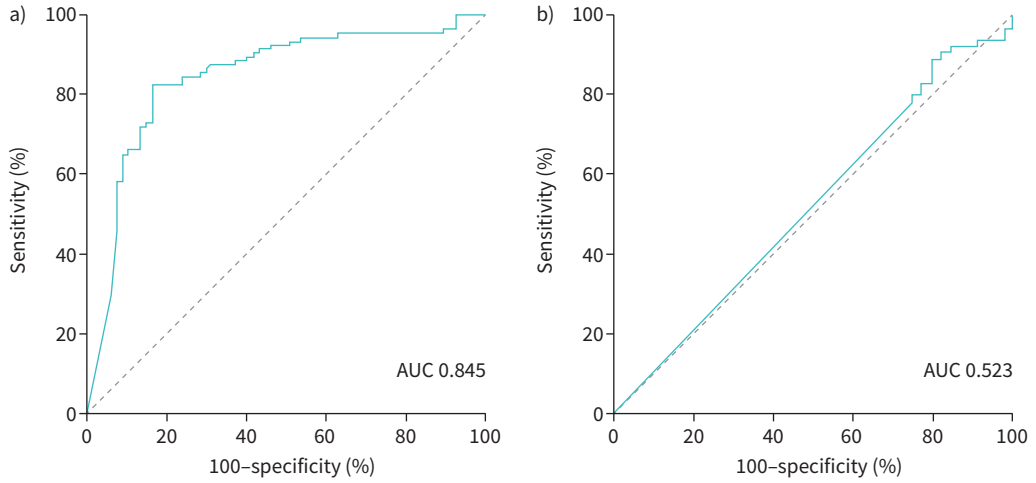

FIGURE 2 Diagnostic value of the pepsin concentration for gastroesophageal reflux-induced chronic cough (GERC): receiver operating characteristic curves of a) salivary pepsin concentration and b) induced sputum pepsin concentration in predicting GERC.

concentration (DeLong test, p<0.0001). The salivary pepsin concentration had a high diagnostic value for GERC, while the induced sputum pepsin concentration had a limited diagnostic value for GERC.

Predictive diagnostic value of the salivary pepsin concentration for acid and non-acid GERC

The diagnostic value of the salivary pepsin concentration for acid and non-acid GERC is shown in table 4. The salivary pepsin concentration had good diagnostic value for acid and non-acid GERC.

Comparison of the salivary pepsin concentration and GerdQ diagnostic value for GERC

When the GerdQ was used for GERC predictive diagnosis, the AUC was 0.670. The salivary pepsin concentration had a better diagnostic value than the GerdQ in GERC (DeLong test, p=0.0008) (table 5). The

TABLE 4 Prediction of acid and non-acid gastroesophageal reflux-induced chronic cough (GERC) with the salivary pepsin concentration

	Acid GERC (n=22)	Non-acid GERC (n=19)
AUC	0.779	0.830
Cut-off value (ng·mL^{-1})	79.00	76.10
Youden index	0.542	0.609
Sensitivity (%)	81.82	89.47
Specificity (%)	70.73	70.63
Positive predictive value (%)	33.33	31.48
Negative predictive value (%)	95.60	97.80
κ-value	0.329	0.337
p-value in κ-test	<0.001	<0.001

AUC: area under the receiver operating characteristic curve.

TABLE 5 Comparison of the salivary pepsin concentration and Gastroesophageal Reflux Disease Questionnaire (GerdQ) diagnostic value for gastroesophageal reflux-induced chronic cough

	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	κ-value
Salivary pepsin >76.10 ng·mL^{-1}	0.845	83.58	82.69	74.67	88.54	0.640
GerdQ ⩾8	0.670	52.24	81.73	64.81	72.65	0.385

AUC: area under the ROC curve; PPV: positive predictive value; NPV: negative predictive value.

TABLE 6 Comparison of the salivary pepsin concentration and Gastroesophageal Reflux Disease Questionnaire (GerdQ) score diagnostic value for acid and non-acid gastroesophageal reflux-induced chronic cough (GERC)

	Acid GERC		Non-acid GERC	
	Salivary pepsin	GerdQ	Salivary pepsin	GerdQ
AUC	0.779	0.826	0.830	0.533
Sensitivity (%)	81.82	86.36	89.47	36.84
Specificity (%)	70.73	78.86	70.63	69.84
Positive predictive value (%)	33.33	42.22	31.48	15.56
Negative predictive value (%)	95.6	97	97.8	88
κ-value	0.329	0.456	0.337	0.042

AUC: area under the receiver operating characteristic curve.

GerdQ score was higher in patients with acid GERC than non-acid GERC (9.86±2.51 *versus* 7.16±1.21; t=4.277, p<0.001). The salivary pepsin concentration diagnostic value was comparable to the GerdQ score in acid GERC (AUC 0.779 *versus* 0.826; p=0.4199). The salivary pepsin concentration diagnostic value was superior to the GerdQ score in non-acid GERC (AUC 0.830 *versus* 0.533; p<0.0001) (table 6).

Discussion

The prevalence of GERC has increased in recent years. Improving the diagnostic accuracy of GERC has become a clinical priority. However, invasive tests are difficult to perform, and the sensitivity and specificity of anti-reflux therapy are poor, thus limiting the application of these methods in clinical practice.

Peptest specifically detects pepsin A, which is only secreted by the principal cells in the stomach [23, 24] and is an objective indicator for detecting the onset of reflux. Peptest is a non-invasive, easily accessible and cost-effective diagnostic tool, which is the closest test available for clinical implementation [25].

The reflux theory suggests that microaspiration of gastric contents is the main deleterious event in patients with chronic cough [26]. Reflux fluid not only includes acids, but also contains pepsin, which can cause respiratory damage [27]. Previous studies have explored the possibility that pepsin has a damaging pro-inflammatory effect on the respiratory epithelium [28] and that pepsin exacerbates respiratory inflammation, which leads to persistent coughing episodes. Both acid and non-acid gaseous reflux may also cause neuronal hypersensitivity from recurrent aspiration, therefore leading to cough hypersensitivity syndrome in such patients.

Our results showed that a salivary pepsin concentration >76.10 $ng \cdot mL^{-1}$ had high diagnostic value for GERC. A prospective study conducted by Yuksel *et al*. [29] showed that the sensitivity of salivary pepsin for diagnosing GERD was 87% when the concentration was >50 $ng \cdot mL^{-1}$. A study by Wang *et al*. [30] also showed that salivary pepsin facilitated the diagnosis of GERD in patients with predominantly extra-oesophageal symptoms, including cough, pharyngitis and hoarseness, but the cut-off value and the sensitivity and specificity as a diagnostic tool were not determined. Reflux that rises ⩾15 cm above the lower oesophageal sphincter is referred to as proximal reflux, which has been linked to coughing [31]. It is hypothesised that the presence of pepsin in saliva may be a sign of proximal reflux. Additionally, GERC patients had more proximal reflux episodes than non-GERC participants in our earlier study [32]. Therefore, proximal reflux has a significant role in the development of GERC.

We found that the salivary pepsin concentration was significantly higher in patients with acid and non-acid GERC than patients with non-GERC. Moreover, there was no significant difference in the salivary pepsin concentration between patients with acid and non-acid GERC. A study by Dy *et al*. [33] also showed no significant difference in the distribution of reflux variables, such as acid *versus* non-acid, in patients with a salivary pepsin concentration ⩾75 $ng \cdot mL^{-1}$. The salivary pepsin concentration was not effective in differentiating between acid and non-acid reflux compared to MII-pH. However, the saliva required for the Peptest is more easily available, does not require a catheter in the gastrointestinal tract and does not disrupt normal life. Patients with low salivary pepsin levels were less likely to have GERC.

The pepsin concentration in induced sputum was low in all chronic cough patients in the current study and did not differ significantly between patients with different chronic cough aetiologies. A low pepsin

concentration in induced sputum was because only a small portion of pepsin refluxed to the oral cavity refluxes to the airway, and coughing prevents pepsin from entering the airway [34]. In contrast, dithiothreitol (DTT) has some protease activity when dissolving induced sputum, leading to a decrease in the protease concentration in DTT-treated sputum [35]. The reflex theory is considered another pathogenic process underlying GERC. Distal oesophageal mucosal receptors are directly stimulated by reflux, which then passes through the oesophageal mucosa to stimulate the cough centre, thus triggering the bronchial cough reflex. According to Shuai and Xie [36], the expression of the c-Fos gene in the medulla of rats increased after stimulation of the oesophagus by pepsin and gastric acid. This finding raises the possibility that pepsin stimulation of the distal oesophagus may cause a central cough. At the same time, incomplete clearance of gastric reflux (caused by oesophageal dysmotility) may lead to oesophagobronchial reflexes, which may lead to vagal hypersensitivity or sequelae of vagal neuron pathology (*e.g.* cough hypersensitivity syndrome). The study by Sykes *et al*. [37] has shown that 66% of patients with chronic cough have oesophageal dysmotility. Therefore, it is challenging to use induced sputum pepsin as a trustworthy marker because numerous factors influence the concentration of pepsin in the sputum.

In the current study, we showed that salivary pepsin has better diagnostic value than the GerdQ for GERC. The results of Norder Grusell *et al*. [38] also showed that the sensitivity of the GerdQ was lower in GERD patients with atypical symptoms, such as cough, dysphagia and hypochondriasis, as the main symptoms. The GerdQ consists of six main items (reflux, heartburn, nausea, insomnia, epigastric pain and medication use) and items to assess cough are not included. Cough is usually the only or main complaint in patients with GERC, and other reflux-related symptoms (*e.g.* acid reflux and heartburn) may be rare or absent [39]. Most acid reflux and heartburn are caused by acid reflux [40]. Patients with non-acid GERC lack symptoms of acid reflux or heartburn due to significant acid reflux. Thus, the GerdQ has limited diagnostic value in non-acid GERC [4]. The results of the current study suggest that the salivary pepsin concentration has a high diagnostic value in patients with acid and non-acid GERC. Therefore, the salivary pepsin concentration has a better complementary role in patients with non-acid GERC. Thus, Peptest can be used in all patients with suspected GERC, especially those with non-acid GERC.

This study has some limitations. 1) We chose to apply a clinically practical method of collecting a single fasting sample, but lacked the results of salivary pepsin testing at different time intervals, which prevented us from determining the time-point with the best diagnostic value. 2) The sample sizes were small when analysing patients with acidic and non-acidic GERC. 3) There was a lack of treatment based on the salivary pepsin test after the diagnostic follow-up data on outcomes. We are conducting corollary studies.

Conclusion

The salivary pepsin concentration has a high diagnostic value for GERC. The diagnosis of GERC should be considered when the salivary pepsin concentration is >76.10 $ng \cdot mL^{-1}$. Induced sputum pepsin has a limited diagnostic value for GERC. Salivary pepsin has a better diagnostic value for GERC than the GerdQ score and is a good addition in the diagnostic evaluation of non-acid GERC.

Provenance: Submitted article, peer reviewed.

Data availability: The data and/or related materials of this study are available from the corresponding author on reasonable request.

This study is registered at Chinese Clinical Trials Register with identifier number ChiCTR1800020221.

Ethics statement: The study procedure was approved by the Ethics Committee of Tongji Hospital (2018-LCYJ-013). Written informed consent was obtained from all participants.

Author contributions: W. Gu: conceptualisation; data curation; formal analysis; investigation; methodology; project administration; software; validation; visualisation; and writing (original draft, review and editing). W. Chen: conceptualisation; data curation; formal analysis; methodology; project administration; supervision; validation; and writing (original draft, review and editing). T. Zhang: formal analysis; investigation; methodology; resources; software; validation; visualisation; and writing (original draft, review and editing). Y. Zhu: investigation; methodology; validation; visualisation; and writing (original draft). W. Li: data curation; investigation; resources; and writing (original draft). W. Shi: investigation; methodology; validation; visualisation; and writing (original draft). N. Li: data curation; formal analysis; investigation; methodology; and writing (original draft, review and editing). S. Wang: data curation; investigation; resources; visualisation; and writing (original draft). X. Xu: conceptualisation; data curation; formal analysis; funding acquisition; investigation; methodology; project

administration; resources; software; supervision; validation; and writing (original draft, review and editing). L. Yu: conceptualisation; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; software; supervision; validation; and writing (original draft, review and editing).

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References

1 Lai K, Chen R, Lin J, *et al.* A prospective, multicenter survey on causes of chronic cough in China. *Chest* 2013; 143: 613–620.
2 Kahrilas PJ, Altman KW, Chang AB, *et al.* Chronic cough due to gastroesophageal reflux in adults: CHEST Guideline and Expert Panel Report. *Chest* 2016; 150: 1341–1360.
3 Zhu Y, Zhang T, Wang S, *et al.* Mean nocturnal baseline impedance (MNBI) provides evidence for standardized management algorithms of nonacid gastroesophageal reflux-induced chronic cough. *Int J Clin Pract* 2023; 2023: 7992062.
4 Xu X, Chen Q, Liang S, *et al.* Comparison of gastroesophageal reflux disease questionnaire and multichannel intraluminal impedance pH monitoring in identifying patients with chronic cough responsive to antireflux therapy. *Chest* 2014; 145: 1264–1270.
5 Samuels TL, Johnston N. Pepsin as a marker of extraesophageal reflux. *Ann Otol Rhinol Laryngol* 2010; 119: 203–208.
6 Rosen R, Johnston N, Hart K, *et al.* The presence of pepsin in the lung and its relationship to pathologic gastro-esophageal reflux. *Neurogastroenterol Motil* 2012; 24: 129-e85.
8 Hayat JO, Gabieta-Somnez S, Yazaki E, *et al.* Pepsin in saliva for the diagnosis of gastro-oesophageal reflux disease. *Gut* 2015; 64: 373–380.
9 Strugala V, Woodcock AD, Dettmar PW, *et al.* Detection of pepsin in sputum: a rapid and objective measure of airways reflux. *Eur Respir J* 2016; 47: 339–341.
10 Yadlapati R, Kaizer A, Greytak M, *et al.* Diagnostic performance of salivary pepsin for gastroesophageal reflux disease. *Dis Esophagus* 2021; 34: doaa117.
11 Asthma Group of the Respiratory Diseases Branch of the Chinese Medical Association. [Guidelines for the diagnosis and treatment of cough (2021)]. *Zhonghua Jie He He Hu Xi Za Zhi* 2022; 45: 13–46.
12 Zhu Y, Tang J, Shi W, *et al.* Can acid exposure time replace the DeMeester score in the diagnosis of gastroesophageal reflux-induced cough? *Ther Adv Chronic Dis* 2021; 12: 20406223211056719.
13 Asthma Group of the Respiratory Diseases Branch of the Chinese Medical Association. [Guidelines for the diagnosis and treatment of cough (2015)]. *Zhonghua Jie He He Hu Xi Za Zhi* 2016; 39: 323–354.
14 Qiu Z, Yu L, Xu S, *et al.* Cough reflex sensitivity and airway inflammation in patients with chronic cough due to non-acid gastro-oesophageal reflux. *Respirology* 2011; 16: 645–652.
15 Xu X, Yu L, Chen Q, *et al.* Diagnosis and treatment of patients with nonacid gastroesophageal reflux-induced chronic cough. *J Res Med Sci* 2015; 20: 885–892.
16 Bai Y, Du Y, Zou D, *et al.* Gastroesophageal Reflux Disease Questionnaire (GerdQ) in real-world practice: a national multicenter survey on 8065 patients. *J Gastroenterol Hepatol* 2013; 28: 626–631.
17 Yu L, Wei W, Wang L, *et al.* Upper-airway cough syndrome with latent eosinophilic bronchitis. *Lung* 2010; 188: 71–76.
18 Hsu JY, Stone RA, Logan-Sinclair RB, *et al.* Coughing frequency in patients with persistent cough: assessment using a 24-hour ambulatory recorder. *Eur Respir J* 1994; 7: 1246–1253.
19 Crapo RO, Casaburi R, Coates AL, *et al.* Guidelines for methacholine and exercise challenge testing – 1999. *Am J Respir Crit Care Med* 2000; 161: 309–329.
20 Fujimura M, Sakamoto S, Kamio Y, *et al.* Effects of methacholine induced bronchoconstriction and procaterol induced bronchodilation on cough receptor sensitivity to inhaled capsaicin and tartaric acid. *Thorax* 1992; 47: 441–445.
21 Buderer NM. Statistical methodology: I. Incorporating the prevalence of disease into the sample size calculation for sensitivity and specificity. *Acad Emerg Med* 1996; 3: 895–900.
22 Malhotra RK, Indrayan A. A simple nomogram for sample size for estimating sensitivity and specificity of medical tests. *Indian J Ophthalmol* 2010; 58: 519–522.
23 Rao Y-F, Wang J, Cheng DN, *et al.* The controversy of pepsinogen A/pepsin A in detecting extra-gastroesophageal reflux. *J Voice* 2023; 37: 748–756.
24 Abdallah AF, El-Desoky T, Fathi K, *et al.* Clinical utility of bronchoalveolar lavage pepsin in diagnosis of gastroesophageal reflux among wheezy infants. *Can Respir J* 2016; 2016: 9480843.

25 Samuels TL, Johnston N. Pepsin in gastroesophageal and extraesophageal reflux: molecular pathophysiology and diagnostic utility. *Curr Opin Otolaryngol Head Neck Surg* 2020; 28: 401–409.
26 Durazzo M, Lupi G, Cicerchia F, *et al.* Extra-esophageal presentation of gastroesophageal reflux disease: 2020 update. *J Clin Med* 2020; 9: 2559.
27 Bathoorn E, Daly P, Gaiser B, *et al.* Cytotoxicity and induction of inflammation by pepsin in acid in bronchial epithelial cells. *Int J Inflam* 2011; 2011: 569416.
28 Hunt EB, Sullivan A, Galvin J, *et al.* Gastric aspiration and its role in airway inflammation. *Open Respir Med J* 2018; 12: 83.
29 Yuksel ES, Hong SK, Strugala V, *et al.* Rapid salivary pepsin test: blinded assessment of test performance in gastroesophageal reflux disease. *Laryngoscope* 2012; 122: 1312–1316.
30 Wang YJ, Lang XQ, Wu D, *et al.* Salivary pepsin as an intrinsic marker for diagnosis of sub-types of gastroesophageal reflux disease and gastroesophageal reflux disease-related disorders. *J Neurogastroenterol Motil* 2020; 26: 74–84.
31 Herregods TVK, Pauwels A, Jafari J, *et al.* Determinants of reflux-induced chronic cough. *Gut* 2017; 66: 2057–2062.
32 Wang S, Wen S, Bai X, *et al.* Diagnostic value of reflux episodes in gastroesophageal reflux-induced chronic cough: a novel predictive indicator. *Ther Adv Chronic Dis* 2022; 13: 20406223221117455.
33 Dy F, Amirault J, Mitchell PD, *et al.* Salivary pepsin lacks sensitivity as a diagnostic tool to evaluate extraesophageal reflux disease. *J Pediatr* 2016; 177: 53–58.
34 Decalmer S, Stovold R, Houghton LA, *et al.* Chronic cough: relationship between microaspiration, gastroesophageal reflux, and cough frequency. *Chest* 2012; 142: 958–964.
35 Wang F, He B. The effect of dithiothreitol on chemotactic factors in induced sputum of chronic obstructive pulmonary disease patients. *Respiration* 2009; 78: 217–222.
36 Shuai XW, Xie PY. Expression and localization of c-Fos and NOS in the central nerve system following esophageal acid stimulation in rats. *World J Gastroenterol* 2004; 10: 2287–2291.
37 Sykes DL, Crooks MG, Hart SP, *et al.* Investigating the diagnostic utility of high-resolution oesophageal manometry in patients with refractory respiratory symptoms. *Respir Medi* 2022; 202: 106985.
38 Norder Grusell E, Mjörnheim A-C, Finizia C, *et al.* The diagnostic value of GerdQ in subjects with atypical symptoms of gastro-esophageal reflux disease. *Scand J Gastroenterol* 2018; 53: 1165–1170.
39 Irwin RS, French CL, Curley FJ, *et al.* Chronic cough due to gastroesophageal reflux. Clinical, diagnostic, and pathogenetic aspects. *Chest* 1993; 104: 1511–1517.
40 Vela MF, Camacho-Lobato L, Srinivasan R, *et al.* Simultaneous intraesophageal impedance and pH measurement of acid and nonacid gastroesophageal reflux: effect of omeprazole. *Gastroenterology* 2001; 120: 1599–1606.