The Laryngoscope

Saliva Pepsin Detection and Proton Pump Inhibitor Response in Suspected Laryngopharyngeal Reflux

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Objectives/Hypothesis: To evaluate the prediction value of saliva pepsin detection for an 8-week proton pump inhibitor (PPI) response in patients with a Reflux Symptoms Index (RSI) score ≥13, which indicates possible laryngopharyngeal reflux.

Study Design: Prospective individual single-cohort study.

Methods: Patients were recruited who had experienced chronic laryngopharyngeal symptoms (RSI score ≥13) for more than 3 months after excluding other etiologies. The patients received PPI (40 mg of esomeprazole once daily) treatment for 8 weeks. Prior to treatment, the patients submitted saliva/sputum samples that were collected during the time symptoms were observed. The samples were taken for pepsin detection, and performed using the commercially available Peptest lateral flow device. The association of the Peptest results and PPI response were statistically analyzed with the χ^2 test.

Results: Seventy-four patients completed the study, and upon completion of PPI treatment, the mean RSI score was significantly reduced from 19.22 ± 5.18 to 8.99 ± 5.69. Forty-four (59.5%) patients exhibited a good response as defined by an RSI score reduction ≥50%. The results of the Peptest were semiquantitatively graded as 0, 1, 2, 3 (negative, weak positive, moderate positive and strong positive, respectively) based upon the visual intensity of the test sample line as compared to the control line. Twenty-four patients (32.4%) exhibited grade 3 strong positive results. The Peptest strong positive results ($P < .05$) were significantly associated with a good PPI response, with the positive predictive value being 79.2%.

Conclusions: Analysis of strong positive results for pepsin detection in saliva/sputum samples may be a useful, noninvasive method for predicting better PPI response in patients with suspected reflux induced chronic laryngopharyngeal symptoms.

Key Words: Laryngopharyngeal reflux, pepsin, proton pump inhibitor, saliva, sputum.

Level of Evidence: 2

***Laryngoscope*, 129:709–714, 2019**

INTRODUCTION

Patients expressing major complaints regarding chronic laryngopharyngeal symptoms are commonly encountered in clinics. Upon excluding variable causes such infections, allergies, smoking, and drinking, along with other chronic irritants and tumors, acid reflux should be considered as a potential etiology that induces a patient's symptoms. Reflux of gastric acid contents into the upper aerodigestive tract is referred to as laryngopharyngeal reflux (LPR), and has been reported in up to 10% of patient who present themselves in otolaryngologist clinics.[1] Although there is an increased understanding of LPR as a cause of chronic laryngopharyngeal symptoms, methods in which to confirm the diagnosis and offer appropriate medical management still remain controversial.[2,3] As a case in point, laryngoscopic findings are widely used to investigate the mucosal changes that will raise the suspicion of LPR. However, a review study has demonstrated that individual laryngopharyngeal signs are highly prevalent in both acid reflux patients and healthy people.[4] Although 24-hour pH monitoring, which detects acid reflux to the laryngopharynx, could offer the evidence necessary to support the diagnosis of LPR, the low sensitivity that pH monitoring offers is still questioned by some experts. Vaezi suggested that when treating patients with LPR using a proton pump inhibitor (PPI), treatment should not be preceded by pH monitoring.[5,6] Recently combined multichannel intraluminal impedance (MII) and pH monitoring (MII–pH) has provided an advance in LPR diagnostics; however, the procedure is still too invasive to be a convenient tool for screening LPR in clinics.[7]

Based upon a careful study of pH monitoring used to confirm LPR cases, Belafsky et al.[8] developed the Reflux

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Editor's Note: This Manuscript was accepted for publication on July 12, 2018

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The study was supported by grants from the Ministry of Science and Technology, Taiwan (Project No. NSC101-2314-B-075A-004-MY3).

The authors have no other funding, financial relationships, or conflicts of interest to disclose.

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DOI: 10.1002/lary.27502

Symptom Index (RSI), a self-administered nine-item questionnaire that helps physicians assess the severity of a patient's LPR symptoms both before and after treatment. According to their article, patients with an RSI score ≥13 were considered to be potential LPR sufferers. In some articles, those patients have been recommended to undergo an empirical PPI treatment trial to suppress their acid reflux. A favorable response could also be used alternatively to diagnose LPR.[2,9] However, it is sometimes difficult to persuade patients to undergo empirical PPI treatment without offering them objective evidence of its value. Pepsin, secreted into gastric juices, is an excellent marker of reflux.[10] Using various methods to detect pepsin in saliva/sputum has reportedly provided a noninvasive tool for assisting in the assessment of LPR.[11–13] Recently, a rapid lateral flow test (Peptest) to detect pepsin in saliva/sputum has been developed, which offers a strong predictive value for diagnosing gastroesophageal reflux disease, according to the report published by Saritas Yuksel et al.[14] However, the association between Peptest results in patients suspected of LPR and the PPI treatment response remains undetermined. In this study, we aimed to clarify the prediction value of saliva pepsin detection for proton pump inhibitor response in patients displaying suspected reflux-induced chronic laryngopharyngeal symptoms.

MATERIALS AND METHODS

The present study was approved by the institutional review board (IRB) of Taichung Veterans General Hospital, Taichung City, Taiwan (IRB# CF12124A) and was sponsored by the Ministry of Science and Technology, Taiwan (Project No. NSC101-2314-B-075A-004-MY3). From August 2012 to November 2016, 83 patients with a suspicion of LPR were prospectively recruited to become members of the research group after reading and signing informed consents. All patients had visited our clinic expressing the chief complaint of having chronic laryngopharyngeal symptoms for more than 3 months. The nine-item RSI questionnaire, which had been developed and validated by Belafsky et al.,[8] was used to record symptoms both before and after treatment. Patients had to fulfill the inclusion criteria of being >20 years old, having an RSI score ≥13, and not having received any antireflux medication for at least 3 months prior to entering the study. Because PPI is not covered by the National Health Care System in Taiwan, and although our patients may have used other antacids or H2 blockers long before the study, they were all naïve with regard to PPI treatment; therefore, any possible rebounding effect to PPI treatment was avoided. The patients were carefully evaluated through their medical history review and a flexible laryngoscopy inserted via the nostril. Any patients who claimed a history of long-term smoking and drinking, allergic rhinitis, asthma, chronic sinusitis, chronic tonsillitis, or any other disorders that might cause chronic laryngopharyngeal symptoms were excluded from the study. Any patient with a laryngoscopy that detected any upper aerodigestive tract abnormality, such as epiglottic cysts, tonsil hypertrophy, or papilloma were also excluded. Therefore, by default, acid reflux was considered to be the most probable reason for the etiology of their symptoms.

The recruited patients were asked to collect their saliva/sputum samples for pepsin analysis during the time they were experiencing their major symptoms. According to the manual of the Peptest lateral flow device (RD Biomed Ltd., Hull, United Kingdom),[15] an expectorated saliva/sputum sample of at least 1 mL was collected into a 30-mL universal sample collection tube containing citric acid to preserve the action of any pepsin present. The collection should be taken within 15 minutes of the patient experiencing their chief complaint symptoms and then stored in the refrigerator. Within a week of collection, approximately 0.5 mL of the samples presented to our lab were transferred into an empty, blue, micro centrifuge tube using a 1-mL graduated pipette. The micro tube was then centrifuged at 4000 rpm for at least 5 minutes, until a clear supernatant layer was seen in the tube. A dual bulb pipette was used to draw out 80 μL from the surface layer of the micro, centrifuged sample, with that 80 μL sample then being transferred to a clear screw-top micro tube containing 240 μL of a migration buffer solution. The sample was then mixed using a vortex mixer for 10 seconds. Another dual bulb pipette was used to draw out the sample prior to applying it to the circular well of the Peptest device. When a blue line appeared under the letter C (control) of the device, it was an indication that the lateral flow was working. If a second blue line appeared under the letter T (test) between 5 and 15 minutes after applying the sample, it indicated pepsin was present in the sample with a positive result (Fig. 1). The test has the ability to detect pepsin down to 16 ng/mL. If the results did not reach that level, the test was considered negative. After the test, the Peptest results were both photographed and recorded by a research assistant; however, both patients and physicians remained blind to the results. Similar to the method outlined by Yadlapati et al.,[16] the results of the Peptest were semiquantitatively classified to the grades of 0, 1, 2, and 3 according to the blue color density of the line under the letter T. Grade 0 indicates a negative result, with a pepsin concentration <16 ng/mL (Fig. 1A). Grade 1 indicates a weak positive result, with a pepsin concentration from 25 ng/mL to 100 ng/mL (Fig. 1B). Grade 2 indicates a moderate positive result, with a pepsin concentration from 100 ng/mL to 250 ng/mL (Fig. 1C), and grade 3 indicates a strong positive result with a pepsin concentration >250 ng/mL. The blue line of the test looks similar to the control line when the test displays a strong positive result (Fig. 1D).

The recruited patients were then treated with 40 mg oral esomeprazole (Nexium; AstraZeneca Pharmaceuticals, Södertälje,

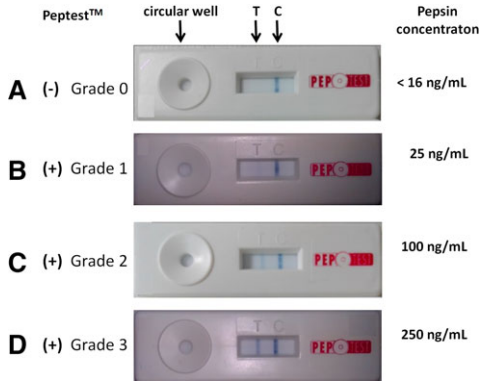

Fig. 1. Test results of Peptest lateral flow device according to a semiquantitative estimate of pepsin concentration in the saliva/sputum samples. (A) Grade 0 (negative) indicated a pepsin concentration <16 ng/mL, with no line shown under the letter T (test). (B) Grade 1 (weak positive) indicated a pepsin concentration from 25 ng/mL to 100 ng/mL, with a subtle line shown under the letter T. (C) Grade 2 (moderate positive) indicated a pepsin concentration from 100 ng/mL to 250 ng/mL, with a line shown under the letter T, but not as dense as the line under the letter C. (D) Grade 3 (strong positive) indicated a pepsin concentration >250 ng/mL, with the line under the letter T looking similar to the line under the letter C (control). [Color figure can be viewed at www.laryngoscope.com.]

Sweden) once daily. Each patient's RSI result was recorded prior to the treatment, and then also at 4 weeks and 8 weeks after the treatment to analyze the outcome. If a patient's RSI score reduced ≥50% from the baseline, then the treatment was defined as a PPI good response. The Friedman test was then used to compare the RSI score for each patient both before and after treatment. The χ^2 test was used to analyze the association between the Peptest results and a PPI good treatment response. Analyses were performed using the Statistical Package for the Social Science (version 22.0; IBM Corp., Armonk, NY). A P value <.05 indicated the result was statistically significant.

RESULTS

Eighty-three patients were recruited in the outpatient clinic after signing the necessary consent form to join the study. However, nine patients withdrew either before or during the PPI treatment, and their data were subsequently excluded in the analysis. The 74 (48 female, 26 male) remaining patients completed the PPI treatment; their ages ranged from 20 years to 67 years, with a mean age of 47 years. The nine-item RSI scores recorded prior to PPI treatment ranged from 13 to 33, with a mean ± standard deviation of 19.22 ± 5.18. The last item included in the RSI involved "heart burn, chest pain, indigestion, or stomach acid coming up," all of which represent the severity of typical acid reflux symptoms. We also evaluated the data of RSI items 1 to 8 (RSI 1–8), which indicated only laryngopharyngeal symptoms. Prior to treatment, 16 (21.6%) patients displayed negative results from their Peptest, whereas 58 (78.4%) patients experienced positive results. However, 24 (32.4%) patients had a grade 3, dense, blue-colored line under the letter T of the device, which indicated a strong positive for pepsin in their saliva/sputum specimen. We also dichotomized the Peptest results to determine whether they were strong positive or not. This was done to evaluate the association of the Peptest results and PPI treatment response. The demography of the studied patients and their baseline data from the RSI and Peptest prior to treatment are listed in Table I.

After 2 months of treatment with oral esomeprazole, 40-mg tablet daily, the RSI 1–9 scores were significantly reduced from 19.22 ± 5.18 to 8.99 ± 5.69 (P < .001). Similarly, upon evaluating atypical reflux symptoms reflected in the data of the RSI 1–8, the scores were significantly reduced from 17.11 ± 4.80 to 8.23 ± 5.31 (P < .001) (Table II). Overall, after PPI treatment for 8 weeks, more than 50% of the patients exhibited a good response (reduction of RSI scores ≥50% when compared to baseline) (Table III).

Using the χ^2 test, gender had no association with a good PPI response for both RSI 1–9 and RSI 1–8 at 4 weeks (P = .984, P = .399) and 8 weeks (P = .984, P = .657), respectively. Similarly, if we dichotomized the Peptest results to positive and negative, the results exhibited no association with a good PPI response for both RSI 1–9 and RSI 1–8 at 4 weeks (P = .994, P = .656) and 8 weeks (P = 1.000, P = 1.000), respectively. However, after dichotomizing the Peptest results to a strong positive and others, we discovered that Peptest strong positive results had a statistically significant association with a good PPI treatment response for RSI 1–9 at 8 weeks

TABLE I.
Clinical Characters, Baseline RSI Data, and Peptest Results Prior to Proton Pump Inhibitor Treatment of the Studied Patients.

	Mean	SD	Median	Minimum	Maximum	No.	%
Age, yr	47.19	11.84	50	20	67		
RSI baseline							
RSI 1	2.58	1.46	3	0	5		
RSI 2	3.28	1.12	3	0	5		
RSI 3	2.54	1.50	2.5	0	5		
RSI 4	1.24	1.34	1	0	5		
RSI 5	1.50	1.26	1	0	5		
RSI 6	1.31	1.33	1	0	5		
RSI 7	1.32	1.42	1	0	5		
RSI 8	3.32	1.10	3	0	5		
RSI 9	2.11	1.53	2	0	5		
RSI 1–9	19.22	5.18	18.5	13	33		
RSI 1–8	17.11	4.80	16	10	29		
Gender							
Female						48	64.9%
Male						26	35.1%
Peptest							
Negative (grade 0)						16	21.6%
Positive (grade 1, 2, 3)						58	78.4%
Others (grade 0, 1, 2)						50	67.6%
Strong positive (grade 3)						24	32.4%

RSI = Reflux Symptoms Index; SD = standard deviation.

TABLE II.
The Data of RSI Including (RSI 1–9) and Excluding (RSI 1–8) Typical Reflux Symptoms Before and After Treatment Using a Proton Pump Inhibitor.

	Mean	SD	Median	Minimum	Maximum	*P* Value
RSI 1–9						<.001*
Baseline	19.22	5.18	18.5	13	33	
4 weeks	11.12	5.16	11	4	25	
8 weeks	8.99	5.69	8	1	25	
RSI 1–8						<.001*
Baseline	17.11	4.80	16	10	29	
4 weeks	10.04	4.72	9	3	23	
8 weeks	8.23	5.31	7	1	23	

*$P < .01$, Friedman test.
RSI = Reflux Symptoms Index; SD = standard deviation.

($P = .032$) and RSI 1–8 at 4 and 8 weeks ($P = .037$, $P = .009$), respectively (Table IV). The specificity was 83.3% and the positive predictive value was 79.2% for a strong positive Peptest result necessary for predicting a good response to PPI treatment.

DISCUSSION

LPR is an extraesophageal reflux manifestation of gastroesophageal reflux disease, which is defined as the reflux of gastric contents into the laryngopharynx. LPR is thought to be associated with various upper airway diseases and chronic laryngopharyngeal symptoms. According to the study performed by Francis et al.,[17] LPR is not only an annoying condition for patients, but also causes a high economic burden on the US healthcare system. Altman et al.[18] reported a 500% increase in visits to otolaryngologists due to LPR between 1990 and 2001. Clearly, LPR has become a prevalent and concerning disorder that needs to be managed more efficiently in the modern era.

The diagnosis of LPR is initially based on the symptoms and laryngoscopic findings of the patient.[8,19] A range of patient-completed questionnaires is useful for recording symptoms over time and the effects therapy may have. One of the most commonly used questionnaires is the RSI, where patients with an RSI score ≥13 were considered to be probable LPR sufferers according to the article that validated this questionnaire.[8] However, the PPI treatment response for patients with an RSI score ≥13 still requires further investigation, and we trust that our research will add aluable information regarding this issue. According to Ford,[2] the most common symptoms of LPR are hoarseness, excessive throat clearing, coughing, and a lumpy sensation in the throat. The results of Ford's study are quite similar to our observations. The mean value of each RSI item for our patients was higher in items 1, 2, 3, and 8 (hoarseness, clearing of throat, excess mucus, and throat lump sensation) (Table I). After 8 weeks of esomeprazole 40-mg tablet/daily treatment, the RSI scores were significantly reduced in our study cohort. These results suggest that our inclusion criteria may serve as a screening tool when selecting proper patients for a short-term PPI treatment trial. However, our RSI scores still ranged from 1 to 25 after treatment. Some patients still recorded a high RSI score, whereas others displayed a good response to the PPI trial. In our previous study,[20] ambulatory 24-hour pH monitoring was found to be useful in predicting a positive esomeprazole response in patients of LPR who did not exhibit typical reflux symptoms. Twenty-four–hour pH monitoring is not routinely used due to its invasiveness, cause for discomfort, cost, and time consumption. The ability to predict who will be a good responder to PPI treatment through the use of a noninvasive method validates this prospective Peptest study. Peptest is a rapid, lateral-flow, reflux diagnostic saliva device that has been registered for sale with the US Food and Drug Administration (FDA).

Pepsin has the potential to damage mucosal tissues and is present in all refluxate. It is only produced in the stomach, and is thus a specific biomarker for gastric reflux. Furthermore, detecting pepsin in saliva/sputum is a noninvasive diagnostic procedure, which is more acceptable when used as a routine application. Potluri et al.[21] used the fibrinogen digestion method to compare saliva/sputum pepsin assay with 24-hour esophageal pH monitoring, for detection of gastric reflux into the proximal esophagus, oropharynx, and lungs. Several different assays for detecting pepsin in saliva/sputum, including Western blot[12] and enzyme-linked immunosorbent

TABLE III.
Number and Percentage of the Patients Who Displayed a Good Response (Reduction of RSI Score ≥50%) After Proton Pump Inhibitor Treatment at 4 and 8 Weeks.

		No.	%		No.	%
4 weeks	ΔRSI 1–9			ΔRSI 1–8		
	<50%	44	59.4%	<50%	45	60.8%
	≥50%	30	40.6%	≥50%	29	39.2%
8 weeks	ΔRSI 1–9			ΔRSI 1–8		
	<50%	30	40.5%	<50%	33	44.6%
	≥50%	44	59.5%	≥50%	41	55.4%

RSI = Reflux Symptoms Index.

TABLE IV.
Cross-Table of Peptest Strong Positive Results and Good Response After Proton Pump Inhibitor Treatment at 4 and 8 Weeks.

Peptest Strong (+)	Good Response at 4 Weeks					Good Response at 8 Weeks				
	RSI 1–9					RSI 1–9				
	No (n = 44)		Yes (n = 30)			No (n = 30)		Yes (n = 44)		
	No.	%	No.	%	$P = .057$	No.	%	No.	%	$P = .032$*
No	34	77.3%	16	77.3%		25	83.3%	25	56.8%	
Yes	10	22.7%	14	22.7%		5	16.7%	19	43.2%	
	RSI 1–8					RSI 1–8				
	No (n = 45)		Yes (n = 29)		$P = .037$*	No (n = 33)		Yes (n = 41)		$P = .009$†
	No.	%	No.	%		No.	%	No.	%	
No	35	77.8%	15	51.7%		28	84.8%	22	53.7%	
Yes	10	22.2%	14	48.3%		5	15.2%	19	46.3%	

*$P < .05$, χ^2 test.
†$P < .01$, χ^2 test.

assay,[22] have been used in the lab. However, these methods are both laborious and time consuming. The pepsin immunoassay has been adapted as a lateral flow–based test (Peptest) with results available within minutes.[23] According to the meta-analysis performed by Wang et al.,[24] diagnostic values of pepsin detection varied markedly in studies involving different study designs.[25–29] The heterogeneity of the studies included sample size, diagnostic criteria (pH monitoring/symptoms/signs), assay type (Peptest vs. others), timing of the collection of saliva/sputum, number of pepsin tests performed, and pepsin concentration cutoff values among other factors. The team concluded that current evidence for salivary pepsin use is insufficient, and that further investigations are required.

In our study, the 74 studied patients were one of the largest cohorts ever assembled. Because an RSI score ≥13 is a practical clinical indicator, we used symptoms (RSI score ≥13) as our LPR suspects after carefully excluding any other possible etiologies. A positive PPI response after treatment further confirmed the diagnosis of LPR in good responders. With regard to the collection of saliva/sputum samples, Fortunato el al.[22] discovered that the concentration of salivary/sputum pepsin had covered a wide range when observed in individuals over 24 hours, and that saliva/sputum samples must be obtained soon after a reflux event. Kim et al.[12] also suggested that saliva/sputum samples be collected at the time of symptoms to provide a sensitive test for LPR. According to the manual of the Peptest lateral flow device,[15] samples with pepsin >16 ng/mL could be detected and shown as a positive result. In our study, the samples collected at the time of symptoms showed a positive (pepsin >16 ng/mL) rate of 78.4%. However, a positive Peptest did not associate with a PPI response showing a symptoms reduction of ≥50% after treatment. Yadlapati et al.[16] discovered that neither oropharyngeal pH testing nor a positive Peptest (pepsin >16 ng/mL) were able to diagnose LPR. However, one positive sample >210 ng/mL of pepsin suggested the presence of reflux with 98.2% specificity. Similarly, we found that a strong positive Peptest result (by visual semiquantification) demonstrated a significant association with a good PPI response. Therefore, a strong positive Peptest result may be recognized as a valuable diagnostic tool for confirming LPR with both satisfactory specificity (83.3%) and positive predictive value (79.2%).

This study had several limitations. First, the optimal timing of saliva sampling was not addressed in this article. For example, Na et al.[30] reported that patients suffering from reflux average pepsin level upon waking was higher than that measured at any other time, including when LPR symptoms occurred. Whether other saliva sampling timing offers better treatment response prediction value is worthy of further investigation in the future. In addition, the correlation between our patients' testing to a recent meal time was not recorded. Therefore, we did not establish whether the reflux symptoms usually occurred before or after a meal. Second, we excluded patients who were habitual smokers and drinkers, even though these patients are commonly encountered in the clinic. According to our previous study, some symptoms in the RSI questionnaire were more related to smoking and drinking, but were not endoscopy-confirmed reflux esophagitis sufferers within the general population.[31] This is why we strictly avoided including patients who most likely experienced chronic inflammation caused by other irritants and not reflux. However, for patients who are habitual smokers and drinkers, the Peptest could be used on them for further studies in the future. Third, the grading of Peptest results was subjectively quantified through visual evaluation in accordance with the report of Yadlapati et al.[16] Recently there has been an electronic lateral-flow device reader available on the market for quantitatively measuring pepsin levels in saliva/sputum. This product may facilitate further studies on this topic, but was unfortunately unavailable to us when we began our research. Fourth, the twice-daily use of a PPI is usually reserved for patients who are suspected of experiencing LPR. However, it is neither FDA approved nor based upon the results of any controlled studies.[32] The meta-analysis also attempted to show whether PPIs twice daily are therapeutically more efficient than PPIs once daily, although this still needs to be determined.[33] In our study, a once-daily PPI treatment was prescribed, with the

resultant significant reduction in either the patient's RSI 1–9 or RSI 1–8 score, supporting the application of this regimen in Taiwanese patients. Furthermore, does PPI offering only a placebo effect for LPR make it controversial in the literature.[9] Our study was not aimed at answering this question, and so we therefore did not include a placebo group. However, no matter whether the response was caused by a placebo effect or truly by pharmacologic mechanism, a strong positive result of pepsin detection still indicates a better response.

CONCLUSION

In this prospective, single-cohort, noncontrolled study, after excluding other etiologies regarding chronic laryngopharyngeal symptoms, patients with an RSI score ≥13 displayed a high possibility of LPR, which could be improved through an 8-week, once-daily, 40 mg esomeprazole treatment. In those patients, strong positive results of pepsin detection in saliva/sputum by the Peptest indicated a good response (≥50% symptoms reduction) to PPI treatment, with an acceptable positive predictive value of 79.2%. We suggest that the Peptest is a useful tool for managing patients with suspected LPR, and its application is worthy of further investigation in the future.

ACKNOWLEDGMENTS

The authors thank the Biostatistics Task Force of Taichung Veterans General Hospital for assisting with the statistical analysis. The study was supported by grants from the Ministry of Science and Technology, Taiwan (Project No. NSC101-2314-B-075A-004-MY3), to whom we also offer our thanks. The authors also thank Ms. Chin-Fang Hung and Ms. Fu-Ying Wu for their help in collecting the clinical data for analysis.

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