

Diagnostic Utility of Salivary Pepsin as Compared With 24-Hour Dual pH/Impedance Probe in Laryngopharyngeal Reflux

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Abstract

Objective. Laryngopharyngeal reflux (LPR) is defined as the retro propulsion of gastric contents into the larynx, oropharynx, and/or nasopharynx. The 24-hour combined hypopharyngeal-esophageal multichannel intraluminal impedance with dual pH probe (24h-HEMII-pH) is currently the gold standard in LPR diagnosis; however, it is invasive, user dependent, and not always tolerated. This study assesses the diagnostic utility of salivary pepsin (Peptest) at different thresholds and during symptomatic periods as compared with the 24h-HEMII-pH probe in diagnosing LPR.

Study Design. Prospective cohort study.

Setting. Private laryngology clinic in Melbourne, Australia.

Subjects and Methods. Thirty-five patients with a clinical history and endoscopic findings of LPR were recruited and simultaneously evaluated for LPR via 24h-HEMII-pH probe and salivary pepsin analysis at 5 key time points over the same 24-hour period.

Results. Salivary pepsin was 76.9% sensitive and had a positive predictive value (PPV) of 87.0% at a threshold of 16 ng/mL when compared with the 24h-HEMII-pH probe. If the pathologic pepsin threshold was raised to 75 ng/mL, salivary pepsin had a sensitivity of 57.7%, a specificity of 75.0%, and a PPV of 93.8%. Symptomatic testing conferred a superior specificity at 16 ng/mL (66.7%) and 75 ng/mL (100.0%) and a superior PPV at 16 ng/mL (92.3%) and 75 ng/mL (100.0%).

Conclusion. Salivary pepsin detection is a simpler, more cost-effective, and less traumatic universal first-line alternative to 24h-HEMII-pH probe in diagnosing LPR. Superior specificities conferring greater diagnostic value may be achieved with higher thresholds and symptomatic testing. If clinical suspicion remains high following negative salivary pepsin analysis, a 24h-HEMII-pH study could provide further diagnostic information.

Keywords

laryngopharyngeal reflux, extraesophageal reflux, salivary pepsin, 24 hour dual pH impedance probe

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Laryngopharyngeal reflux (LPR) is defined as the retro propulsion of gastric contents into the larynx, oropharynx, and/or nasopharynx. It is one of the most common otolaryngology presentations¹ and has consistently shown significant negative impacts on quality of life.² As it can cause substantial laryngeal inflammation, the clinical presentation of LPR differs from gastroesophageal reflux disease: it is not often associated with heartburn and regurgitative symptoms; rather, it manifests as throat clearing, persistent cough, hoarseness of voice, and globus pharyngeus.³ As a result, LPR cannot be accurately diagnosed through the same modalities as gastroesophageal reflux disease, with upper gastrointestinal endoscopy and barium swallow yielding a low sensitivity.³

The first step in diagnosing LPR is clinical assessment and evaluation. Scoring systems such as the Belafsky Reflux Symptom Index, a 9-item patient-reported validated outcome measure, allow patients to quantify symptoms to aid diagnosis (**Table 1**).⁴ Although not diagnostic of LPR, a Reflux Symptom Index score >13 is considered abnormal, indicating the need for further investigation.⁵ The current gold standard investigation for the diagnosis of LPR is a 24-hour combined hypopharyngeal-esophageal multichannel intraluminal impedance with dual pH probe (24h-HEMII-pH), which allows monitoring of hypopharyngeal, proximal, and

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Table 1. Reflux Symptom Index.^{4,a}

1. Hoarseness or a problem with your voice	0	1	2	3	4	5
2. Clearing your throat	0	1	2	3	4	5
3. Excess throat mucus or postnasal drip	0	1	2	3	4	5
4. Difficulty swallowing food, liquids or pills	0	1	2	3	4	5
5. Coughing after you ate or after lying down	0	1	2	3	4	5
6. Breathing difficulties or choking episodes	0	1	2	3	4	5
7. Troublesome or annoying cough	0	1	2	3	4	5
8. Sensations of something sticking in your throat or a lump in your throat	0	1	2	3	4	5
9. Heartburn, chest pain, indigestion, or stomach acid coming up	0	1	2	3	4	5

^a“Within the last month, how did the following problems affect you? Circle the appropriate response.” 0, no problem; 5, severe problem.

distal esophageal pH/acid exposure and impedance over a 24-hour period.⁶ However, it is invasive and costly and is often poorly tolerated during insertion or the ensuing 24-hour period that it is in situ.⁷ It is also a highly user-dependent process with variable data entry and compliance, which result in an inconsistent diagnostic accuracy.

Current clinical practice includes dietary and lifestyle modification, as well as use of alginates, proton pump inhibitors (PPIs), or other antireflux medications, without an objective diagnosis of LPR. With the long-term use of PPIs being associated with multiple potential adverse effects—including collagenous colitis, *Clostridium difficile* infection, drug interactions with hepatic drug metabolites, acute interstitial nephritis, chronic kidney disease, and osteopenia⁸—and with the discovery of carcinogenic substances in the common anti-H2 receptor antagonist ranitidine, there is a growing focus on a formal diagnosis of LPR before the commencement of treatment.

Oral salivary pepsin detection has been suggested as an alternative diagnostic modality. Pepsin is synthesized only by chief cells in the gastric mucosa in the form of pepsinogen A, which is subsequently cleaved to form pepsin in the acidic environment of the stomach. If pepsin is present in oral saliva, reflux of gastric contents into the upper aerodigestive tract is assumed to have occurred.⁹ Peptest (RD Biomed Limited) is a commercially available immunoserologic test that provides qualitative and quantitative measures of oral salivary pepsin for the diagnosis of LPR. There are promising data suggesting that oral salivary pepsin detection can be used as an alternative diagnostic test.^{10,11} To our knowledge, no prospective study has simultaneously evaluated the accuracy of pepsin detection, including accuracy of symptomatic pepsin samples, against the current gold standard of the 24h-HEMII-pH probe across the same 24-hour period in symptomatic patients.

Methods

Ethics approval for this study was obtained from Monash Health (NMA HREC ref HREC/18/MonH/22). Patients referred to a laryngologist who had a clinical history suggestive of LPR were invited to participate in the study, and

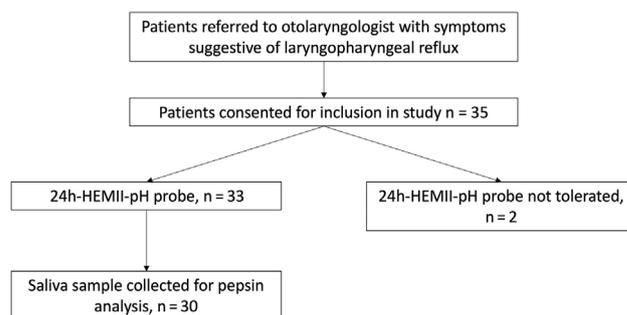


Figure 1. Flow diagram of the study procedure.

voluntary consent was obtained. All patients underwent assessment by a fellowship-trained laryngologist and speech-language pathologist, including videostroboscopy to exclude other laryngeal pathologies. Patients were not eligible for this study if they were unable to consent, had comorbid laryngeal pathology, were unable to tolerate the 24h-HEMII-pH probe, or had prior antireflux surgery.

Patients were asked to nominate their key LPR-related symptoms of concern for 24h-HEMII-pH probe testing and were counseled regarding the testing process, including the need for placement of a nasal catheter; the need to maintain usual daily diet, activities, and medications; and the requirement to wear a digital recording device for a 24-hour continuous period. They were advised to carefully register upright and supine periods, mealtimes, and self-nominated symptoms. Patients who were not taking antireflux medication (PPIs, H2 antagonists, alginates, etc) remained off these medications. Patients who reported reflux symptoms despite antireflux therapy were not asked to suspend their medications or undergo a washout period before enrollment into the trial. This ensured that results were not confounded by PPI cessation-related rebound acidity, and it prevented false-negative results, maintaining ecologic validity. Patients concurrently underwent 24h-HEMII-pH probe and saliva collection (**Figure 1**) across the same 24-hour period.

This study used a Sandhill-Zephyr 24h-HEMII-pH probe, ZAI-BL-55 or ZAI-BL-56, depending on the patient's

Table 2. Saliva Sample Collection Times.

Sample	Collection time
1	After waking up and standing up but before breakfast and brushing teeth
2	1 h after lunch
3	1 h after dinner
4	When experiencing a pre-nominated symptom of LPR
5	When experiencing a second pre-nominated symptom of LPR

Abbreviation: LPR, laryngopharyngeal reflux.

height. The 20- to 22-cm probe (esophageal length) was used in patients <180 cm tall and the 23- to 25-cm probe in patients >180 cm. This 24h-HEMII-pH probe included a pH sensor 1.0 cm above/proximal to the upper esophageal sphincter (UES) and impedance sensors 0.6 and 1.4 cm above/proximal to the UES. It also included a pH sensor 21.0 cm distal to the UES and impedance sensors 5.0, 7.0, and 21.0 cm distal to the UES (and 23.0 cm, depending on probe length). The probe was calibrated with standard manufacturer buffer solutions and inserted by a single trained laryngologist and speech pathologist. Topical nasal decongestant/anesthetic spray (phenylephrine/lignocaine) was applied to both nostrils, and the probe was advanced transnasally under vision into the stomach, confirmed by a pH drop <4.0. The probe was then withdrawn until the upper pH/impedance sensor array was directly visualized with flexible laryngoscopy in the hypopharynx. It was then advanced into the post-cricoid segment to the point where it was fully covered by mucosa, placing the proximal pH and impedance sensor array slightly above the UES, as described by Cumpston et al.¹² This also positioned the lower esophageal pH sensor between 5 and 10 cm above the physiologic lower esophageal sphincter depending on the patient's height. The probe was then removed in office the next day, and recording data were retrieved from the device.

Patients were instructed to collect their saliva at 3 to 5 key time points into collection tubes containing 0.5 mL of 0.01M citric acid. Patients collected the first sample after waking and standing in the morning but before breakfast and brushing teeth. Patients collected the second and third samples 1 hour after lunch and dinner and a further 2 samples (fourth and fifth) within 15 minutes of experiencing one of the nominated LPR-related symptoms (**Table 2**). All samples were refrigerated at 4 °C and processed with manufacturer testing kits and according to manufacturer protocols.

A positive pepsin test result was defined as any of the fasting, postmeal, or symptom-related samples collected recording >16 ng/mL of pepsin. Analysis of the salivary pepsin data was performed by 2 clinicians who were not present at the initial consultation and who were both trained through a standardized technique according to the analysis protocol. They were blinded to each patient's symptoms, endoscopic findings, and probe results.

Table 3. Demographics of Included Patients (N = 30).^a

	Age, y	Height, cm	Weight, kg	BMI, kg/m ²
Median	55.5	170	76.5	25.2
25th percentile	40.0	163	65.3	21.8
75th percentile	64.3	178	89.5	29.3
Interquartile range	24.3	15	23.8	7.5

Abbreviation: BMI, body mass index.

^aSex: male, n = 16; female, n = 14.

A positive 24h-HEMII-pH probe study result was defined as ≥ 2 episodes of pharyngeal reflux (PR) or ≥ 6 episodes of proximal esophageal reflux (PER) in accordance with normative data published by Zerbib et al.¹³ PER was defined as reflux that reached the impedance site 5 cm below/distal to the UES but did not reach the hypopharyngeal ring set. PR was defined as reflux that reached the impedance array located 0.6 cm or 1.4 cm above the UES. To ensure that swallowing or gaseous events were not falsely classified as reflux events, the 24h-HEMII-pH data were manually analyzed and independently cross-checked by 3 trained physician reviewers blinded to the patient's symptoms, endoscopic findings, and salivary pepsin results.

Demographic data were collected from patient records, including age, sex, height, weight, body mass index, and relevant investigations. 24h-HEMII-pH probe analysis and Peptest results were collated and analyzed. Sensitivity, specificity, and positive and negative predictive values (PPV/NPV) were calculated for the Peptest with the gold standard 24h-HEMII-pH probe being used as a true result reference. Patients who were unable to tolerate the probe or did not return their saliva samples were excluded from the analysis.

Statistical Analyses

All statistical calculations were performed with Jamovi,¹⁴ an open-source statistical platform with sensitivity, specificity, PPV, and NPV expressed as percentages. Confidence intervals for sensitivity and specificity were calculated as Clopper-Pearson confidence intervals. Confidence intervals for predictive values were determined by standard logit calculations per Mercaldo et al.¹⁵ McNemar's test was performed to test strength of association, with a statistically significant result defined as $P < .05$.

Results

Thirty-five patients were recruited into the study. Two patients did not tolerate the 24h-HEMII-pH probe, and 3 did not return their saliva samples. These patients were excluded from analysis. **Table 3** portrays the demographics of the 30 patients included in the analysis. Their median age was 55.5 years, and 53.3% were male. There were no statistically significant associations between levels of oral salivary pepsin and age, sex, or body mass index.

Of the 30 patients who completed the 24h-HEMII-pH probe, 26 tested positive for LPR, with 24 and 25 fulfilling

Table 4. Salivary Pepsin Analysis vs 24h-HEMII-pH.

Salivary pepsin	pH/impedance probe		Total
	Positive	Negative	
≥ 16 ng/mL	20	3	23
< 16 ng/mL	6	1	7
Total	26	4	30

Abbreviation: 24h-HEMII-pH, 24-hour hypopharyngeal/esophageal multichannel intraluminal impedance with dual pH probe.

Table 5. Sensitivity, Specificity, and Positive and Negative Predictive Values of Salivary Pepsin at 16 and 75 ng/mL.^a

Any salivary pepsin	Value	95% CI	
		Lower	Upper
≥ 16 ng/mL			
Sensitivity	76.9	56.4	91.0
Specificity	25.0	0.63	80.6
Positive predictive value	87.0	69.3	96.2
Negative predictive value	14.3	2.6	51.2
≥ 75 ng/mL			
Sensitivity	57.7	36.9	76.7
Specificity	75.0	19.41	99.4
Positive predictive value	93.8	72.7	98.8
Negative predictive value	21.4	11.7	36.0

^aValues are presented as percentages.

the criteria of ≥6 episodes of PER and ≥2 episodes of PR, respectively. Of the 30 patients who returned samples for salivary pepsin detection, 23 returned pepsin results ≥16 ng/mL (**Table 4**).

There was strong evidence that salivary pepsin >16 ng/mL correlated with a positive 24h-HEMII-pH study result ($\chi^2 = 15.4$, $df = 1$, $P < .001$). When compared with the 24h-HEMII-pH probe, salivary pepsin was 76.9% sensitive (95% CI, 56.4%-91.0%) and had a PPV of 87.0% (95% CI, 78.5%-92.4%) at a cutoff of 16 ng/mL (**Table 5**). When the pathologic pepsin threshold was raised to 75 ng/mL, salivary pepsin was 57.7% sensitive (95% CI, 36.9%-76.7%) and 75.0% specific (95% CI, 19.4%-99.4%) and had a PPV of 93.8% (95% CI, 72.7%-98.8%).

All patients who recorded salivary pepsin ≥16 ng/mL tested positive either prior to breakfast or 1 hour after lunch/dinner. Thirty-six symptomatic samples were submitted by 23 patients, and there was strong evidence suggesting correlation between positive symptomatic pepsin level and a positive 24h-HEMII-pH study result ($\chi^2 = 11.5$, $df = 1$, $P < .001$). Testing during symptomatic periods conferred superior specificity at 16 ng/mL (66.7%) and 75 ng/mL (100.0%) and a superior PPV at 16 ng/mL (92.3%) and 75 ng/mL (100.0%) but inferior sensitivity (**Table 6**).

Table 6. Sensitivity, Specificity, and Positive and Negative Predictive Values of Symptomatic Salivary Pepsin at 16 and 75 ng/mL.^a

Symptomatic salivary pepsin	Value	95% CI	
		Lower	Upper
≥ 16 ng/mL			
Sensitivity	60.0	36.1	80.9
Specificity	66.7	9.4	99.2
Positive predictive value	92.3	70.0	98.4
Negative predictive value	20.0	8.7	39.6
≥ 75 ng/mL			
Sensitivity	50.0	27.2	72.8
Specificity	100.0	29.2	100.0
Positive predictive value	100.0	—	—
Negative predictive value	23.1	16.2	31.7

^aValues are presented as percentages.

Discussion

LPR is common and can account for up to 10% of otolaryngology consultations.¹⁶ Most cases are currently diagnosed on the basis of clinical signs and symptoms, with the Reflux Symptom Index⁴ used to establish the presence of common laryngopharyngeal symptoms. However, reported symptoms, such as hoarseness of voice, cough, and globus pharyngeus, are not specific to LPR and may indicate other laryngeal pathology. As such, patient-reported symptomatology alone is not sufficient for the diagnosis of LPR, with endoscopic laryngopharyngeal findings being similarly nonspecific.¹⁷

The current gold standard, the 24h-HEMII-pH probe, allows for objective assessment of pH and impedance proximal to the UES and throughout the oesophagus, with changes in pH corresponding to episodes of reflux of gastric acid into the proximal or distal probe. Pharyngeal pH analysis alone is not a sensitive test, given the role of alkaline and weakly acidic refluxate in LPR and the acid-neutralizing properties of the alkaline environment in the proximal aerodigestive tract.¹⁸ Multichannel intraluminal impedance monitoring allows for the detection and measurement of retrograde esophageal and pharyngeal movement, with impedance decreasing with electrolyte-rich fluids. Decreases in impedance starting from the distal probe and ending at the proximal probe indicate a reflux event,¹⁸ and when combined with pH monitoring, the 24h-HEMII-pH probe is a highly specific test.¹⁸

However, the 24h-HEMII-pH probe is expensive and invasive and can be poorly tolerated in patients.¹⁷ In this study, 2 of 35 patients (5.7%) were unable to tolerate the probe and self-removed it prior to the completion of the 24-hour period. The 24h-HEMII-pH probe's diagnostic utility is also dependent on data entry, with patients having to log when they are supine or standing, when they begin and finish meals, and when they experience their nominated 3 symptoms. Impedance and pH data during mealtimes are

discarded during analysis by the proprietary software, as the acidic substances would confound data and may be falsely interpreted as a reflux event. This means that accuracy of data entry, especially around mealtimes, is important for accurate detection of potential reflux events.

The proprietary software that analyzes pH and impedance data from the recording device compares recorded data with the normative data published by Hoppo et al,¹⁸ which defined abnormal pharyngeal acid exposure as ≥ 4 episodes of PER or ≥ 1 episode of PR. In this area, the literature is heterogenous, as a complementary study by Zerbib et al¹³ suggested that ≥ 6 episodes of PER or ≥ 2 episodes of PR are abnormal, and these were the criteria that we adopted in this study.

Cumpston et al,¹² using the normative data published by Xiao et al,¹⁹ described yet another set of 24h-HEMII-pH LPR diagnostic criteria. LPR was defined as any significant pharyngeal acid episodes (ie pH < 4.0 at the proximal pH sensory array) or > 40 episodes of proximal extent impedance events. The differing “normative” data from Zerbib et al,¹³ Hoppo et al,¹⁸ and Xiao et al¹⁹ illustrate a lack of consensus in the published literature regarding the normative data against which 24h-HEMII-pH probe results are to be analyzed, thereby affecting the practicality, utility, and reliability of this diagnostic modality.

With no clear, reliable, and universally tolerable diagnostic method, clinicians have favored empiric treatment with a 3-month trial of medications, diet, and lifestyle change, where a positive response retrospectively validates LPR as a diagnosis for the patient’s symptoms. However, multiple randomized controlled studies have showed no statistical difference in the improvement of symptoms between PPI treatment and placebo in the management of presumed LPR.^{20,21} Additionally, the risks of long-term PPI use include collagenous colitis, drug interactions with hepatic drug metabolites, acute interstitial nephritis, chronic kidney disease, and osteopenia.^{8,22} As such, there is a growing focus on the formal diagnosis of LPR through less invasive and more cost-effective means before the commencement of management.

Oral salivary pepsin detection has been proposed as a much simpler, cost-effective, and minimally invasive alternative. It is difficult to evaluate the diagnostic value of pepsin as a solitary diagnostic testing modality, as the gold standard true-result reference is poorly tolerated and inconsistent and there is a lack of consensus surrounding its interpretation. With some degree of reflux being physiologic and small quantities of pepsin identified in healthy patients,¹⁷ the optimal timing of saliva collection and the pepsin threshold yielding the most diagnostic accuracy are still unknown. We initially defined the threshold for a positive study result as ≥ 16 ng/mL, per the manufacturer’s specifications to account for “physiologic” levels of pepsin detection. We performed additional analyses to evaluate whether a threshold ≥ 75 ng/mL and whether sampling during symptomatic periods conferred any additional diagnostic advantage.

Results from our study indicate that salivary pepsin ≥ 16 ng/mL has a sensitivity of 76.9% (95% CI, 56.4%-91.0%)

and a PPV of 87.0% (95% CI, 78.5%-92.4%) in the diagnosis of LPR. In comparison with existing literature, our sensitivity is similar to previously reported figures, with a pooled sensitivity of 64% (95% CI, 43%-80%).²³ Increasing the pathologic pepsin level to ≥ 75 ng/mL yields a specificity of 75.0% and a PPV of 93.8%; however, the sensitivity decreases to 57.7% (**Table 5**). Hayat et al²⁴ drew similar conclusions when comparing salivary pepsin with distal esophageal pH/impedance monitoring, indicating that an increase in the pepsin threshold results in increased specificity but decreased sensitivity. This suggests that there may exist one threshold as a highly sensitive screening test and a different higher threshold as a highly specific test with greater diagnostic value.

Our data show far better specificity and PPV if testing occurs during symptomatic periods. At a threshold of 16 ng/mL, specificity is 66.7% and PPV is 92.3%, as compared with 25.0% and 87.0%, respectively. At a threshold of 75 ng/mL, specificity and PPV are both 100.0%, as opposed to 75.0% and 93.8%, respectively. This suggests that a superior specificity may be achieved during symptomatic sampling.

With the simplicity, cost-effectiveness, and atraumatic nature of saliva collection, salivary pepsin analysis holds promise as a first-line screening test. Our data suggest that if patients have unconvincing history and examination results and unremarkable nasoendoscopy findings, repeated negative salivary pepsin tests would possibly exclude LPR as a diagnosis. If no salivary pepsin is detected and patients have a highly suspicious history and endoscopic changes consistent with LPR, our data suggest that perhaps symptomatic saliva sampling or 24h-HEMII-pH probe would be the next diagnostic modality of choice.

To our knowledge, this is one of the first studies to simultaneously assess the utility of salivary pepsin, at symptomatic times and at 2 thresholds, against the 24h-HEMII-pH probe. The other strengths of this study lie in its prospective design, ability to concurrently compare salivary pepsin with 24h-HEMII-pH probe in real time for the diagnosis of LPR, multiple saliva sample analyses, and collection during symptomatic periods. Any confounders in dietary variation or environmental changes are mitigated in the simultaneous assessment of pepsin against 24h-HEMII-pH monitoring, allowing an accurate, real-time comparison of the 2 modalities. Collecting multiple samples (1 hour prior to breakfast and 1 hour after lunch/dinner) and during symptomatic periods allows a more comprehensive assessment of LPR. There are studies in the literature that derive diagnostic utility results from a singular pepsin sample; however, our study indicates that saliva collection at multiple time points, as well as during symptomatic periods, does vary within individuals and may confer a greater level of diagnostic accuracy.

Limitations of this study include the small sample size and the homogeneity of the study population. The lack of asymptomatic controls and the limited number of negative results on 24h-HEMII-pH probe did not allow for accurate analysis of specificity and NPV. More data are required to completely assess the diagnostic utility of oral salivary

pepsin detection against the gold standard 24h-HEMII-pH probe. Future studies could include larger cohorts across several sites, introduce an asymptomatic control group, and perform a receiver operator characteristic curve analysis to reach a consensus regarding the optimal timing and minimum thresholds for both pathologic oral salivary pepsin and 24h-HEMII-pH results; thereby improving the diagnostic accuracy of both testing modalities.

Conclusion

Salivary pepsin detection is a simpler, more cost-effective, and less traumatic universal first-line alternative to 24h-HEMII-pH probe in diagnosing LPR. Superior specificities conferring greater diagnostic value may be achieved with higher thresholds and symptomatic testing. If clinical suspicion remains high following negative salivary pepsin analysis, a 24h-HEMII-pH study could provide further diagnostic information.

Author Contributions

Michael Zhang, formulating protocol and study design, ethics submission and approval, designing patient information forms, consent forms, analysis of saliva and 24-hour probe data, write-up and editing of manuscript, and presentation of research; **Clemente Chia**, analysis of saliva and 24-hour probe data, write-up and editing of manuscript, and presentation of research; **Claire Stanley**, patient recruitment, assessment of recruited patients, data collection, analysis of saliva, and speech pathologist for patients recruited into study; **Debra J. Phyland**, research supervisor, editing of manuscript; **Paul M. Paddle**, study idea, formulation of patient protocol and study design, ethics submission and approval, chief clinician of study, analysis of 24-hour probe data, manuscript editor.

Disclosures

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