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Journal

Gullet, 36(4)

Authors

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Publication Date

2023-03-30

DOI

10.1093/dote/doac063

Peer reviewed

Diseases of the Esophagus (2023), **36**, 1–8
https://doi.org/10.1093/dote/doac063

DISEASES OF THE ESOPHAGUS

ISDE The International Society for Diseases of the Esophagus

Original Article

Diagnostic thresholds and optimal collection protocol of salivary pepsin for gastroesophageal reflux disease

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*SUMMARY.***Gastroesophageal reflux disease (GERD) is primarily diagnosed based on symptoms and response to a proton-pump inhibitor (PPI) trial. Gold standard testing requires an invasive endoscopic procedure, often with ambulatory pH monitoring. Salivary pepsin is a potential noninvasive modality for GERD diagnosis. This study aimed to assess diagnostic performance of salivary pepsin thresholds for GERD and determine optimal collection protocol of saliva in an external validation cohort. Over 10 months, adults with symptoms of GERD undergoing esophagogastroduodenoscopy with wireless pH-monitoring off PPI were enrolled. Saliva was self-collected by participants over 4 days across three different time points: fasting ante meridiem (AM), post-prandial, and bedtime (PM). Pepsin levels were calculated via Peptest. Pepsin variability and agreement were determined using linear mixed effects models and intraclass correlation. Validation of diagnostic threshold and performance characteristics were evaluated by receiver–operator curve analysis. Twenty participants enrolled in the study; 50% with physiologic acid exposure (acid exposure time < 4% no GERD) and 50% with elevated acid exposure (GERD). Mean pepsin concentrations were significantly lower in the AM (22.6 ± 25.2 ng/mL) compared to post-prandial (44.5 ± 36.7 ng/mL) and PM (55.4 ± 47.0 ng/mL). Agreement between pepsin concentrations across 3 days was substantial for AM samples (kappa 0.61), with lower agreement for post-prandial and PM samples. A single AM pepsin concentration of 25 ng/mL was 67% accurate for GERD with 56% sensitivity and 78% specificity. This validation study highlights fair accuracy and performance characteristics of a single fasting AM salivary pepsin concentration for the diagnosis of GERD.**

KEY WORDS: **biomarkers, Bravo, erosive esophagitis, functional heartburn, reflux monitoring.**

INTRODUCTION

Gastroesophageal reflux disease (GERD) is extremely common in the United States, affecting up to 30% of adults and accounting for over 7 million ambulatory visits annually.[1,2] Currently, GERD is primarily a clinical diagnosis based on patient reports of typical and/or atypical symptoms such as heartburn, regurgitation, chest pain, cough, or laryngeal complaints. First-line diagnosis and management typically involves an empiric trial of proton pump inhibitor (PPI) therapy.[3] However, approximately 50% of patients on PPI therapy do not achieve symptomatic relief and subsequent ambulatory reflux testing often results in normal findings or the absence of GERD.[1,4] Current evidence also suggest that PPIs are overused in up to 70% of cases and have been associated with potential adverse effects such as increased risk of *Clostridium difficile* infection.[5,6] At present, the gold standard for diagnosis of pathologic GERD is upper GI endoscopy followed by ambulatory reflux monitoring in the absence of erosive findings of GERD on endoscopy.[7,8] However, this approach is invasive, costly, time-consuming, and not always readily available to patients. Therefore, a quick, noninvasive, and cost-effective diagnostic tool for GERD is critically needed.

Salivary pepsin, an endopeptidase originating from gastric chief cells, has been proposed as a potential diagnostic tool for GERD.[9–13] Peptest (RD Biomed,

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Cottingham, UK) is a lateral flow device (LFD) test registered with the US Food and Drug Administration, which contains two antibodies to human pepsin and can rapidly quantify the concentration of pepsin in saliva. A previous study performed by our group demonstrated that a single fasting salivary pepsin level had modest diagnostic performance for patients with objective GERD, where a concentration of 24.9 ng/mL optimized the true negative rate with 86% sensitivity and a concentration of 100.0 ng/mL optimized the true positive rate with 72% specificity.[12] This data outperform the traditional use of an empiric PPI trial (78% sensitivity and 54% specificity).[14] However, further research on ideal time for collection, single versus multiday collection, and validation of threshold cut-off points is needed prior to routine clinical use of salivary pepsin. In order to address remaining gaps regarding the clinical protocol and validity of these prior findings, this current study aimed to assess diagnostic performance of salivary pepsin thresholds for GERD and determine optimal collection protocol of saliva in an external validation cohort.

METHODS

Study design & setting

This prospective observational study enrolled subjects over 10 months (January 2021 to September 2021) at a single tertiary care center. The study was approved by the Institutional Review Board.

Study Population

Adult patients (18–89 years) with symptoms of GERD undergoing upper endoscopy with prolonged wireless pH-monitoring off acid-suppression therapy were prospectively enrolled. Participants experienced at least 8 weeks of heartburn, regurgitation, non-cardiac chest pain, with or without extra-esophageal symptoms. Participants were required to have access to a standard refrigerator at home as well as a reliable means of communication. Exclusion criteria included patients who were unable to consent, complete questionnaires, provide unstimulated salivary samples, and those who had erosive esophagitis (LA grade C or D), were imprisoned, required legal adult representation, pregnant, mentally disabled, non-English speakers, and under the age of 18 years of age.

Study protocol

All enrolled participants provided informed consent. Salivary samples were collected at multiple timepoints during the day in alignment with 96-hour wireless pH monitoring (Fig. 1).

Saliva collections

During the study, unstimulated expectorated salivary samples were collected and processed for salivary pepsin concentration analysis. The first collection occurred on the morning of the scheduled upper endoscopy by the study team after participants fasted overnight. Subsequent salivary samples were then self-collected by participants over 4 days across three different time points: fasting morning (AM), post-prandial (PP), and 30-minute prior to bedtime (PM). Patients were contacted every night (via telephone or email) by the study team to ensure samples were properly collected/stored throughout the day and to confirm their future duties/appointments. Salivary samples were collected into 15-mL sterile plastic tubes containing 0.5 mL of 0.01 mol/L citric acid at pH 2.5. Participants were instructed to transfer samples promptly to their at-home refrigerator, stored between 2 and 8°C. At the end of their collection period, salivary samples were returned in the provided cooler and processed.

Upper endoscopy with wireless pH monitoring

On day 1 of the study participants underwent their standard of care scheduled sedated upper endoscopy. In the absence of severe erosive esophagitis on endoscopy, the wireless pH probe delivery catheter (Bravo; Medtronic, Minneapolis, MN) was introduced transorally and the pH capsule was positioned 6 cm proximal to the endoscopically identified squamocolumnar junction, corresponding to 5 cm above the proximal border of the lower esophageal sphincter. Once the catheter was in the appropriate position, the external portable vacuum pump was switched on to apply suction to the well of the capsule and suck in adjacent esophageal mucosa. After 30 seconds, the plastic safety guard was removed and the activation button was depressed. Participants were instructed to continue usual daily activities and meals, remain off PPI, and log symptoms/meals in a written and electronic diary, while remaining within 3 feet of the pager-sized receiver at all times. Participants returned the wireless pH study receiver 96 hours later, following which data were downloaded and analyzed (Reflux Reader, Medtronic, Minneapolis, MN).

Patient reported symptoms

Research participants also completed two validated questionnaires, the GerdQ and reflux symptom index (RSI). GerdQ is a six-item questionnaire with scores ranging from 0 to 18 that evaluates reflux symptoms,[15] while RSI is a nine-item questionnaire with scores ranging from 0 to 45 that evaluates laryngeal symptom burden; for both validated instruments, higher scores indicated more severe symptoms.[16]

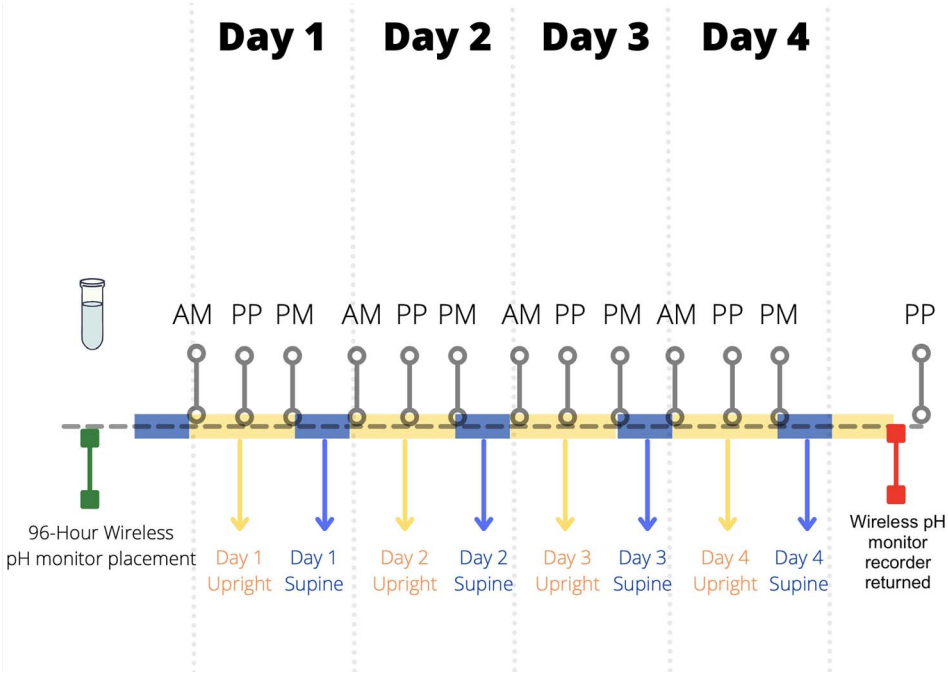

Fig. 1 Timeline of salivary collection and pH monitor recordings for study participants.

Data collection & management

Data for all participants were entered and stored as de-identified datasets on Research Electronic Data Capture (REDCap). Data collected included salivary pepsin concentrations, reflux monitoring data, demographics, and endoscopic findings.

Salivary pepsin analysis

Pepsin was measured using the Peptest LFD (RD Biomed Ltd). Within seven days of collection, samples were centrifuged for 5 minutes at 4,000 rpm in a bench top centrifuge and the supernatants were collected. 80 μL of the supernatants layer was then mixed with 240 μL of migration buffer solution and vortexed for 10 seconds. 80 μL of the mixture was then added to the well of the LFD. The LFD was transferred to the Peptest recorder which provides a quantified concentration of pepsin in ng/mL. Peptest has the ability to detect pepsin concentrations of 16 ng/mL or greater. Concentrations between 16 and 24.9 ng/mL are quantified as 16 < 25 ng/mL by the recorder.

Wireless reflux monitoring

Prolonged wireless ambulatory reflux monitoring tracings were manually interpreted using manufacturer software (Reflux Reader; Medtronic, Minneapolis, MN) by an esophageal specialist (RY) in a blinded fashion. Data from these tracings included acid exposure time (AET), upright AET, supine AET, and post-prandial AET through the overall monitoring period and day-by-day. AET < 4.0% was considered physiologic. In the primary analysis, GERD was defined as an AET > 4.0%, and in the sensitivity analysis GERD was defined as an AET > 6.0% based on the Lyon Consensus.[17]

Sample size, outcomes, and data/statistical analysis

Sample size: Based on the previously reported results for an AUC of 0.74 (95% CI: 0.65, 0.83),[12] a sample size of 9 per group is needed to achieve an AUC with a lower limit of the 95% confidence interval that is greater than 0.5.

The primary outcome was salivary pepsin concentration. Salivary pepsin variability throughout the day and across days were determined using linear mixed effects modeling with a random intercept for each participant. The trends in salivary pepsin was visualized by plotting mean pepsin values over the study duration with 95% bootstrap percentile confidence intervals estimated via 10,000 bootstrap resamples and all estimates smoothed with Loess curves for visualization. Optimal salivary collection protocol was evaluated by intraclass correlation coefficient with Fleiss' Kappa interpretation to determine agreement between pepsin level and objective GERD diagnosis determined by wireless ambulatory pH monitoring. Validation of diagnostic threshold of salivary pepsin for classifying GERD versus no GERD was evaluated by receiver–operator curve (ROC) analysis with area under the curve (AUC) and 95% confidence intervals estimated using DeLong's method. Objective GERD was defined as a total AET (time spent with a pH of <4.0) >4.0%. Additionally, we performed a sensitivity validation analysis for a higher AET threshold of 6.0% for GERD, based on the Lyon

Table 1 Summary of baseline characteristics, salivary pepsin level, and wireless pH monitor measurement for study participants; values are mean (SD) or *N* (%)

Component	Overall (*N* = 18)	GERD (*N* = 9)	No GERD (*N* = 9)
Characteristic			
Male	4 (22.2%)	3 (33.3%)	1 (11.1%)
Age (Years)	40.9 (13.4)	45.0 (16.2)	36.9 (8.9)
BMI (kg/m^2)	29.7 (13.6)	30.4 (8.7)	29.0 (17.7)
Presence of Hiatal Hernia	6 (33.3%)	6 (66.6%)	0 (0.0%)
Race/Ethnicity:			
White/Caucasian	9 (50.0%)	4 (44.4%)	5 (55.6%)
Asian	4 (22.2%)	2 (22.2%)	2 (22.2%)
Hispanic	1 (5.6%)	1 (11.1%)	0 (0.0%)
Other	4 (22.2%)	2 (22.2%)	2 (22.2%)
Surveys			
GerdQ	8.0 (2.5)	8.4 (3.2)	7.5 (1.5)
RSI	18.4 (8.9)	21.3 (9.4)	15.4 (7.8)
Salivary Pepsin Level			
Day 1 – PP	53.6 (63.5)	68.6 (79.0)	40.3 (46.6)
Day 1 – PM	73.9 (72.9)	75.4 (76.2)	72.5 (74.4)
Day 2 – AM	25.9 (33.1)	25.0 (25.0)	26.7 (41.2)
Day 2 – PP	43.9 (53.3)	33.6 (46.3)	55.5 (61.3)
Day 2 – PM	50.0 (54.7)	43.1 (60.8)	56.1 (51.6)
Day 3 – AM	22.2 (31.3)	35.9 (38.7)	8.6 (12.9)
Day 3 – PP	51.7 (64.2)	40.8 (36.5)	62.7 (84.5)
Day 3 – PM	51.1 (68.5)	62.1 (85.3)	40.0 (49.3)
Day 4 – AM	20.7 (30.2)	27.8 (37.0)	12.8 (19.5)
Day 4 – PP	33.5 (54.0)	42.5 (72.6)	24.4 (27.1)
Day 4 – PM	50.6 (85.0)	61.8 (105.2)	39.5 (63.3)
Mean AM Pepsin	22.6 (25.2)	29.6 (29.0)	15.5 (19.9)
Mean PP Pepsin	44.5 (36.7)	44.4 (41.4)	44.5 (34.0)
Mean PM Pepsin	55.4 (47.0)	58.7 (59.2)	52.1 (34.2)
pH Monitor Measurement			
Total AET (%)	4.4 (3.1)	6.9 (2.4)	1.9 (0.9)
Total Number Reflux	86.8 (46.0)	127 (19.9)	46.8 (22.0)

Consensus.[17] Confidence intervals for sensitivity and specificity estimates were based on the exact binomial approach. All figures created and analyses were conducted using Rv4.1.0 (Vienna, Austria).

RESULTS

Baseline characteristics (Table 1)

A total of 20 participants enrolled over the 10-month period, and a total of 18 participants completed the entire protocol: 4 (22%) male, mean age 40.9 years ±13.4, and mean body mass index 29.7 kg/m^2 ± 13.6. Nine had a physiologic acid exposure time (AET < 4.0%). The data analysis included a total of 210 saliva collections.

Pepsin variability throughout the day (Table 2 ; Fig. 2)

Mean pepsin concentrations were as follows in the AM (22.6 ± 25.2 ng/mL), PP (44.5 ± 36.7 ng/mL), and PM (55.4 ± 47.0 ng/mL). PP and PM pepsin were significantly higher compared to AM pepsin values by 22.3 ng/mL (95% CI: 4.6, 40.0; $P = 0.015$) and 33.4 ng/mL (95% CI: 15.7, 51.2; $P < 0.001$), respectively (Table 2). There were no significant differences between PP and PM pepsin levels.

Table 2 Linear mixed effects models with a random intercept for the outcome of average pepsin measurement. Model 1 represents estimated pepsin when AM pepsin measurements were the reference category (e.g., PP pepsin is 22.3 ng/mL higher on average than AM pepsin). Model 2 represents AM pepsin measurements for someone without GERD as the reference categories

Parameter	Estimate	95% CI	*P*-value
Model 1			
Intercept	22.5	(3.3, 41.6)	0.026
PP	22.3	(4.6, 40.0)	0.015
PM	33.4	(15.7, 51.2)	<0.001
Model 2			
Intercept	15.2	(−11.9, 42.2)	0.288
PP	29.6	(4.4, 54.7)	0.023
PM	36.9	(11.8, 61.9)	0.005
GERD	14.4	(−23.7, 52.5)	0.472
PP × GERD	−14.3	(−49.7, 21.1)	0.431
PM × GERD	−6.7	(−42.0, 28.8)	0.714

Pepsin variability across days (Table 3 ; Fig. 2)

The agreement between pepsin concentration from day-to-day were assessed. AM pepsin values had substantial agreement (ICC agreement 0.460 [95% CI: 0.159, 0.730]; Fleiss' kappa 0.61). PP pepsin values had fair agreement (ICC agreement 0.145 [95% CI: −0.062, 0.456]; Fleiss' kappa 0.31) and PM pepsin

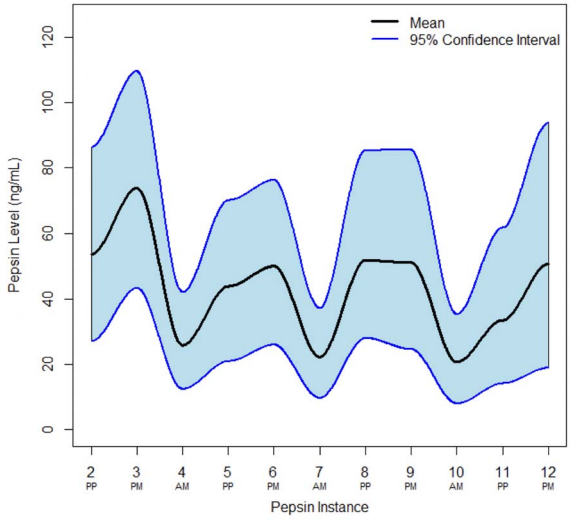

Fig. 2 Mean trend of salivary pepsin levels across four days and three different time points: fasting AM, PP, and 30-minutes prior to bedtime (PM).

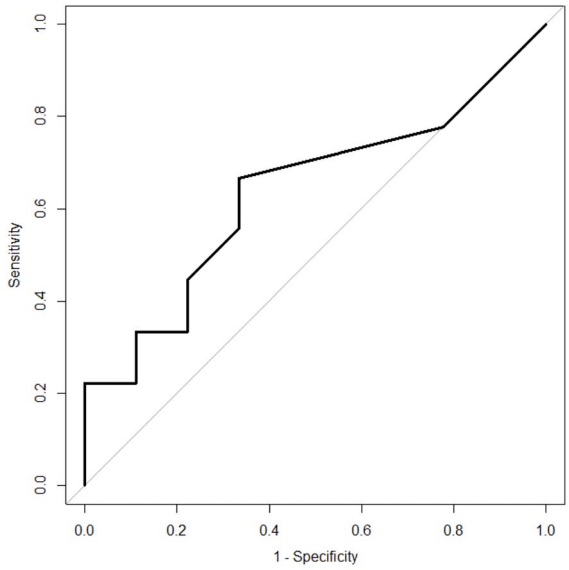

Fig. 3 ROC for fasting AM salivary pepsin threshold of 25 ng/mL. AUC 0.636 (95% CI: 0.364, 0.908).

Table 3 Agreement across AM/PP/PM pepsin readings (note: day 1 AM is excluded). Fleiss' Kappa suggests level of agreement (i.e., <0 is poor, 0–0.20 is slight, 0.21–0.40 is fair, 0.41–0.60 is moderate, 0.61–0.80 is substantial, 0.81+ is almost perfect agreement)

Timing	ICC Agreement (95% CI)	Fleiss' Kappa
AM	0.460 (0.159, 0.730)	0.61
PP	0.145 (−0.062, 0.456)	0.31
PM	0.250 (0.017, 0.558)	0.16

Table 4 Table predicting GERD if average AM pepsin over days 2/3/4 > 25 ng/mL

		GERD	
		Yes	No
Pepsin ≥ 25 ng/mL	Yes	4	2
	No	5	7

values had slight agreement (ICC agreement 0.250 [95% CI: 0.017, 0.558]; Fleiss' kappa 0.16).

Validation of pepsin threshold of 25 ng/mL for diagnosis of GERD (AET > 4.0%) (Table 4 ; Fig. 3)

A single AM pepsin > 25 ng/mL accurately identified GERD (defined as AET > 4.0%) in 66.7% (95% CI: 35.7%, 82.7%) of cases with 55.6% sensitivity (95% CI: 21.2%, 82.3%) and 77.8% specificity (95% CI: 40.0%, 97.2%). When considering AM pepsin values across three days of testing, an average AM salivary pepsin >25 ng/mL accurately identified GERD in 61.1% of cases (95% CI: 35.7%, 82.7%) with 44.4% sensitivity (95% CI: 13.7%, 78.8%) and 77.8% specificity (95% CI: 40.0%, 97.2%). The area under the curve for average AM pepsin concentrations was 0.64 (95% CI: 0.36, 0.91).

A single post-prandial pepsin >25 ng/mL accurately identified GERD in 50% (95% CI: 26.0%, 74.0%) of cases with 55.6% sensitivity (95% CI: 21.2%, 86.3%) and 44.4% specificity (95% CI: 13.7%, 78.8%). A single PM pepsin >25 ng/mL accurately identified GERD in 38.9% of cases (95% CI: 17.3%, 64.3%) with 55.6% sensitivity (95% CI: 21.2%, 86.3%) and 22.2% specificity (95% CI: 2.8%, 60.0%).

Sensitivity validation analysis of pepsin threshold of 25 ng/mL for AET of 6.0%

When considering the diagnostic performance of salivary pepsin in identifying AET greater than or lower than 6.0%, a single AM pepsin was 60.0% sensitivity (95% CI: 14.7%, 94.7%) and 69.2% specificity (95% CI: 38.6%, 90.9%) with area under the curve of 0.68 (95% CI: 0.34, 1.00). A single post-prandial pepsin >25 ng/mL accurately identified AET > 6.0% in 33.3% (95% CI: 13.3%, 59.0%) of cases with 60.0% sensitivity (95% CI: 14.7%, 94.7%) and 23.1% specificity (95% CI: 5.0%, 53.8%). A single PM pepsin >25 ng/mL accurately identified AET >6.0% in 38.9% of cases (95% CI: 17.3%, 64.3%) with 100.0% sensitivity (95% CI: 47.8%, 100%) and 15.4% specificity (95% CI: 1.9%, 45.4%).

DISCUSSION

GERD is a prevalent disorder afflicting a wide variety of patients. Overdiagnosis in the ambulatory setting along with overuse of PPIs imparts a large burden of cost on the US healthcare system.[5] With healthcare costs continuing to rise, an affordable, efficient, office-

based diagnostic tool is needed to rapidly screen for GERD. In this study of 20 patients with GERD symptoms undergoing 96-hour wireless reflux monitoring off PPI, we identified fair accuracy (67%) for objective GERD (AET > 4.0%) with a single fasting AM salivary pepsin threshold of 25 ng/mL (56% sensitivity and 78% specificity). A single fasting AM pepsin performed comparably to three consecutive fasting AM pepsin concentrations, and outperformed postprandial or PM pepsin concentrations. Further, sensitivity analysis identified excellent positive predictive value of a PM saliva concentration for elevated levels of AET where a single PM salivary pepsin threshold of >25 ng/mL was 100% sensitive for an AET > 6.0%. This study highlights that a single AM fasting collection of saliva yields reliable and reproducible pepsin concentrations, with good specificity in distinguishing between physiologic AET (<4.0%) or non-physiologic AET (>4.0%), with potential as an office-based diagnostic tool for GERD.

This study is consistent with prior studies exploring salivary pepsin. In November 2020, Guo *et al*. assessed patients with suspected GERD who had PPI-refractory symptoms. All participants underwent upper endoscopy, 24-hour pH monitoring, and provided salivary samples in the AM and PP (2 hours after breakfast) for pepsin testing. Instead of using LFD testing (i.e., Peptest), their group used enzyme-linked immunosorbent assay (ELISA) analysis that has a minimum pepsin detection level of <0.93 ng/mL. With this modality, their group was able to show that a salivary pepsin level of 4.21 ng/mL had an AUC on the ROC of 0.76 with a sensitivity of 76.36% and a specificity of 63.41% in the detection of conclusive GERD.[18] While our study did not use ELISA testing, which objectively has greater abilities in delineating lower levels of pepsin, our team produced similar data with LFD testing that is generally quicker, less expensive, and more readily available for clinicians. Applying the same LFD assay as in our study, Wang *et al*. performed a study from January 2015 to November 2018 where patients with varying GERD subtypes and healthy controls underwent upper endoscopy, 24-hour pH monitoring, and provided a single salivary sample for Peptest analysis. They found that a cutoff value of 75 ng/mL positively identified GERD-related disorders in 65.0–76.3% of objectively diagnosed GERD.[11] Another study performed by Hayat *et al*. identified even higher cutoff values ranging from >100 to >210 ng/mL in order to highlight improved specificity in diagnosing GERD.[13] The higher diagnostic threshold in these studies may be related to the fact that many of their samples were collected post-prandially or after patients had their first onset of symptoms. However, it is important to keep in mind that higher salivary pepsin thresholds dramatically hindered its sensitivity/positive predictive value and highlights the need to balance diagnostic yield and a reasonable cutoff value. Overall, our study identified similar diagnostic accuracy, sensitivity, and specificity with prior groups. Advantages of our study design include our multi-day and multi-timepoint collection protocol, the novel concept of overlapping it with prolonged pH monitoring, and our characterized cohorts. This cohort included patients with GERD symptoms who did not respond to PPI, had no erosive findings on esophagogastroduodenoscopy, and those who had either functional heartburn (physiologic AET) or non-erosive GERD (elevated AET) based on reflux monitoring. Thus, our study focuses on real-world clinical dilemma in GI practice—how to identify non-erosive GERD from functional heartburn.

Given the advantage of our study design to assess pepsin concentrations across four days and at three distinct timepoints each day, we identified variability in pepsin concentration throughout the day, similar to what has been observed with acid exposure. Multiple studies have previously shown that there is significant day-to-day variability of esophageal acid reflux exposure, including studies that highlight discordance between day 1 versus 2 of 48-hour pH monitoring[19,20] and improved diagnostic yield with prolonged testing.[21–24] Confounding factors that could affect this variability include sleep deprivation,[25,26] dietary/eating habits,[27–29] acute stress,[30,31] and high-intensity exercise.[32–34] Salivary pepsin levels are also likely influenced by multiple confounding factors (e.g., recent meal, type of food intake, and activity level) with similar day-to-day and within-day variability. Presumably, fasting AM salivary pepsin has the lowest level of influence from confounding factors and is most representative of a patient's gastroesophageal reflux physiology. This is reflected in the substantial agreement of the AM salivary pepsin values (Fleiss's kappa of 0.61) compared to reduced day-to-day agreement of PP/PM salivary pepsin values. This likely occurred because the number of confounder variables, such as the patient's dietary habits and activity/stress levels, increase as the day goes on.

Current clinical practices employ a wide variety of techniques to presumptively and noninvasively diagnose GERD. A detailed history often leads clinician to initially suspect GERD. Although GERD can present with a plethora of different symptoms, the most sensitive and specific symptoms appear to be heartburn and regurgitation.[7] One meta-analysis performed in patients with erosive esophagitis found that sensitivities ranged from 30% to 76% and specificities ranged from 62% to 96%.[7,14] Once clinicians have an index of suspicion for GERD, an empiric PPI trial is often employed to confirm or reject the presence of GERD. The pooled sensitivity of this method is 78% with a specificity of 56%.[7,35] Finally, the advent of validated questionnaires such as the GerdQ, RSI, and

Mayo-GERD questionnaires sought to find a more standardized method to assess GERD. Many research teams have examined the performance characteristics of these questionnaires. Dent *et al*. found that the GerdQ had a sensitivity of 62% and specificity of 67% in the diagnosis of GERD.[36] Another study by, Chan *et al*., using the Mayo-GERD questionnaire, found that it had a sensitivity of 68% and specificity of 72%.[37] Our current and previous work shows that salivary pepsin performs similarly in the diagnosis of GERD with our current study showing that a single fasting AM pepsin threshold of 25 ng/mL had a sensitivity of 56% and specificity of 78%. However, it should be noted that our previous study showed that a threshold of 24.9 ng/mL could have a sensitivity up to 86%.[12] Although additional studies need to be done, this highlights the fact that the work done on salivary pepsin currently shows comparable performance characteristics to commonly used noninvasive diagnostic methods with the additional benefit of objective data and the avoidance of unnecessary medication trials.

There are several strengths to this study. The study cohort is well characterized with robust objective data (endoscopic findings and prolonged ambulatory reflux monitoring) and represented a balance between patients with functional heartburn versus non-erosive GERD. Further, we analyzed a large number of saliva samples which were collected in a standardized rigorous protocol. Limitations of this study include the inability to perform further sub-group analyses given the smaller sample size. Participants in our study also only underwent wireless pH monitoring and did not complete impedance–pH testing, with potential to miss cases of non-acid reflux. An unavoidable potential limitation is the challenges in controlling external confounding factors (e.g., sleeping patterns, collection times, stress levels, and activity levels) that can influence salivary pepsin and/or esophageal acid burden. Similarly, dietary habits and the content of each participants' diet were not controlled. Diet and eating habits can vary from person-to-person and may impact day-to-day variability of salivary pepsin levels. However, these limitations were minimized since both acid exposure data and salivary pepsin concentrations were measured in conjunction and given the fact that this protocol is representative of routine clinical practice.

In conclusion, a single fasting AM salivary pepsin level showed substantial day-to-day agreement, fair accuracy, and modest performance in the objective diagnosis of GERD. Salivary pepsin continues to show promise as a potential rapid, noninvasive, and affordable office-based tool that can aid in the diagnosis of GERD and potentially lessen the healthcare cost of this prevalent disease. Salivary pepsin has even entered the realm of emergency medicine where elevated values for patients presenting with chest pain, in addition to negative cardiac biomarkers and unremarkable ECGs, help aid in ruling out acute coronary syndrome.[38] Likewise, if salivary pepsin levels are low for patients presenting with GERD-related symptoms, it may prompt the practitioner to suspect and manage for non-GERD related etiologies.

CONFLICT OF INTEREST

SM: none; VP: none; JR: none; MG: none; AK: none; RY: consultant for Medtronic, Phathom Pharmaceuticals, StatLinkMD; Medscape; Research Support: Ironwood Pharmaceuticals; Advisory Board with Stock Options: RJS Mediagnostix.

WRITING ASSISTANCE

None.

FINANCIAL SUPPORT

RY is supported by NIH (K23 DK125266; PI: Yadlapati).

SPECIFIC AUTHORS' CONTRIBUTIONS

Study concept and design: RY; study oversight: RY; acquisition of data: RY, MG, SM, VP, JR; analysis and interpretation of data: RY, AK, MG, SM, VP; drafting of manuscript: SM, VP; critical revision of the manuscript for important intellectual content: RY, AK, MG, SM, VP, JR; finalization of manuscript: RY, AK, MG, SM, VP, JR.

References

1. Delshad S D, Almario C V, Chey W D *et al.* Prevalence of gastroesophageal reflux disease and proton pump inhibitor-refractory symptoms. Gastroenterology 2020; 158(5): 1250–61.
2. Peery A F, Crockett S D, Barritt A S *et al.* Burden of gastrointestinal, liver, and pancreatic diseases in the United States. Gastroenterology 2015; 149(7): 1731–1741.e3.
3. Katz P O, Gerson L B, Vela M F. Guidelines for the diagnosis and management of gastroesophageal reflux disease. Am J Gastroenterol 2013; 108(3): 308–28.
4. Abdallah J, George N, Yamasaki T *et al.* Most patients with gastroesophageal reflux disease who failed proton pump inhibitor therapy also have functional esophageal disorders. Clin Gastroenterol Hepatol 2019; 17(6): 1073–80.
5. Shaheen N J, Hansen R A, Morgan D R *et al.* The burden of gastrointestinal and liver diseases, 2006. Am J Gastroenterol 2006; 101(9): 2128–38.
6. Jaynes M, Kumar A B. The risks of long-term use of proton pump inhibitors: a critical review. Ther Adv Drug Saf 2019; 10: 2042098618809927.
7. Katz P O, Dunbar K B, Schnoll-Sussman F H *et al.* ACG clinical guideline for the diagnosis and management of gastroesophageal reflux disease. Am J Gastroenterol 2021; 117(1): 27–56.
8. Bello B, Zoccali M, Gullo R *et al.* Gastroesophageal reflux disease and antireflux surgery-what is the proper preoperative work-up? J Gastrointest Surg 2013; 17(1): 14–20.

9. Du X, Wang F, Hu Z *et al.* The diagnostic value of pepsin detection in saliva for gastro-esophageal reflux disease: a preliminary study from China. BMC Gastroenterol 2017; 17(1): 1–9.
10. Saritas Yuksel E, Hong S K S, Strugala V *et al.* Rapid salivary pepsin test: blinded assessment of test performance in gastroesophageal reflux disease. Laryngoscope 2012; 122(6): 1312–6.
11. Wang Y J, Lang X Q, 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(1): 74.
12. Yadlapati R, Kaizer A, Greytak M *et al.* Diagnostic performance of salivary pepsin for gastroesophageal reflux disease. Dis Esophagus 2021; 34(4): doaa117.
13. Hayat J O, Gabieta-Somnez S, Yazaki E *et al.* Pepsin in saliva for the diagnosis of gastro-oesophageal reflux disease. Gut 2015; 64(3): 373–80.
14. Numans M E, Lau J, de Wit N J *et al.* Short-term treatment with proton-pump inhibitors as a test for gastroesophageal reflux disease: a meta-analysis of diagnostic test characteristics. Ann Intern Med 2004; 140(7): 518–27.
15. Jones R, Junghard O, Dent J *et al.* Development of the GerdQ, a tool for the diagnosis and management of gastro-oesophageal reflux disease in primary care. Aliment Pharmacol Ther 2009; 30(10): 1030–8.
16. Belafsky P C, Postma G N, Koufman J A. Validity and reliability of the reflux symptom index (RSI). J Voice 2002; 16(2): 274–7.
17. Gyawali C P, Kahrilas P J, Savarino E *et al.* Modern diagnosis of GERD: the Lyon Consensus. Gut 2018; 67(7): 1351–62.
18. Guo Z, Wu Y, Li L *et al.* The role of salivary pepsin in the diagnosis of gastroesophageal reflux disease (GERD) evaluated using high-resolution manometry and 24-hour multichannel intraluminal impedance-pH monitoring. Med Sci Monit 2020; 26: e927381–1.
19. Ayazi S, Lipham J C, Portale G *et al.* Bravo catheter-free pH monitoring: normal values, concordance, optimal diagnostic thresholds, and accuracy. Clin Gastroenterol Hepatol 2009; 7(1): 60–7.
20. Pandolfino J E, Richter J E, Ours T *et al.* Ambulatory esophageal pH monitoring using a wireless system. Am J Gastroenterol 2003; 98(4): 740–9.
21. Hasak S, Yadlapati R, Altayar O *et al.* Prolonged wireless pH monitoring in patients with persistent reflux symptoms despite proton pump inhibitor therapy. Clin Gastroenterol Hepatol 2020; 18(13): 2912–9.
22. Patel R, Kumar A, Jacobs J W *et al.* Su1096-96-hour esophageal ph monitoring: the tiebreaker for abnormal demeester score and symptom index. Gastroenterology 2018; 154(6): S-486–7.
23. Tseng D, Rizvi A Z, Fennerty M B *et al.* Forty-eight-hour pH monitoring increases sensitivity in detecting abnormal esophageal acid exposure. J Gastrointest Surg 2005; 9(8): 1043–52.
24. Yadlapati R, Ciolino J D, Craft J *et al.* Trajectory assessment is useful when day-to-day esophageal acid exposure varies in prolonged wireless pH monitoring. Dis Esophagus 2019; 32(3): doy077.
25. Fass R. The relationship between gastroesophageal reflux disease and sleep. Curr Gastroenterol Rep 2009; 11(3): 202–8.
26. Yamasaki T, Quan S F, Fass R. The effect of sleep deficiency on esophageal acid exposure of healthy controls and patients with gastroesophageal reflux disease. Neurogastroenterol Motil 2019; 31(12): e13705.
27. Cheng Y, Kou F, Liu J *et al.* Systematic assessment of environmental factors for gastroesophageal reflux disease: an umbrella review of systematic reviews and meta-analyses. Dig Liver Dis 2021; 53(5): 566–73.
28. Fox M, Barr C, Nolan S *et al.* The effects of dietary fat and calorie density on esophageal acid exposure and reflux symptoms. Clin Gastroenterol Hepatol 2007; 5(4): 439–444.e1.
29. Salvia G, De Vizia B, Manguso F *et al.* Effect of intragastric volume and osmolality on mechanisms of gastroesophageal reflux in children with gastroesophageal reflux disease. Am J Gastroenterol 2001; 96(6): 1725–32.
30. Holtmann G, Kriebel R, Singer M V. Mental stress and gastric acid secretion. Dig Dis Sci 1990; 35(8): 998–1007.
31. Konturek P C, Brzozowski T, Konturek S. Stress and the gut: pathophysiology, clinical consequences, diagnostic approach and treatment options. J Physiol Pharmacol 2011; 62(6): 591–9.
32. Clark C S, Kraus B B, Sinclair J *et al.* Gastroesophageal reflux induced by exercise in healthy volunteers. JAMA 1989; 261(24): 3599–601.
33. Herregods T V, Van Hoeij F B, Oors J M *et al.* Effect of running on gastroesophageal reflux and reflux mechanisms. Am J Gastroenterol 2016; 111(7): 940–6.
34. Schoeman M N, Tippett M D, Akkermans L M *et al.* Mechanisms of gastroesophageal reflux in ambulant healthy human subjects. Gastroenterology 1995; 108(1): 83–91.
35. Gyawali C P, Carlson D A, Chen J W *et al.* ACG clinical guidelines: clinical use of esophageal physiologic testing. Am J Gastroenterol 2020; 115(9): 1412–28.
36. Dent J, Vakil N, Jones R *et al.* Accuracy of the diagnosis of GORD by questionnaire, physicians and a trial of proton pump inhibitor treatment: the Diamond Study. Gut 2010; 59(6): 714–21.
37. Chan K, Liu G, Miller L *et al.* Lack of correlation between a self-administered subjective GERD questionnaire and pathologic GERD diagnosed by 24-h esophageal pH monitoring. J Gastrointest Surg 2010; 14(3): 427–36.
38. Saviano A, Petruzziello C, Brigida M *et al.* Use of the PEPTEST™ tool for the diagnosis of GERD in the Emergency Department. Am J Emerg Med 2019; 37(11): 2115–6.