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Original Article

Juice Test for Identification of Nonerosive Reflux Disease in Heartburn Patients

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Background/Aims
Evaluation of esophageal clearance by orange juice swallowing could be useful to identify different categories of gastroesophageal reflux disease. We determined whether a juice test at the beginning of esophageal pH monitoring can identify nonerosive reflux disease (NERD) among heartburn patients.

Methods
Multiple swallows of orange juice (pH 3) were performed at the beginning of esophageal pH monitoring in 71 heartburn patients off acid-suppressive therapy. The area between pH drop below 5 and recovery to 5 was calculated from pH tracings and named Delta5 (mmol·L^{-1}·sec). Fifteen healthy subjects served to determine Delta5 cutoff (95th percentile). Patients were classified as NERD, non-NERD (a mix of reflux hypersensitivity, functional heartburn, and undetermined), and erosive disease depending on acid exposure, reflux symptom analysis, and upper endoscopy.

Results
Delta5 cutoff in healthy subjects was 251 mmol·L^{-1}·sec. Among 71 patients, 23 had NERD, 26 had non-NERD, and 22 had erosive disease. Compared to non-NERD, Delta5 was higher in both NERD (median [interquartile range]: 316 [213-472] vs 165 [105-225]; $P < 0.01$) and erosive disease (310 [169-625] vs 165 [105-225]; $P < 0.01$). An elevated Delta5 (> 251 mmol·L^{-1}·sec) showed sensitivity of 74% and specificity of 81% for identification of NERD. Positive and negative likelihood ratios were 3.84 and 0.32 respectively, whereas test accuracy was 78%.

Conclusions
A juice test with calculation of Delta5 helps in the identification of true NERD among heartburn patients with endoscopy-negative reflux disease. In these patients, an elevated Delta5 could make prolonged reflux testing unnecessary.
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Key Words
Delta5; Esophageal pH monitoring; Juice test; Nonerosive reflux disease

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Introduction

Gastroesophageal reflux disease (GERD) is an expensive entity.[1,2] Patients with reflux symptoms are often referred for complementary investigation with diverse diagnostic techniques.[3] Among them, ambulatory reflux testing with standard pH monitoring is often requested, particularly for patients with nonerosive reflux disease (NERD) off acid suppressive medications.[4] Such technique allows calculation of esophageal acid exposure as well as analyses of the association between acid reflux and symptoms.[5] However, esophageal pH monitoring may present limitations in terms of diagnostic accuracy, with false negative and false positive results.[6,7]

The process of esophageal clearance is carried out by 2 mechanisms: esophageal peristalsis and saliva swallowing.[8] Under physiological conditions, once the luminal esophageal pH is decreased either by acid reflux or acidic bolus swallowing, such as orange juice, the volume present in the lumen is quickly propelled into the stomach by the esophageal peristalsis, and then the esophagus pH is restored after a number of saliva swallowing. In pathological conditions, processes that compromise either saliva production or esophageal peristalsis may prolong the contact of the esophageal mucosa with acidified material.[9,10] Such phenomena are often observed in patients with erosive reflux disease (ERD) or Barrett's metaplasia.[11] This also could be the case in true NERD patients, in whom pathological reflux could deteriorate esophageal motility.[12] In contrast, patients with reflux hypersensitivity and functional heartburn have their symptoms explained by visceral hypersensitivity and hypervigilance in the context of normal or borderline esophageal function.[13]

Theoretically, the ingestion of an acidified solution along with esophageal pH monitoring could provide an idea about esophageal clearance function. Evaluation of esophageal function after luminal acidification could include pH drop magnitude, length of pH recovery and area under the curve of pH drop and recovery. We hypothesized that a test with orange juice during esophageal pH monitoring might be helpful to estimate esophageal clearance and improve reflux testing accuracy in recognize different categories of GERD, such as true NERD. Hence, the aims of our study are: (1) to develop a 'juice test' combined with esophageal pH monitoring; (2) to evaluate its ability in predicting esophageal acid clearance and exposure; and (3) to establish juice test performance in identifying true NERD among heartburn patients with endoscopy-negative reflux disease.

Materials and Methods

Subjects

In this retrospective study, patients referred for esophageal pH monitoring between June 2011 and June 2012 were invited to participate. Inclusion criteria were: (1) age > 18 years; (2) heartburn as the main complaint, combined or not with regurgitation and atypical symptoms; (3) reflux testing off acid suppressive medications; and (4) agreement to participate. Patients were excluded if they have achalasia, history of gastroesophageal surgery, any technical problem in the juice test or pH monitoring, absence of heartburn or lack of endoscopy examination. Patients replied to a validated GERD symptom's questionnaire and performed a juice test in the beginning of the pH monitoring. Data regarding endoscopy off acid suppressive medications performed in our institution in the last 6 months were registered.

A group of 15 healthy subjects underwent esophageal pH monitoring with juice test. These subjects denied typical GERD symptoms and presented normal acid exposure at pH monitoring. Data from this group served to calculate reference values of the juice test.

The study was conducted according to principles of the Helsinki Declaration and was approved by the Ethical Committee of the Universidade de Passo Fundo (number 058/2012).

Clinical Assessment and Upper Endoscopy

Patients replied to a GERD symptom's questionnaire (GERD-SQ) validated to the native language,[14] followed by measurement of weight, height, and body mass index. Additional information including typical GERD symptoms, use of acid-suppressive medications and endoscopic data were recorded in a standardized chart. Based on GERD-SQ, heartburn and regurgitation were rated between 0 (no symptom) and 5 (severe symptom). Patients were endoscopically examined in our institution under endovenous sedation with midazolam (0.03 mg/kg) after cessation of acid-suppressive therapy (at least 30 days). Sliding hiatal hernia was measured in centimeters. Esophageal breaks suggestive of reflux esophagitis were described according to Los Angeles classification.[15]

Juice Test and Esophageal pH Monitoring

Patients were evaluated after an overnight fast. Esophageal pH monitoring was performed with a portable pH recorder (Sandhill Scientific, Inc.; Highlands Rach, CO, USA). A catheter was cali-

brated in pH 4.0 and 7.0 solutions and inserted transnasally. The pH sensor was positioned in the distal esophagus, either 5 cm above the lower esophageal sphincter when manometrically determined or 5 cm above the pH turning point, as defined by a gastroesophageal pH mapping.[16] Patients in the sitting position were instructed to swallow orange juice (Suvalan, Natural Products Ltda, Brazil; pH ~3) as follows: 10 mL offered with a syringe, and simultaneous monitoring of pH drop in the recorder display, followed by 5 rapid multiple swallows of juice soon after recovery of pH to 5 from the initial 10 mL swallow. Potential adverse reactions related to the juice test were registered. Patients remained sitting for 10 minutes while a diary card was explained. They were instructed to keep habitual daily activities and to record symptoms, food and fluid consumption and posture changes. On the following day, the pH catheter was removed and the tracings analyzed (BioView pH, Sandhill Scientific Inc). Acid suppressive medications, including proton pump inhibitors (PPIs) and H2 blockers, were stopped at least 7 days before reflux testing. Prokinetics were stopped 3 days before. Symptom reflux association was analyzed by means of symptom index, considered abnormal if > 50%. Reflux was considered symptomatic when a symptom occurred within the 2-minute time window of the preceding reflux episode.

Study Protocol and Juice Test Analysis

Although patients were identified prior to the publication of Rome IV criteria,[13] it was possible to update the categorization of GERD according to Rome IV using endoscopic and pH monitoring data off acid suppressive therapy. For assessment of juice test performance, heartburn patients were classified into 3 groups: NERD (abnormal reflux test with total acid exposure ≥ 4.2% regardless of symptom reflux association), non-NERD (mix of patients with reflux hypersensitivity, functional heartburn, and heartburn patients with normal acid exposure and no symptom during reflux testing), and erosive disease (ERD). Whilst reflux hypersensitivity and functional heartburn are 2 different categories according to Rome IV, for the purpose of this manuscript we decided to classify them as non-NERD due to the rarity of patients with reflux hypersensitivity in our series.

The analysis of the juice test tracings was carried out considering the pH drop related to rapid juice swallows. The tracings corresponding to pH drop after 10 mL swallow were not analyzed, but served to stabilize the baseline pH near 5 before multiple swallows. Three parameters were calculated: (1) Delta5 in mmol·sec·L^{-1}, ie, area under the curve between pH drop from 5 to pH recovery to 5; (2) absolute pH drop in mmol.L^{-1}; and (3) time in seconds from pH drop to recovery to 5. Juice test tracings were analyzed using the software AutoCAD (Autodesk, Inc. San Rafael, CA, USA). Initially, we identified 6 points in the tracing of multiple swallows, named P0, P1, P2, P3, P4, and P5 (Fig. 1). P0 was the starting point, which corresponded to pH near 5, immediately before juice swallow. P1 was the pH nadir, at the lowest value of pH drop after juice intake. P2, P3, and P4 corresponded to the quartiles 25%, 50%, and 75% between P1 and P5; while P5 was the tracing point where pH recovered to 5. A line connecting all points allowed calculation of Delta5. Absolute pH drop was the value between P0 and P1, whereas pH recovery was the time between P1 and P5. Normal values of these parameters were first calculated from a control group, using the 95th percentile for each parameter. Resulting cutoff were then applied for comparison of patients with different categories of GERD. Juice test was considered undefined when acid reflux was presumed to occur during pH recovery from juice swallow. In such a case, an abrupt pH fall > 1 point was indicative of superimposed reflux.

Eleven consecutive patients repeated the juice test at the end of the pH monitoring in the supine position, in order to determine both the effect of gravity and test reproducibility.

Statistical Methods

Qualitative data are presented as absolute frequency and percentage, whereas quantitative data are presented as median and interquartile range (25-75%), or when otherwise stated. Groups of quantitative data were first analyzed in regard to normality and

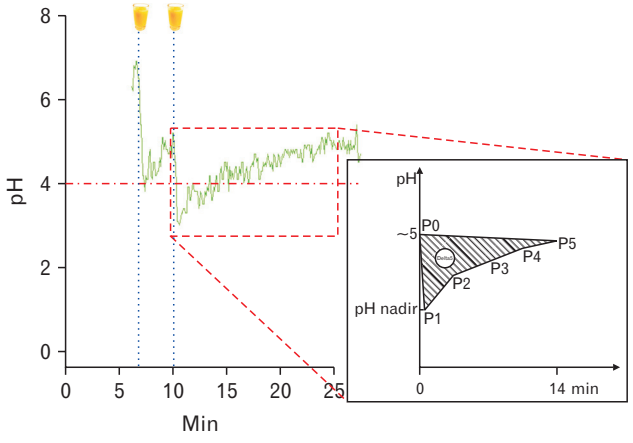

Figure 1. Schematic figure showing juice test tracing on pH monitoring (left) and its corresponding tracing build in AutoCAD (right). It is possible to observe how Delta5 was calculated, including pH drop and recovery to 5 (P0 to P5). In this example, Delta5 was 696 mmol·L^{-1}·sec.

homogeneity assumptions and then compared using t tests, one way ANOVA or non-parametric Kruskal-Wallis test accordingly. *P*-values were adjusted for pairwise multiple comparisons between groups. Categorical variables were analyzed using chi-square or exact Fisher tests. Reference values obtained from the control group were calculated on the basis of the 95th percentile. Concordance of repeated juice tests was determined using paired *t* test. Calculation of test performance included sensitivity, specificity, positive and negative likelihood ratio (LR), accuracy, and receiver operating characteristics (ROC) curves. The analyses were carried out with the software Graph Prism version 5 (GraphPad Software, Inc, San Diego, CA, USA), WinPEPI version 11.23 (Abramson, 2011) and SPSS version18 (IBM, Armonk, NY, USA). A *P*-value < 0.05 was indicative of statistical significance.

Results

Subjects

Fifteen healthy subjects served to define the normality cutoff for the juice test (age 34 ± 14 years old, weight 25 ± 5.7 kg/m^2, 66% women). In these subjects, the median (interquartile range [IQR]) total acid exposure was 1.7 (0.3-2.3).

A total of 109 patients performed the juice test and esophageal pH monitoring. Among them, 38 were excluded: 2 patients showed undetermined tracings during the juice test, 2 patients reported a history of anti-reflux surgery, 8 patients had acid reflux during pH recovery after juice intake, 10 patients denied typical reflux symptoms, and 16 patients had no endoscopy data.

The final study population was composed of 71 heartburn patients: 22 (31%) had ERD and 49 patients (69%) had endoscopy-negative reflux disease, being 23 NERD and 26 non-NERD (3 reflux hypersensitivity, 9 functional heartburn, and 14 undetermined [no symptoms during reflux testing]). There was no difference among groups in regard to age and gender distribution (Table 1). Patients with NERD had a body mass index significantly higher than those with non-NERD, but did not differ as compared to ERD patients. Sliding hiatal hernia was more common in ERD patients (10 out of 22 patients, between 2 cm and 6 cm), and equally distributed between NERD (2 cases, 3 cm and 4 cm) and non-NERD patients (3 cases, all with 2 cm). Heartburn and regurgitation scores were similar between groups.

Table 1. Characteristics of Non-nonerosive Reflux Disease, Nonerosive Reflux Disease, and Erosive Reflux Disease Patients (N = 71)

Variable	Non-NERD (n = 26)	NERD (n = 23)	ERD (n = 22)	*P*-value
Age in years (mean ± SD)	45.5 ± 14.7	43.2 ± 14.0	42.8 ± 10.9	0.751
Women (n [%])	18 (69)	12 (52)	9 (41)	0.137
BMI in kg/m^2 (mean ± SD)	24.9 ± 3.4	28.3 ± 3.9	26.5 ± 3.8	0.008[a]
Sliding hiatal hernia (n [%])	3 (9)	2 (10)	10 (45)	0.003
Symptoms score (median [IQR])				
Heartburn[b]	2 (0-3)	2 (1-3)	2.5 (2-3)	0.210
Regurgitation[b]	2 (2-2)	2 (1-3)	2 (0-2)	0.162

[a]Nonerosive reflux disease (NERD) vs non-NERD.
[b]Score generated by gastroesophageal reflux disease symptom's questionnaire (GERD-SQ), ranging between 0 (no symptom) to 5 (severe symptom).
Non-NERD, mix of reflux hypersensitivity, functional heartburn, and heartburn patients with normal acid exposure and no symptoms during reflux testing; ERD, erosive reflux disease; BMI, body mass index.

Table 2. Juice Test Parameters in Controls and Gastroesophageal Reflux Disease Categories

Variable (median [IQR])	Control (n =15)	Non-NERD (n = 26)	NERD (n = 23)	ERD (n = 22)
Delta5 in mmol·L^{-1}·sec[a]	123 (72-168)	165 (105-225)	316 (213-472)	310 (169-625)
pH recovery to 5 in sec[b]	151 (100-213)	248 (126-310)	258 (221-423)	239 (143-610)
pH drop in mmol·L^{-1}[c]	1.9 ± 0.6	2.1 ± 0.5	2.3 ± 0.7	2.2 ± 0.5

[a]$P < 0.001$ control vs NERD and ERD; $P < 0.01$ non-NERD vs NERD and ERD.
[b]$P < 0.05$ control vs NERD.
[c]Data presented as mean ± SD.

Juice Test and pH Monitoring

The juice test was performed easily and without adverse reactions. From 15 healthy subjects, Delta5 was 128 ± 58 mmol·L^{-1}·sec on average. A Delta5 cutoff calculated as the 95th percentile was 251 mmol·L^{-1}·sec, which served to establish the performance of the juice test in GERD patients. Juice test parameters were first compared between healthy subjects and the entire group of patients with GERD. Patients presented higher Delta5 (median [IQR]: 252 [149-382] vs 123 [72-168]; $P < 0.001$) and longer pH recovery time in seconds than healthy subjects (median [IQR]: 254 [150-387] vs 151 [100-213]; $P < 0.01$), whereas pH drop did not differ between groups (mean ± SD: 2.2 ± 0.6 vs 1.9 ± 0.5; $P = 0.101$).

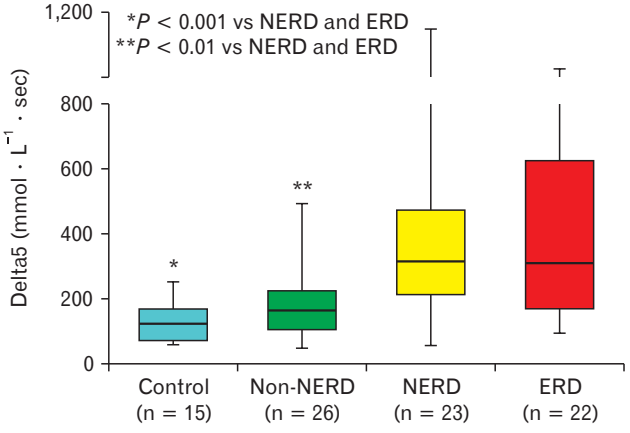

Figure 2. Whiskers graph presenting Delta5 data from controls and patients with non-nonerosive reflux disease (non-NERD), NERD, and erosive reflux disease (ERD).

In the comparison of GERD categories and controls, Delta5 was significantly higher in both NERD and ERD compared to either non-NERD or controls (Table 2 and Fig. 2). NERD patients also showed prolonged recovery of pH to 5 in comparison to controls, while pH drop was similar between the 4 groups. No statistical difference was observed in these parameters after comparison of ERD and NERD patients. Delta5 was the parameter elected to address the performance of the juice test. Figure 3 shows examples of the juice tests obtained from patients with NERD and non-NERD. In 11 patients who repeated juice test, Delta5 in upright was similar as compared to Delta5 in supine position (146.2 ± 83.8 vs 138.2 ± 62.4; $P = 0.622$).

Juice Test Performance

The performance of the juice test for identification of NERD was calculated taking into account the parameter Delta5 and the prevalence of NERD found in the study population (47%). The purpose of an elevated Delta5 (> 251 mmol·L^{-1}·sec) was to identify NERD patients. Test accuracy was 78%. As presented in Table 3,

Table 3. Juice Test Performance for Identification of Nonerosive Reflux Disease, Based on Elevated Delta5 (> 251 mmol·L^{-1}·sec)

Test performance	Juice test
Sensitivity (% [95% CI])	74 (53-87)
Specificity (% [95% CI])	81 (62-91)
Positive likelihood ratio[a]	3.84 (1.69-8.76)
Negative likelihood ratio[b]	0.32 (0.16-0.66)
Accuracy (%)	78

[a]Elevated Delta5.
[b]normal Delta5.
Area under the receiver operating characteristics curve: 80.3%; $P < 0.001$.

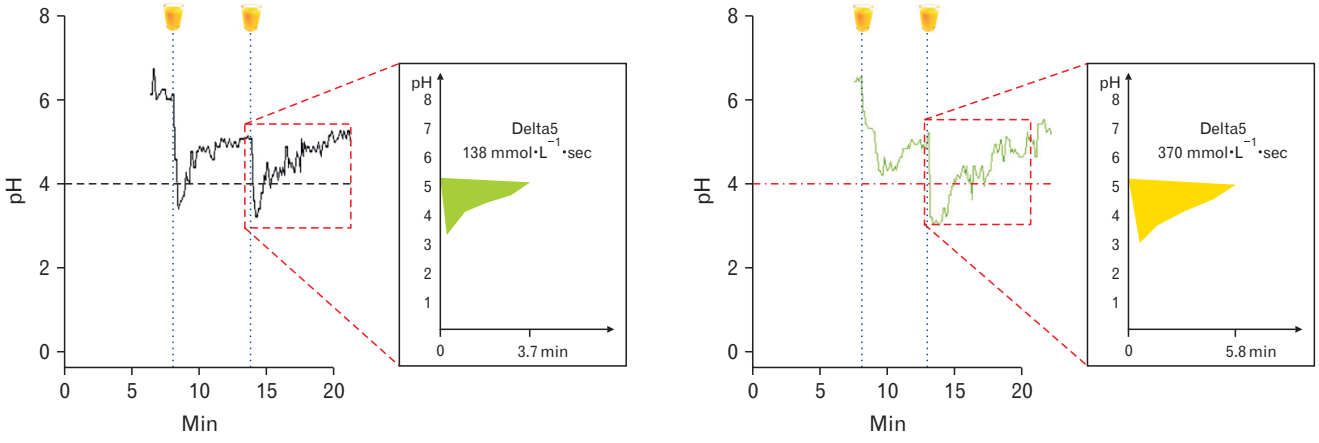

Figure 3. Examples of the juice tests obtained from patients with functional heartburn (representing non-nonerosive reflux disease [non-NERD] group, left) and NERD (right). Note that Delta5 was double in the patient with NERD.

juice test showed high sensitivity and specificity for identification of NERD patients. Accordingly, Delta5 showed high positive LR and low negative LR values for recognition of NERD among patients with endoscopy-negative reflux disease. The area under the ROC curve was 80.3% ($P < 0.001$) and suggested the cutoff of 251 mmol·L^{-1}·sec as the best point of performance in terms of sensitivity (74%) and specificity (81%). However, in order to decrease the chance of a false diagnosis of non-NERD, a Delta5 higher than 285 mmol·L^{-1}·sec would provide a specificity of 85%, with sensitivity of 70%.

Discussion

The development of new diagnostic strategies is fundamental to help in the management of relevant diseases such as GERD. This is particularly important for the category of patients with endoscopy-negative reflux disease, in which the differentiation between NERD, reflux hypersensitivity, and functional heartburn is crucial for therapeutical outcomes. Taking this into account, we first assessed whether a simple juice test could be useful to predict reflux in GERD patients investigated with esophageal pH monitoring. Furthermore, we estimated test performance in the identification of NERD among heartburn patients with endoscopy-negative reflux disease.

The main findings of this study were: (1) the juice test was easily performed, well tolerated, and useful for estimation of esophageal clearance by calculating a parameter named Delta5; (2) NERD patients had the greatest Delta5 among participants with endoscopy-negative reflux disease, similar to those of ERD patients; and (3) in patients with endoscopy-negative reflux disease, an elevated Delta5 showed high sensitivity and specificity for identification of NERD. It was also possible to provide values of negative and positive LR.

To our knowledge, this is the first study applying a juice test for evaluation of esophageal physiology in patients with GERD. The juice test was easily performed, well tolerated, and useful for estimation of esophageal clearance by calculating a parameter named Delta5. The rationale for performing a juice test during esophageal pH monitoring is to challenge the capacity of the esophagus in restoring luminal pH which occurs after an acid reflux. Hence, Delta5 is calculated based on the magnitude of pH drop below 5 and the time lasting for pH recovery to 5, reflecting the ability of the esophagus in clearing acid contents. The choice of pH 5 was arbitrary due to the fact that pH 4 would result in a small Delta area, whereas pH 6 might distort such an area because of common pH fluctuations around 6 during pH recovery. It is known from studies in normal subjects that almost all volumes are emptied from the esophagus by esophageal peristalsis after an episode of acid reflux, followed by luminal pH recovery after saliva swallowing.[8] Such process of clearance may be compromised in GERD patients, particularly in those with reflux esophagitis and/or Barrett esophagus.[17,18] Indeed, Booth and Colleagues validated an acid clearing test and demonstrated that the ability of the distal esophagus in clearing instilled acid is compromised in GERD patients compared to healthy subjects.[19] However, the authors did not provide information regarding NERD patients. Here we indicate that esophageal clearance is delayed also in NERD, as compared to patients with endoscopy-negative reflux disease and normal acid exposure. This may occur due to esophageal micro-inflammation related to acid reflux, with mucosal production of interleukins able to inhibit esophageal muscle contraction.[12]

Additional mechanisms that may influence esophageal clearance are contractions after multiple rapid swallows (MRS),[20,21] as performed here during the juice test, and post-reflux swallow induced peristaltic wave (PSPW) index.[22] In normal subjects, MRS provokes a cholinergic response with esophageal contraction after neural inhibition of the esophagus during rapid swallows. However, the exact role of MRS in the esophageal clearance of patients with endoscopy-negative reflux disease deserves further studies. Analysis of PSPW index combined with mean nocturnal baseline impedance showed high sensitivity in identifying different categories of GERD, increasing the accuracy of impedance-pH monitoring compared with pH-only data.[22] Finally, Savarino and Colleagues have shown that esophageal clearance and bolus transit abnormalities increase in parallel with the severity of GERD.[23]

Our findings confirm the hypothesis that a simple juice test can help in the differentiation between organic and functional disease in the complex group of NERD patients. One may question the lack of utilization of impedance-pH monitoring in our study. It has been recently demonstrated that the diagnostic gain of impedance-pH over pH only testing is modest in patients off acid suppressive therapy,[24,25] Nevertheless, we recognize the lack of impedance as a limitation of our study and suggest the need of future studies with impedance technique to provide data regarding volume clearance during juice test, provided that the orange juice pH is standardized close to 3.

The criteria here employed for categorization of GERD in erosive and nonerosive disease were based on symptoms and complementary evaluation with endoscopy and reflux testing. According to guidelines,[4,26] endoscopy-negative reflux disease patients are further classified has having true NERD, reflux hypersensitivity and func-

tional heartburn. In response to our limited number of participants, we grouped patients with reflux hypersensitivity and functional heartburn into one category and named them as non-NERD. On one hand, the symptom in reflux hypersensitivity is clearly associated with reflux and in functional heartburn it is not. On the other hand, at the moment there is no clear different treatment for both conditions. Further studies are needed to evaluate the role of the juice test in patients with either reflux hypersensitivity or functional heartburn.

In endoscopy-negative reflux disease patients, an increased Delta5 showed high sensitivity and specificity for identification of NERD. In such cases, either positive or negative likelihood ratio could be useful in clinical practice to estimate post-test probability according to disease prevalence. Test accuracy approached 80%, meaning that approximately four-fifths of the patients presenting an abnormal juice test would be correctly identified has having NERD. Based on these findings, we are prone to accept that the performance of the juice test is superior to that of a PPI test, in which the specificity ranges between 24% and 65%.[3] However, case-control studies may overestimate diagnostic accuracy of new tests and it likely introduces a spectrum bias. Nevertheless, the high performance of the juice test in identifying NERD among patients with endoscopy-negative reflux disease provides support for further studies that test the cancellation of prolonged reflux testing in case of elevated Delta5 indicating NERD, while patients with normal Delta5 as represented here by a heterogeneous group named non-NERD would need to continue 24 hours pH ± impedance monitoring to quantify reflux and assess reflux symptom association. Because of this limitation, new studies using impedance-pH technique are still needed to assess juice test performance in patients with hypersensitive esophagus and functional heartburn.

In conclusion, we developed a juice test to help esophageal pH monitoring in the identification of different categories of GERD. Our data suggest that a juice test with calculation of Delta5 may help in the differentiation between NERD and non-NERD among heartburn patients with endoscopy-negative reflux disease. Four-fifths of these patients who presented an elevated Delta5 where correctly classified as having increased acid exposure after pH monitoring, being correctly identified as true NERD. Additional studies on outcome data are still needed to further validate the juice test, and assessing its ability to predict response to PPIs or surgery compared to acid exposure or symptom association analysis.

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Conflicts of interest: None.

Author contributions: Fernando Fornari planned the study; Fernando Fornari, Michel R Fernandes, and Sidia M Callegari-Jacques collected and analyzed the data; and Fernando Fornari, Michel R Fernandes, Marina de Oliveira, Sidia M Callegari-Jacques, and Gissele V R Gonçalves discussed the results and wrote the paper.

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