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“ANXIETY CAN SIGNIFICANTLY EXPLAIN BOLUS PERCEPTION IN THE CONTEXT OF HYPOTENSIVE ESOPHAGEAL MOTILITY: RESULTS OF A LARGE MULTICENTER STUDY IN ASYMPTOMATIC INDIVIDUALS”

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ABSTRACT

Background: Previous studies have not been able to correlate manometry findings with bolus perception. The aim of this study was to evaluate correlation of different variables, including traditional manometric variables (at diagnostic and extreme thresholds), esophageal shortening, bolus transit, automated impedance manometry (AIM) metrics and mood with bolus passage perception in a large cohort of asymptomatic individuals.

Methods: High resolution manometry (HRM) was performed in healthy individuals from nine centers. Perception was evaluated using a 5-point Likert scale. Anxiety was evaluated using Hospitalized Anxiety and Depression scale (HAD). Subgroup analysis was also performed classifying studies into normal, hypotensive, vigorous and obstructive patterns.

Key Results: 115 studies were analyzed (69 using HRM and 46 using high resolution impedance manometry (HRIM)). 3.5% swallows in 9.6% of volunteers were perceived. There was no correlation of any of the traditional HRM variables, esophageal shortening, AIM metrics nor bolus transit with perception scores. There was no HRM variable showing difference in perception when comparing normal versus extreme values (percentile 1 or 99). Anxiety but not depression was correlated with perception. Among hypotensive pattern, anxiety was a strong predictor of variance in perception (R^2 up to 0.70).

Conclusion: Bolus perception is less common than abnormal motility among healthy individuals. Neither esophageal motor function nor bolus dynamics evaluated with several techniques seem to explain differences in bolus perception. Different mechanisms seem to be relevant in different manometric patterns. Anxiety is a significant predictor of bolus perception in the context of hypotensive motility.

KEYWORDS

Anxiety. Automated Impedance Manometry. Bolus perception. High resolution esophageal manometry. Hypotensive motility.

KEY POINTS

- To date, no study has been able to correlate bolus passage perception with esophageal manometric findings. The aim of this study was to evaluate predictors of perception, including new developed metrics and mood.
- We could not demonstrate any correlation between high resolution manometry or automated impedance manometry variables and perception. Anxiety seems to be a strong predictor of perception among individuals with hypotensive motility
- Anxiety should be considered in future studies and in the management of patients with dysphagia in the context of hypomotility disorders.

INTRODUCTION

Dysphagia is present in 3 to 9% in general population ^{1,2}, and up to 20% in older than 50 years ³. Esophageal manometry is considered the gold standard test in these cases ⁴. The finding of esophagogastric junction (EGJ) obstruction leads to an effective treatment recommendation. As such, high resolution manometry (HRM) represents a prominent advance, as it has higher sensitivity for obstructive disorders, such as achalasia ^{5, 6}. On the contrary, findings such as hypo- or some hypertensive patterns do not lead to a clear therapeutic option. This could be due to lack of effective treatments, but also because such manometric patterns may not be causally related to symptoms. The use of standard pressure-only manometry has repeatedly shown no correlation with bolus passage perception, both in symptomatic and asymptomatic individuals^{7,8}. Despite the better spatiotemporal discrimination of HRM, its findings could not be correlated with perception in a large dysphagia cohort ⁹. This lack of correlation could have several reasons. It could be that the evaluated mechanism is nonspecific for dysphagia. An example is incomplete bolus transit, which has been reported in healthy volunteers in 40% for liquid and in 80% for solid swallows ¹⁰. When a putative mechanism is highly prevalent in healthy individuals, its positive predictive value diminishes. In fact, dichotomous determination of bolus transit has never been correlated with perception, either evaluated with impedance ⁷ or fluoroscopy ¹¹. These findings highlight the importance of including normals in the study of a certain mechanism. Unfortunately, most studies are small, making the evaluation of infrequent patterns difficult.

It is possible that the putative mechanism to explain symptoms (e.g. wall tension) is correct, but the metric used (e.g. distal contractile integral-DCI) is not. Using the recently developed automated impedance manometry (AIM), several new metrics have been described that characterize flow/pressure

dynamics during a swallow^{12, 13}. These metrics have shown some promising results in predicting perception in small samples^{12, 14}.

Another reason could be that the mechanism and metric are correct, but the cutoff value is not. An epidemiological-derived threshold (e.g. percentile 95) could not be a good symptom predictor. For example, in Chicago Classification 3.0 (CC3.0), the diagnostic DCI criteria for hyper-contractile disorders have been steadily revised upwards from 5000 (percentile 95) to 8000 mmHg cm⁻¹sec⁻¹, as there is no apparent clinical significance of a contraction with a DCI between these values¹⁵. This suggests that the capacity of the more extreme manometric thresholds (beyond percentile 5 or 95) to generate symptoms should be tested.

Some esophageal symptoms, like heartburn and chest pain have been related to contraction of longitudinal muscles^{16, 17}. HRM only evaluates circular muscle function. Nevertheless, some studies has evaluated EGJ movement as a surrogate of longitudinal muscle contraction^{18, 19}. Mittal et al have recently showed a good correlation between EGJ movement measured by HRM and measured using a piezo-electrical assembly²⁰.

It could be speculated that perception is driven by different mechanisms among different manometric patterns (e.g. by wall hypertension in Jackhammer's esophagus and by bolus stasis in ineffective esophageal motility-IEM). To date, no study has evaluated separately different patterns.

Finally, perception can be modulated by mood. Anxiety has been demonstrated to modulate gastrointestinal symptoms in healthy²¹ and symptomatic individuals²². Sharma et al showed that anxiety increases acid-induced esophageal hyperalgesia²³. To our knowledge, mood has never been included in predictor models related to esophageal symptom generation during manometry.

Thus, the aim of this study is to evaluate, in a large cohort of healthy subjects, the correlation between HRM and AIM variables (including extreme cutoff values and subgroup analysis of different manometric patterns), mood and symptom generation during a traditional manometric protocol.

METHODS

Subjects

One hundred and fifteen volunteers were recruited from nine centers between August 2012 and February 2014. Participant centers were Badalona-Spain, Madrid-Spain, Mexico DF-Mexico, Veracruz-Mexico, Rio do Janeiro-Brazil, Bogota-Colombia, Quito-Ecuador, Buenos Aires-Argentina and Santiago de Chile-Chile. None of the individuals had any history of gastroesophageal/swallowing complaints or had undergone gastroesophageal surgery. All individuals gave written informed consent for their participation. The study protocol was approved by the Human Research Ethics Committee of each participant center.

Data acquisition

Before HRM, all individuals gave epidemiological information and filled in the Hospitalized Anxiety Depression (HAD) scale. This is a 14-item self-reported scale (7 for depression and 7 for anxiety subscales), developed for mood disorders screening in non-psychiatric outpatient individuals. It refers to symptoms occurring during the last seven days. Each item scores 0 to 3 points, giving a maximum of 21 for each subscale. For each anxiety and depression, a level of 0-7 is considered normal ²⁴.

After eight hours fast, HRM was performed using a 4.2 mm 36 channel solid-state probe (Mano-Scan 360; Sierra Scientific Instruments, Mountain View, CA, USA). In the case of high resolution impedance manometry (HRIM), a 4.0-mm diameter probe with 36 pressure sensors and 18 adjoining impedance segments was used (Given Imaging, Los Angeles, CA, USA). The probe was inserted transnasally leaving at least three sensors in the stomach. All the studies were performed in a supine position using ten 5ml

liquid swallows. In the case of traditional HRM studies water was used. In the case of HRIM studies, either 0.45% saline solution or an electrolyte containing soda (Aquarius-Coca Cola Company) was used. After each swallow, individuals reported bolus passage perception using a five point Likert scale (Do you perceive any difficulty while swallowing? 0=None. 1=Mild. 2=Moderate. 3=Very much. 4=It is stuck in the esophagus).

Data analysis

All studies were analyzed by one researcher (DC) using ManoviewESO 3.0 analysis software (Given Imaging, Duluth, GA) to obtain traditional HRM variables as previously suggested^{5, 15, 25}. Esophageal shortening was evaluated by one researcher (HM) using the same software. The proximal margin of the EGJ was determined using 20 mmHg isobaric contour. Smart mouse tool was used to evaluate its axial movement before and at the maximal displacement during each swallow. In the case of HRIM studies, bolus entry was defined as a drop in impedance of 50% from baseline and bolus exit as the return to this 50%, as previously described^{26, 27}. Bolus transit was considered complete when all the evaluated channels that showed bolus entry showed bolus exit. AIM analysis was performed by one researcher (CS) using a purpose-designed MATLAB-based (Math-Works, Natick, Massachusetts, USA) analysis program (AIMplot software, version 5.0 2015. Copyright Taher Omari. Adelaide, Australia). AIMplot derived nine esophageal pressure-flow variables, each for the whole, proximal (upper esophageal sphincter-transition zone) and distal (transition zone-lower esophageal sphincter) esophagus. The variables are listed in Table 1 and explained in Figure 1. Figure 2 shows an example of the calculation of individual variables in a certain swallow.

For analysis purposes, any swallow with a score ≥ 1 in this 0-4 scale was considered perceived. Manometry and impedance results were analyzed without knowledge of the perception score. Abnormal anxiety and/or depression levels were considered if the HAD score was ≥ 8 for the respective

subscale. Analyses were done separately in a swallow by swallow and person by person fashion (considering mean variables for each volunteer). In the last case, correlations were done using mean perception scores for the whole set of swallows. In the swallow by swallow analysis, each swallow received the HAD score of the respective individual. The type of analysis was specified in each part of the results section. For subgroup analysis, each swallow was classified in one of four patterns (normal, obstructive, vigorous and hypotensive) using a hierarchical algorithm depicted in Figure 3. For the person by person analysis, the complete set of swallows was classified in the same patterns according to Chicago 2.0 (CC2.0) and 3.0 (CC3.0) classifications, as follows: Normal pattern if the study was regarded as normal in both classifications. Obstructive if it was diagnosed as achalasia or Esophagogastric Junction Outflow Obstruction (EGJOO) in any classification. Vigorous if it was classified as Distal Esophageal Spasm, Hypercontractile Esophagus, Rapid Contraction with Normal Latency or Hypertensive Peristalsis (using CC2.0), or Distal Esophageal Spasm or Hypercontractile Esophagus (using CC3.0). Hypotensive if it was diagnosed as Absent Peristalsis, Weak Peristalsis with either small or large peristaltic defects, Frequent Failed Peristalsis (using CC2.0) or Absent Contractility, Ineffective Esophageal Motility or Fragmented Peristalsis (using CC3.0)^{15, 25}.

Statistical analysis

Data are presented as mean and standard deviation or median and interquartile range. Correlation was evaluated using Spearman rank. Comparison between groups was performed using ANOVA, Mann-Whitney U, Student's or Chi-square test. Evaluation of the independence of the association between several variables was performed using partial correlation and logistic regression (forced or stepwise entry). All p values were considered significant at a 0.05 level (two-tailed). For the AIM related variables analysis, a Bonferroni's correction was applied, and a p value ≤ 0.002 was considered significant (0.05/27 variables). Statistical analysis was done using SPSS 15.0 (SPSS Inc., Chicago, USA).

RESULTS

One hundred fifteen volunteers were recruited between August 2012 and February 2014. Fifty-seven (49.6%) were female. Mean age was 32 years (range 18-69 years). A total of 996 swallows were analyzed. Of them, 320 swallows in 46 volunteers were evaluated using HRIM. In the mood evaluation, mean HAD depression was 2.23 ± 2.18 and mean HAD anxiety was 4.13 ± 2.44 . Abnormal levels (≥ 8) for depression and anxiety were present in 3/115 (2.6%) and 9/115 (7.8%) of volunteers, respectively.

Individuals had a mean perception score of 0.05 ± 0.22 . Of all swallows, 323(32.4%) were manometrically abnormal and 35 (3.5%) were perceived (24 score 1, 10 score 2 and 1 score 3). The percentage of perceived swallows (score ≥ 1) was low (Median 0%. Percentile 95: 30%). Eleven volunteers (9.6%) showed at least one perceived swallow. Among these individuals, the median perceived swallows was 20.0% (IQR 40.0%-14.3%=25.7%). Two individuals perceived all the swallows.

VARIABLES ASSOCIATED WITH PERCEPTION

Epidemiological variables

There was no correlation between perception score and gender or age (p ns), neither in swallow by swallow nor person by person analysis.

Individuals from Bogota and Veracruz showed significantly higher mean perception scores than other centers (0.13 ± 0.44 and 0.44 ± 0.72 , respectively) ($F=19.13$. $p<0.01$). 3/19 (15.8%) and 5/9 (55.6%)

individuals showed any symptomatic swallow in Bogota and Veracruz, respectively. There was significantly more anxiety in some centers (ANOVA $F=12.17$, $p<0.0001$). Mexico DF, Bogota and Veracruz showed the highest anxiety levels (Mean HAD anxiety scores 5.1 ± 2.4 ; 4.5 ± 3.6 ; 6.2 ± 1.5 , respectively). In a logistic regression model that included center of origin and HAD anxiety, only the latter was an independent perception predictor ($\beta=0.51$, $p<0.001$).

In the swallow by swallow analysis, there was a significant positive correlation between perception score and HAD anxiety ($\rho=0.18$, $p<0.0001$) and HAD depression ($\rho=0.11$, $p<0.0001$). When analyzing only symptomatic swallows, neither anxiety nor depression levels were correlated with perception scores (p ns). In the person by person analysis, mean perception scores did not differ significantly between individuals with normal versus abnormal levels of depression (p ns). Mean perception scores were significantly higher in individuals with abnormal compared with normal levels of anxiety (0.45 ± 0.70 vs 0.02 ± 0.06 , $p<0.0001$). In a partial correlation model, when fixing anxiety, depression is no longer associated with perception (p ns). In a logistic regression model that included HAD depression and HAD anxiety, only the latter was independently associated with perception ($\beta=0.49$, $p<0.001$).

Manometric variables. All swallows analysed.

There was no correlation between any traditional HRM variable and perception scores (p ns), neither considering all swallows or only symptomatic ones, in both swallow by swallow and person by person analysis (Table 2).

In the swallow by swallow analysis, 189/995 (19.0%) and 102/995 (10.3%) of swallows showed a DCI < 450 and <100 mmHg $\text{cm}^{-1}\text{sec}^{-1}$, respectively. Neither showed a significant difference in perception scores when compared to swallows with normal DCI (p ns) (Table 3). There was no difference in perception

scores when comparing normal, small (2-5 cm), large peristaltic size defect (>5 cm) and failed swallows ($F=0.275$, p ns). 15/995 (1.5%) of swallows had a DCI > 5000 mmHg cm⁻¹sec⁻¹, including 2 swallows with DCI > 8000 mmHg cm⁻¹sec⁻¹. None of these 15 swallows were perceived.

In the swallow by swallow analysis, 75/995 (7.5%) of swallows had an IRP > 15 mmHg. Only 2/75 (2.7%) of these swallows were perceived. There was no difference in perception when comparing swallows with IRP > and < 15 mmHg, neither when comparing swallows with an IRP > and < 19.6 mmHg (percentile 99) (p ns) (Table 3).

In the swallow by swallow analysis, 23/995 (2.3%) and 10/995 (1.0%) swallows showed a CFV > 9 and > 12 cm/s (percentile 95 and 99, respectively). They showed no difference in perception scores when compared with swallows with normal CFV (Table 3). 4/995 (0.4%) of swallows had a DL < 4.5 s, and they showed no difference in perception scores when compared with swallows with DL > 4.5 s (p ns) (Table 3). There was only one swallow with a DL < 1.9 s (percentile 1) and it was not perceived.

In the person by person analysis, individuals had a mean esophageal shortening of 11.0 ± 3.92 mm. There was no correlation between esophageal shortening and mean perception score (p ns). There was no difference in perception score when comparing studies when shortening < and > 18.5 mm (percentile 95) (p ns).

Using HRIM, 65/320 (20.3%) of swallows had incomplete bolus transit. Among these, 5/65 (7.7%) were perceived. This is not different than the 21/255 (8.2%) of perception among swallows with complete bolus transit ($\chi^2 0.02$ (1), p ns). There was no difference in perception scores when comparing swallows with complete versus incomplete bolus transit (0.11 ± 0.39 vs 0.11 ± 0.43 , respectively, p ns).

There was no correlation between anxiety levels and any of the aforementioned HRM variables.

Manometric variables. Subgroup analysis

Using the person by person analysis, 66/107 (61.7%) of studies were classified as normal, 28/107 (26.2%) as hypotensive, 8/107 (7.5%) as obstructive and 5/107 (4.7%) as vigorous. There was no difference in the percentage of symptomatic swallow nor in the mean perception score when comparing any subgroup to normal (Table 4).

Swallows classified as normal showed a mean perception score of 0.05 ± 0.28 , and 24/650 (3.7%) were perceived. Among these, there was a significant although weak correlation between perception score and HAD anxiety ($\rho=0.17$. $p<0.001$). Swallows in individuals with abnormal levels of anxiety showed a significantly higher perception scores (0.51 ± 0.84 vs 0.02 ± 0.15 . $p=0.001$). When considering only symptomatic swallows, anxiety is no longer correlated with perception (p ns). Among this normal subgroup, none of the manometric variables correlated with perception, neither when considering all or only symptomatic swallows.

Swallows classified as hypotensive had a mean DCI of 194.5 ± 192.6 mmHg $\text{cm}^{-1}\text{sec}^{-1}$. They had a mean perception score of 0.06 ± 0.30 , which was no different from mean perception score among normal swallows (p ns). Among hypotensive swallows, neither DCI, sum of all peristaltic defect lengths (in the swallow by swallow analysis), mean DCI, maximum DCI nor mean peristaltic defect length (in the person by person analysis) was correlated with perception. Among this subgroup, HAD anxiety was significantly correlated with perception ($\rho=0.38$. $P<0.001$). None of the swallows in individuals with normal HAD anxiety levels was perceived, while 9/25 (36.0%) of swallows among anxious individuals were perceived ($p<0.001$). In a logistic regression model, HAD anxiety significantly predicted perception in this subgroup ($R^2=0.43$. $p<0.001$). This predictive capacity was even better when considering only hypotensive swallows from anxious individuals ($R^2=0.73$. $p>0.001$) (Figure 4). 44/59 swallows (74.6%) had

incomplete bolus transit using impedance criteria. Swallows with incomplete bolus transit had a lower perception scores compared to swallows with complete bolus transit, although this was a non-significant trend (0.07 ± 0.25 vs 0.47 ± 0.74 . $p=0.06$).

75 swallows were classified as obstructive. They had a mean IRP of 17.71 ± 2.26 mmHg and a mean perception score of 0.027 ± 0.16 , which is no different from normal swallows (p ns). 2/75 (2.7%) swallows were perceived. Neither HAD anxiety nor HAD depression showed any correlation with perception scores (p ns). IRP was not correlated with perception (p ns). There was no difference in IRP between perceived vs non-perceived swallows (p ns). 12/75 of these obstructive swallows showed any evidence of compartmentalized pressurization and none was perceived. None of the other manometric variables showed any significant correlation with perception, neither when considering separately symptomatic or asymptomatic swallows, neither when comparing extreme (percentile 99 or 1) versus normal values.

38 swallows were classified as vigorous: 15 with $DCI > 5000$ mmHg $cm^{-1}sec^{-1}$ (2 with $DCI > 8000$ mmHg $cm^{-1}sec^{-1}$), 23 with $CVF > 9$ (including 4 with $DL < 4.5$). None of them was perceived.

AIM variables

The analysis was made based on average results per subject (person by person).

HRIM with AIM analysis was made in 41 volunteers, using variables depicted in Table 1. Among these volunteers, mean perception score was 0.11 ± 0.34 . 6/41 (14.6%) individuals perceived at least one swallow. No AIM variable showed a significant correlation with perception. Nadir Impedance/Impedance at Peak Pressure ratio (NI/IPP ratio) in the whole esophagus showed a negative correlation with perception, although this was non-significant after Bonferroni's correction ($\rho = -0.336$ $p = 0.031$).

26/41 (63.4%) studies were classified as normal, 9/41 (21.9%) as hypotensive and 6/41 (14.6%) as obstructive. No AIM variable was correlated with mean perception when these subgroups were analyzed separately. Among hypotensive studies, a logistic regression model showed that HAD anxiety was a very good predictor of mean perception score ($\beta=0.09$. $R^2= 0.71$. $p=0.005$).

HAD depression was positively associated with contraction vigor in terms of Peak Pressure in the distal esophagus ($\rho=0.39$ $p=0.012$). HAD anxiety was negatively associated with intrabolus pressurization, in terms of intrabolus pressure slope ($\rho=-0.37$. $p=0.018$)

DISCUSSION

As expected, healthy individuals perceive the passage of water swallow very infrequently. In this series, only 3.5% of swallows was perceived. This requires large samples to evaluate perception in healthy individuals. On the other hand, this suggests a potential value of documenting the presence of symptomatic swallows during a patient study. Our data suggest that $\leq 5\%$ of healthy individuals perceive more than 30% of swallows during HRM.

Only 9.6% of volunteers from 33.3% of centers perceived liquid swallows, suggesting that the prevalence was skewed to some individuals and locations. Even though this could be explained by cultural differences, regression analysis suggested that anxiety is the main independent explanation, rather than location itself. We found no evidence that depression is a significant independent predictor of heightened bolus passage perception. **This significant concentration of perception among certain individuals is a weakness of this study. A study of symptomatic patients rather than healthy volunteers is likely to overcome this limitation.**

Our findings support the idea that the mechanisms explaining perception are different according to manometric pattern (i.e. one mechanism could be relevant in some patterns but not in others). This implies that to have enough statistical power, there must be a sufficient number of individuals in each

category. Our study only includes normal individuals, so we only had a significant number of normal and hypotensive studies. We found that anxiety was a strong perception predictor among hypotensive studies, but was only weakly correlated with perception among normal studies.

Anxiety has been associated with symptom burden in patients. Kessing et al showed correlation between anxiety and retrosternal pain or heartburn among GERD patients ²⁸. Our data show that the effect over perception does not seem to be secondary to the effects over motility addressed with traditional HRM metrics. Using AIM analysis, we found a positive correlation between depression and contraction vigor and a negative correlation between anxiety and intrabolus pressure dynamics. This correlation was unexpected and weak. The effects we report need to be specifically address by future studies. It seems that the main effect of anxiety is on perception. It has been shown that anxiety could have peripheral (i.e decrease in receptor trigger threshold)²⁹ or central (i.e. increase in vigilance and/or modification in central stimulus processing)²³ effects on perception. Our methodology does not allow us to discriminate the level of the effect of anxiety. We confirmed the modulating effect of increased anxiety, but not depression, on heightened esophageal perception. Finally, due to the small dataset of perceived swallows, anxiety was only related to the dichotomous presence of perception (Yes or No), but not to its degree among symptomatic swallows. With this information, we can hypothesize that the main effect of anxiety is increasing the probability of referring perception, probably due to an effect over vigilance. Nevertheless, the exact mechanism of this association needs to be specifically addressed.

Among individuals with a hypotensive pattern, we found no correlation between any traditional HRM metrics (isobaric defect size, number or DCI) and perception, even at extreme values. This is in concordance with previous reports ⁷⁻⁹. Lazarescu et al failed to demonstrate any correlation between perception and sildenafil-induced hypomotility in healthy volunteers ⁸. In the current study we also could not demonstrate a relation between incomplete bolus liquid transit and perception. This is

consistent with what has been previously described^{8,10,11}. To date, there is no evidence to support that weak peristalsis, nor any of its consequences (peristaltic breaks and incomplete bolus transit), can explain bolus perception. In our series, anxiety independently explained 40-70% variance in perception scores of this subgroup. Only hypotensive swallows that occurred in anxious individuals were perceived. We hypothesize that in the context of a dysfunctional but frequent phenomena as hypotensive motility, an increase in sensitivity (by anxiety, for example) is necessary for perception to occur. Anxiety has been described as a frequent modulator of symptoms among functional gastrointestinal disorders³⁰. Thus, our findings support the decision of the Rome IV esophageal committee to include dysphagia in the context of hypotensive motility into the functional dysphagia category³¹.

Even though we have a large sample of normal studies, we could not find any significant correlation between traditional HRM metrics, bolus transit or AIM variables with perception. Among this subgroup, the role of anxiety seems to be minor (it explains only 2% variance). The determination of a model to explain perception in this setting will require evaluating other mechanisms and/or metrics. Nevertheless, the explanation for perception in asymptomatic individuals with normal HRM studies is not expected to be clinically relevant.

Traditionally, it has been postulated that esophageal perception depends upon esophageal wall tension and/or wall stretch. According to Laplace's Law, wall tension can increase when the esophagus becomes dilated (as when there is residual content due to incomplete bolus transit) and/or when there is a significant muscle contraction (as in hypercontractile/spastic disorders).

We found no perception in the 15/995 (1.5%) of swallows with a DCI > 5000 mmHg cm⁻¹sec⁻¹, including 2 swallows with DCI > 8000 mmHg cm⁻¹sec⁻¹. The lack of correlation could be due to a small number of vigorous peristalsis in our series. Nevertheless, the completely unperceived occurrence of such extreme contraction challenges its role as symptoms triggers. This is in line with other author's opinions^{32,33}. Vigorous peristalsis is only occasionally present in patients with dysphagia or chest pain (never more

than 5% even using HRM)^{34,35}. There is evidence that the correction of this manometric pattern does not necessarily correlate with symptom improvement³⁶ and viceversa³⁷. Finally, several studies that used pharmacological esophageal relaxation have suggested that it is wall stretch (i.e. lumen deformation) rather than wall contraction (i.e. isometric tension) that is the main factor inducing perception³⁸⁻⁴⁰.

We found no correlation between perception and any of the evaluated metrics among the 75/995 (7.5%) of swallows with an IRP> 15 mmHg (obstructive studies). Our initial hypothesis was that variables related to the bolus pressurization dynamics (e.g., the rate of bolus pressurization over time) would correlate with perception. Nevertheless, we found no pressure related AIM variable to be associated with perception. It has been reported that intrabolus pressure correlates with dysphagia among individuals with gastric bands⁴¹ and post fundoplication⁴². Although it has not been specifically addressed, it is the clinical experience that the decrease in swallow-induced intraesophageal pressure correlates with clinical improvement after EGJ decompression in achalasia. Montazeri et al showed that LES pressure and volume retained in time-barium studies (both could be considered surrogate for intrabolus pressure) correlate with clinical improvement after treatment in achalasia⁴³. The lack of correlation found in this study could be due to a small sample or the probability that these findings in healthy individuals could be an artifact. It has been reported that many individuals with EGJOO have a good clinical prognosis⁴⁴, so the use of additional test to confirm it is not an artifact has been suggested⁴⁵. The evaluation of the correlation between obstruction markers and perception in patients with “true” obstruction needs to be done.

We could not demonstrate any correlation between perception and esophageal shortening measured using HRM. Nevertheless, our methodology could have been suboptimal for the evaluation of longitudinal muscle function. Studies using intraluminal ultrasound should be used to specifically address its role.

Our study has the limitation that it includes only asymptomatic controls, leading to only 3.5% swallows being perceived. Nevertheless, as the sample was large, we found sufficient abnormal swallows (32.4%; most of them hypotensive) for meaningful swallow by swallow analysis. We recognise that this type of analysis is less robust when compared to subject averaged data. Subjects were studied using small liquid swallows in a supine position, which is the recommended standard diagnostic protocol. Whether the evaluated variables correlate with perception when using other protocols (such as solid or repetitive swallows) needs to be specifically addressed. To our knowledge this is the first study to describe a model to explain perception, at least in a subgroup of individuals (hypotensive motility). Finally, our findings support the notion that wall tension does not drive symptom generation in the context evaluated here. Future research should focus on other mechanisms, such as anxiety.

In summary, we could not find any correlation between any traditional HRM nor AIM variable and perception in a large sample of healthy individuals, despite a significant number of abnormal swallows. Anxiety seems to be a significant predictor of perception, especially among hypotensive swallows.

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DISCLOSURES

Competing Interests: the authors have no competing interests

SPECIFIC AUTHOR CONTRIBUTIONS:

Daniel Cisternas contributed to conception and study design, recruitment, data acquisition, analysis and interpretation, manuscript drafting, editing, critical revision and final approval. Charlotte Scheerens and Hugo Monrroy contributed to data analysis, critical revision and final approval. Taher Omari contributed to the data analysis and interpretation, manuscript drafting, editing, critical revision and final approval. Claudio Bilder, Andres Ditranto, Jose Maria Remes-Troche, Arturo Meixueiro, Miguel Angel Zavala, Ingrid Marin, Antonio Ruiz de Leon, Julio Perez de la Serna, Albis Hani, Ana Maria Leguizamo, Luiz Abrahao, Ramiro Coello and Miguel Angel Valdovinos contributed to recruitment, data acquisition, manuscript critical revision and final approval. Jordi Serra contributed to the study conception, recruitment, data acquisition and interpretation, manuscript editing, critical revision and final approval.

REFERENCES

ABBREVIATIONS

HRM, high-resolution manometry; AIM, automated impedance manometry; HAD, hospitalized anxiety and depression scale; HRIM, high-resolution impedance manometry; EGJ, esophagogastric junction; CC, Chicago classification; DCI, distal contractile integral; IEM, ineffective esophageal motility; IRP, integrated relaxation pressure; DL, Distal Latency; CFV, Contractile Front Velocity;

Table 1- Variables evaluated using Automated Impedance Manometry (AIM)

	Variable (unit)	Description
Bolus Characterization	<i>Nadir Impedance (NI) (Ohms)</i>	Minimum impedance, located at the center of the bolus
Contraction amplitude	<i>Peak pressure (PP) (mmHg)</i>	Pressure recorded at maximum contractile tension-maximum contact with the probe
Pressurization kinetics	<i>Pressure at Nadir Impedance (PNadImp) (mmHg)</i>	Intrabolus pressure recorded when the esophageal lumen is maximally full of bolus.
	<i>Intrabolus pressure (IBP) (mmHg)</i>	Median pressure recorded during the phase of transition from a full lumen to an occluded lumen
	<i>IBP slope (mmHg)</i>	Rate of change in IBP recorded during the phase of transition from a full lumen to an occluded lumen.
	<i>Time Nadir Impedance-Peak Pressure (TNI-PP) (sec)</i>	Time interval of transition from a maximally full lumen to maximal contractile tension.
	<i>Pressure Flow Index (PF Index)=(IBP×IBPslope)/TNI-PP (mmHg²/sec)</i>	Reflects esophageal emptying difficulties
Impedance at maximal contact with probe	<i>Impedance at Peak Pressure (IPP) (Ohms)</i>	Impedance at the moment of maximal contact with probe
	<i>Nadir Impedance/Impedance at Peak Pressure ratio (NI/IPP ratio)</i>	Marker of incomplete bolus transit.

Table 2. Correlations between perception score and HRM variables

Metric	Correlation (Swallow by swallow)	Correlation (Person by person)	Correlation (Percieved)
DCI	-0.017. p=0.593	0.076. p=0.444	0.165. p=0.344
Peristaltic defect lenght	-0.01. p=0.758	-0.037. p=0.707	-0.039. p=0.824
IRP	0.046. p=0.151	0.104. p=0.290	-0.244. p=0.196
DL	-0.019. p=0.576	0.024. p=0.812	-0.208. p=0.278
CFV	-0.014. p=0.688	-0.046. p=0.647	0.014. p=0.941

Table 3. Perception scores according to different HREM variables thresholds

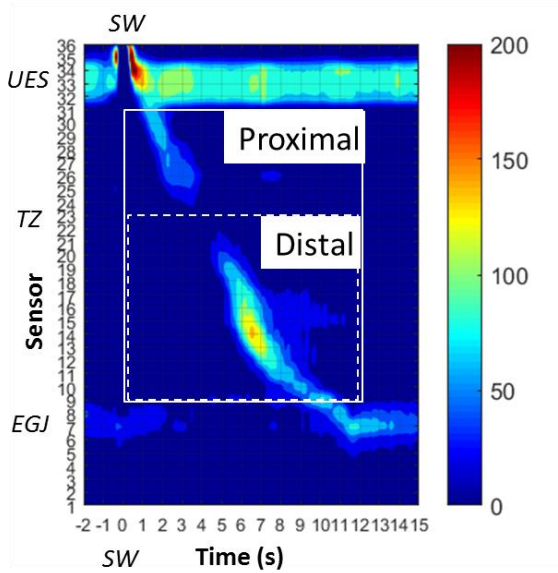
	Perception score	p Value
IRP p95 (>vs<15 mmHg)	0.08 ±0.27 vs 0.05 ±0.27	0.63
IRP p99 (>vs<19.6 mmHg)	0.01 ±0.10 vs 0.05 ±0.27	0.52
DCI (>vs<5000 mmHg/cm/s)	0.02± 0.12 vs 0.05 ± 0.27	0.45
DCI (<vs>450 mmHg/cm/s)	0.05 ±0.27vs 0.20 ±0.40	0.38
DCI (<vs>100 mmHg/cm/s)	0.10 ±0.30 vs 0.05± 0.27	0.86
CFV p95(>vs<9 cm/s)	0.03± 0.16 vs 0.05 ±0.27	0.81
CFV p99(>vs<12 cm/s)	0.01 ±0.10 vs 0.05± 0.27	0.54
DL p5 (< vs > 4.5 sec)	0,01 ±0,07 vs 0,05 ± 0,27	0,71
DL p1 (< vs > 1,9 sec)	0,00 ±0,03 vs 0,0 5± 0,27	0,85

Table 4. Percentage of perceived swallows among different manometric patterns

	Normal 66/107 (61.7%)	Hypotensive 28/107 (26.2%)	Obstructive 8/107 (7.5%)	Vigorous 5/107 (4.7%)
Perceived	13.6 %	3.6 %	0 %	20 %
Non perceived	86.4 (%)	96.4 (%)	100 (%)	80 (%)
Mean perception score	0.06 ± 0.24	0.05 ± 0.25	0.0 ± 0.0	0.03 ± 0.06

Figure 1. Automated Impedance Manometry (AIM) analysis

A. Pressure Topography Plot



B. Pressure-Impedance

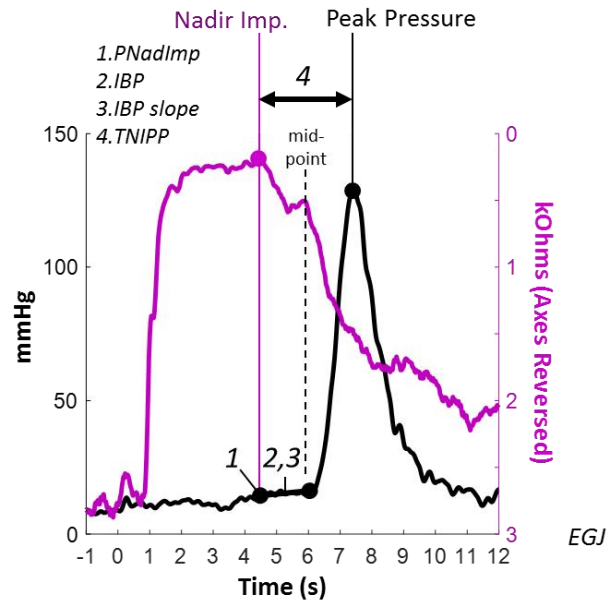


Figure 2. AIM variables along distal esophageal segment

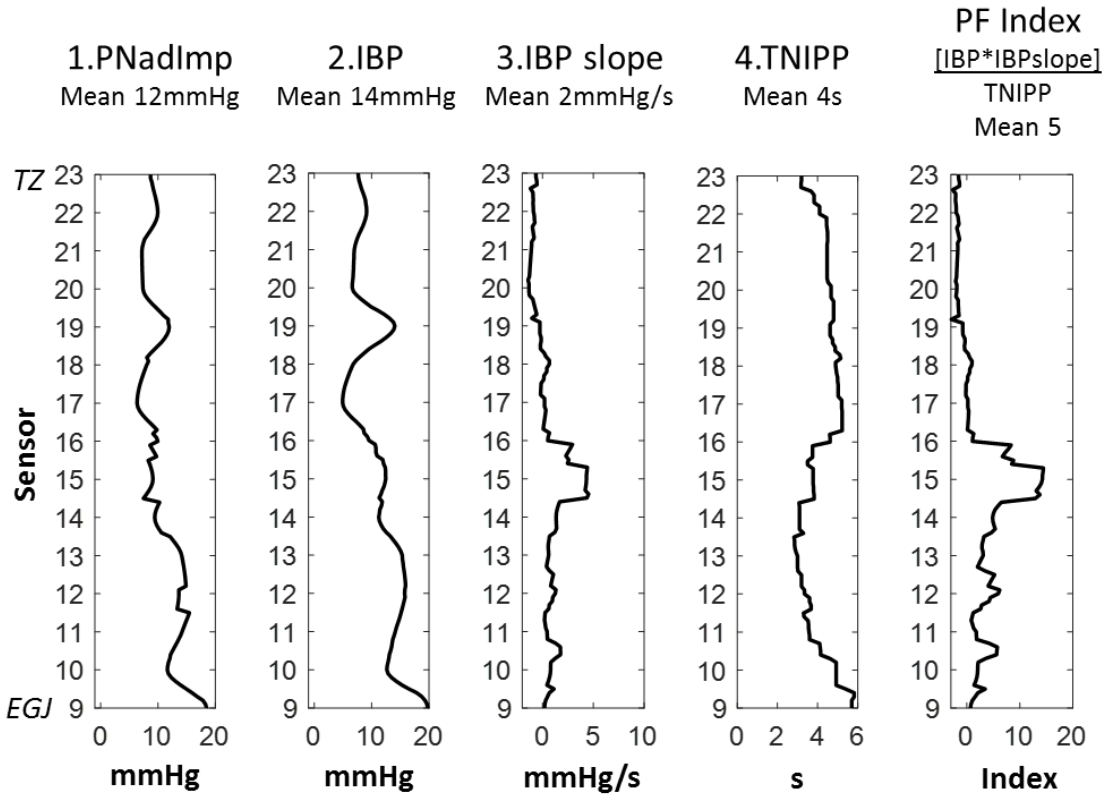


Figure 3. Diagram showing pattern classification in the Swallow by Swallow Analysis

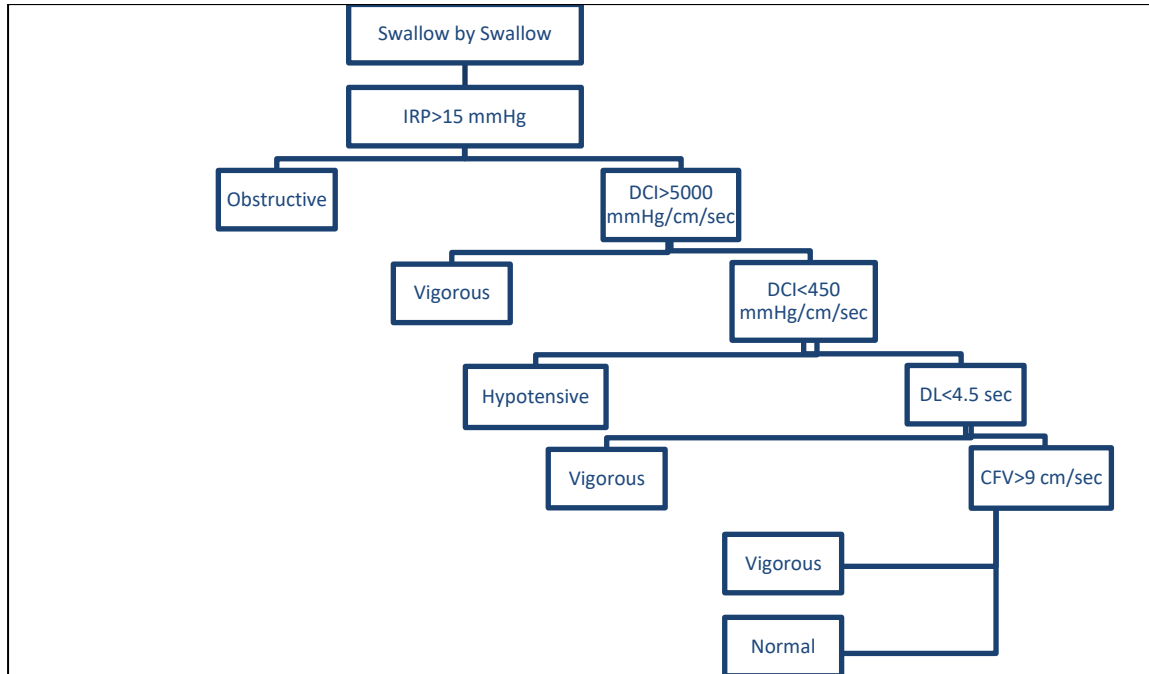


Figure 4. HAD anxiety significantly predicts perception among hypotensive swallows. Logistic regression

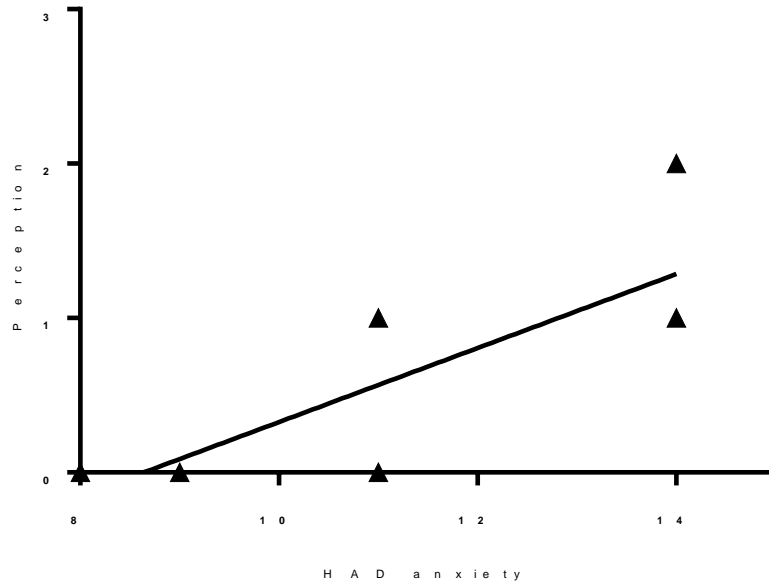


FIGURE AN TABLE LEGENDS

Table 1: Variables evaluated using Automated Impedance Manometry (AIM). Some variables are illustrated in Figure 1B

Figure 1. A. Pressure topography plot of a 5ml liquid swallow. Analysis was done in the whole esophagus (upper esophageal sphincter (UES) to esophagogastric junction (EGJ) and in proximal (UES to transition zone (TZ) and distal esophagus (TZ to EGJ). **B.** Pressure-impedance plot derived in the distal esophagus (at sensor position number 13). Pressure (black line) and impedance (purple line) are shown for a 12s period from swallow onset (0s). Impedance values have been reversed (lowest impedance at the top). Four key pressure-flow variables are displayed: *Pressure at nadir impedance* (1.PNadImp) is the pressure at maximal luminal distension. *Intrabolus pressure* (2.IBP) and *intrabolus pressure slope* (3.IBP slope) are the median pressure and gradient of pressure change respectively during luminal closure (defined by the period from nadir impedance to the midpoint between nadir impedance and peak pressure). *Time from nadir impedance to peak pressure* (4.TNI-PP) corresponds to the latency period from maximum distension to maximum contraction.

Figure 2: Example plots of the four key pressure variables and the *pressure-flow index (PF Index)* composite score at all axial locations along distal esophagus in an individual swallow. For analysis, average values were calculated from individual sensors along the respective segment. Note the marked increase in bolus pressurization and shortening of distention-contraction latency below sensor position number 16. This corresponds to an increase in bolus flow resistance associated with the transition from compartmentalized bolus transport along the esophagus to esophageal emptying across a (variably

resisting) EGJ opening. PNadImp=Pressure at nadir impedance. IBP=Intrabolus pressure. IBP slope=Intrabolus pressure slope. TNIPP=Time from nadir impedance to peak pressure.

Figure 3: Algorithm used for classification of each swallow. IRP=Integrated Relaxation Pressure.

DCI=Distal Contractile Integral. DL=Distal Latency. CFV=Contractile Front Velocity.

Table 2: Correlation coefficients (Spearman's Rho) between perception score and HRM variables.

Swallow by Swallow: Analysing all swallows. Person by person: Using mean values for perception and HRM variables during the whole set of swallows. Percieved: Analysing only swallows with a perception score ≥ 1 .

Table 3: Comparison of perception scores using different thresholds for HRM variables. Swallow by swallow analysis. p=percentile. IRP=Integrated Relaxation Pressure(4sec). DCI=Distal Contractile Integral. CFV=Contractile FrontVelocity. DL=Distal Latency

Table 4: Percentage of percieved swallows (perception score ≥ 1) during HRM according to manometric pattern (person by person analysis). Classification criteria explained in methods section. There was no difference in any category when compared to normal pattern (p ns)

Figure 4: Logistic regression. Swallow by swallow analysis. Only hypotensive swallows from individuals with abnormal anxiety levels (≥ 8) are considered. $R^2=0.73$. $p<0.0001$

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