

Response to reviewers

We gratefully thank the editor and all reviewers for their time spent making their constructive remarks and useful suggestions, which are very helpful for revising and improving the manuscript. We have studied each suggested revision and comment carefully and have made corrections that we hope meet with the approval. The comments of the reviewers are responded point by point and the revisions are indicated in the manuscript (uploaded on supplementary material).

Reviewer 1

General Comments: Li et al. present a retrospective series of patients that underwent high-resolution manometry with the diagnosis of esophagogastric junction outflow obstruction (EGJOO) either by Chicago classification 3.0 or 4.0 and compared to controls with a normal HRM. The topic is interesting and the addition of UES parameters is a good addition; but there are some issues with methodology and data interpretation.

1. **Comment:** Individuals with normal HRM cannot be used as controls. They are not healthy volunteers as far as I understood and they underwent HRM for clinical reasons. Moreover, Ph-monitoring was not showed.

Reply: Thank you for your valuable comment. The control group was obtained from patients who underwent HRM for mild symptoms such as dysphagia, retrosternal pain, regurgitation, or heartburn, with normal HRM results. The symptom composition of the control group was listed in table 1 (page 23). Heartburn was not listed in table 1 because the symptom was not involved in patients with EGJOO. We have neglected to mention that these patients also fulfilled normal results in pH-monitoring and upper gastrointestinal endoscopy, in order to exclude the possibility of organic diseases. Thus, similar to the referenced study ^[1], we think it is reasonable to use the group for comparison. Thank you for kindly reminding, it is not appropriate to name the group as controls and might be more precisely to rename the group as the normal HRM group, as we have modified this expression throughout the manuscript (e.g. line 210). We have added the description about the normal HRM group in the revised manuscript (line 137-141), and shown below as well.

The normal HRM group was obtained from patients who underwent HRM for mild symptoms such as dysphagia, retrosternal pain, regurgitation, or heartburn, with normal HRM results. The patients also fulfilled normal results in pH-monitoring and upper gastrointestinal endoscopy, in order to exclude the possibility of organic diseases.

2. **Comment:** It is unclear if patients with EGJOO based on Chicago 4.0 had also supportive tests and symptoms or only a manometric feature.

Reply: Thank you for your valuable comment. Due to the limitation of our retrospective studies, we are sorry for the lack of complementary tests such as timed barium esophagram or functional lumen imaging probe. In this

study, all patients have excluded motility disorders secondary to hiatal hernia, infiltrative disease, mechanical obstruction, and extrinsic compression by upper gastrointestinal endoscopy or endoscopic ultrasonography. Therefore, the patients in the EGJOO group (CCv4.0) fulfilled the manometric diagnosis of EGJOO based on Chicago 4.0, and we have added the above content in the revised manuscript (line 131-137). As for symptoms, among the 24 patients with manometric diagnosis of EGJOO (CCv4.0), there were 7 with dysphagia and 5 with retrosternal pain that might be clinically relevant, as shown in Table 1 (page 23), and we also add the description in text (line 211-213).

3. **Comment:** All comparisons should be done using groups Chicago 3 and only otherwise it is very confusing to follow the manuscript.

Reply: We appreciate your insightful comment. Indeed, it would be more readable to compare EGJOO (CCv4.0) group with EGJOO (CCv3.0) group. The EGJOO (CCv3.0) group was composed of patients with EGJOO (CCv4.0) and patients with isolated supine IRP elevation that failed to meet the version 4.0 criteria. We consulted with statisticians about grouping and analysis, statistically, it might be more effective to compare among EGJOO (CCv4.0) group, the isolated supine IRP elevated group, and the normal HRM group, because there was no overlap between the groups. In order to describe the grouping and study process more clearly and concisely, we added a flow chart in the revised manuscript (page 22) and shown below as well, hopefully making the manuscript more readable.

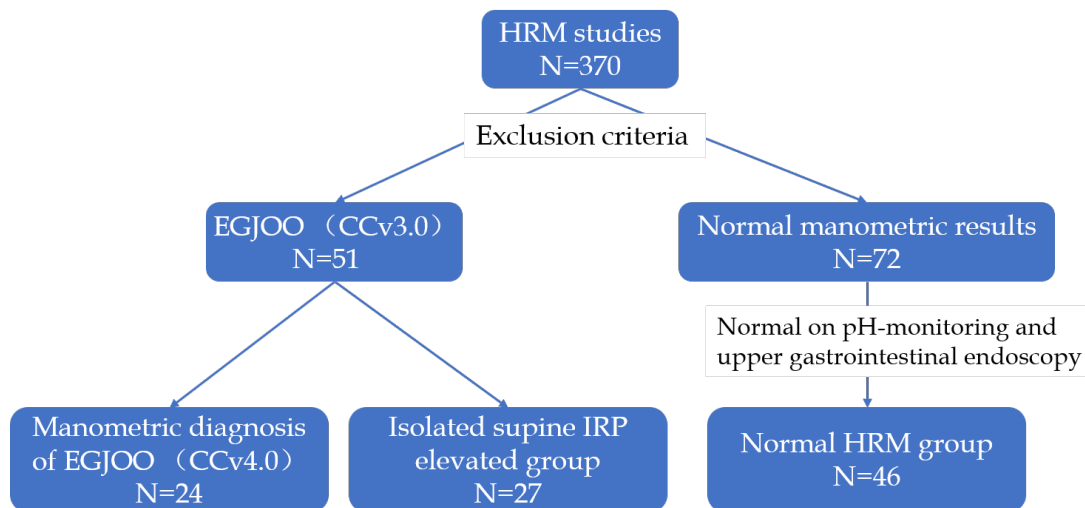


Figure 2. Patient flow. HRM, high-resolution manometry; EGJOO, esophagogastric junction outflow obstruction; CCv3.0, Chicago Classification version 3.0; CCv4.0, Chicago Classification version 4.0; IRP, integrated relaxation pressure.

4. **Comment:** The analysis of symptoms including the whole population is not contributory.

Reply: Thank you for your insightful comment, following your suggestion, we eliminated patients with normal HRM and conducted symptom analysis for the 51 patients with elevated IRP, the results showed PD-UESCI, PCI and PEL were significantly lower in patients with dysphagia than patients without dysphagia, indicating that dysphagia may represent a potential dysfunction of the UES and proximal esophagus in patients with impaired EGJ relaxation. PEL was higher in patients with retrosternal pain, compared with patients without the symptom. We also conducted symptom analysis for the 24 patients with manometric diagnosis of EGJOO (CCv4.0), the results were similar but less significant than the 51-patients analysis, probably due to the limited sample size. Therefore, we revised the text according to the symptom analysis for the 51 patients (line 72-74; line 240-245; line 291-295), and changed the figure in the revised manuscript (attached below).

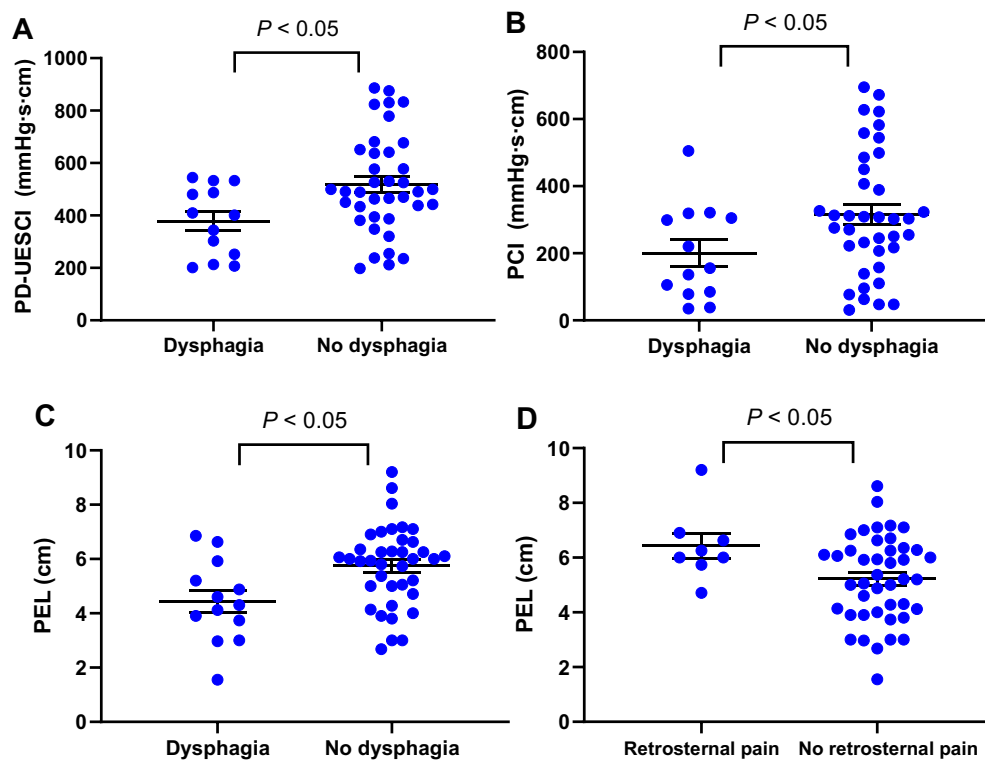


Figure 3 Comparisons of parameters according to symptoms in 51 patients with elevated supine IRP.

- Comment:** The presented ROC curve also is meaningless. How can you diagnosis a manometric disease based on other parameters apart from the ones that define the disease.

Reply: Thank you for your kindly reminding. Indeed, the diagnosis of EGJOO does not depend on the parameters mentioned in ROC analysis.

Based on our initial findings, besides the features listed in the diagnostic criteria, the patients with EGJOO (CCv4.0) revealed multiple abnormalities in the esophagogastric junction and may share common changes in proximal esophagus and UES. Inspired by a previous study that reported the optimal diagnostic threshold of IRP on RDC for diagnosis of achalasia and EGJ dysfunction using ROC analysis [2], we conducted ROC analysis in order to find out characteristic parameters that have high sensitivity and specificity for confirming the diagnosis of EGJOO (CCv4.0). The parameters mentioned in the ROC analysis may provide support in rare cases, for example when patients fail to complete swallows in the upright position, or in borderline cases. Hopefully, the findings may prompt further studies to reveal the underlying pathophysiological mechanism. Thank you for your suggestion, we modified the description in the revised manuscript that the parameters are supportive for confirming EGJOO, rather than identification (e.g. line 77; line 303-305; line374-376).

6. **Comment:** "p value" NOT "P value".

Reply: Thank you so much for your careful check, and the mistake has been corrected in the revised manuscript (e.g. line 68; line 551). We feel sorry for our carelessness.

7. **Comment:** numbers should not be repeated in the text if they are included in the table. This makes the manuscript long and confusing.

Reply: Thank you for your suggestion, we have removed the detailed number throughout the text, and hopefully to make the manuscript more concise.

8. **Comment:** Please clarify what "pharyngeal paresthesia" means.

Reply: Thank you for your comment, we used "pharyngeal paresthesia" referring to inappropriate sensations such as burning, prickling, and tingling at the pharyngeal. With the changes in the analysis of symptoms, there was no significant finding related to pharyngeal paresthesia. Therefore, we eliminated the content to make the article more focused.

Reviewer 2

General Comments: This is an interesting paper which assess the utility of various parameters during the HRM study in an attempt to better define EGJOO. Several paraments have been noted that seem to improve the sensitivity and specificity of identifying this cohort which potentially can be used in clinical practice; however the relevance of these is not clear considering the lack of therapeutic outcome responses.

1. **Comment:** What was the protocol for each position? How many swallows were undertaken when supine and upright?

Reply: Thank you for your valuable comment. The manometric protocol consisted of a landmark phase captured during a quiet rest in the supine position at the beginning, followed by ten 5-mL ambient temperature water swallows in the supine position, then five 5-mL water swallows in the upright position, with 30 s between each swallow, and finally an RDC of 200 mL water in the upright position. We have modified the manuscript in detail accordingly (line 53-55; line 154-158).

2. **Comment:** What is the difference between the designation of EGJOO and isolated supine EGJOO?

Reply: Thank you for your insightful comment. The 24 patients in EGJOO group fulfilled the manometric definitions of EGJOO based on Chicago Classification version 4.0, with an elevated median IRP in supine and upright positions as well as at least 20% of supine swallows with elevated intrabolus pressurization, and evidence of peristalsis (not meeting criteria for achalasia). The 27 patients in the isolated supine IRP elevated group were failed to meet the version 4.0 criteria, with either normal median IRP in upright position or less than 20% of supine swallows with elevated intrabolus pressurization. We have added the description in the revised manuscript (line 60-61; line 205-208). Patients in both groups were ruled out secondary factors such as the effect of hiatal hernia, infiltrative disease, mechanical obstruction, or opioid effect.

3. **Comment:** RDC was measured during the first 30 seconds. But free drinking commonly takes much less than 30 seconds unless the swallowing was interrupted. Was 30 seconds used as a standard for all regardless of the time it took to drink or do the authors mean that if the free drinking was extended they would cut off the measurement at 30 seconds?

Reply: Thank you for your kindly reminding, the IRP on RDC was assessed in the window beginning with deglutitive UES relaxation to the end of EGJ relaxation for free drinking lasting less than 30 seconds, or during the first 30 seconds of the window for free drinking lasting longer. We are sorry for our carelessness and have modified the content in the revised manuscript (line 186-189).

4. **Comment:** What are the CC4 criteria that were used to meeting manometric definitions of EGJOO? It is not clear what the 27 patients who did not meet the criteria were and how they were included as part of the 51 in the first instance.

Reply: Thank you for your insightful comment. Initially, fifty-one patients fulfilling the criteria of EGJOO (CCv3.0) were identified, with elevated supine IRP and evidence of peristalsis. The manometric criteria for 24 patients in EGJOO (CCv4.0) group include elevated median IRP in supine and upright positions, at least 20% of supine swallows with elevated

intrabolus pressurization, and evidence of peristalsis (not meeting criteria for achalasia). The 27 patients in isolated supine IRP elevated group were failed to meet the version 4.0 criteria, with either normal median IRP in upright position or less than 20% of supine swallows with elevated intrabolus pressurization (revised in line 202-209). Following your suggestion, in order to describe the grouping and study process more clearly, we added a flow chart in the revised manuscript and shown below as well, hopefully making the manuscript more readable.

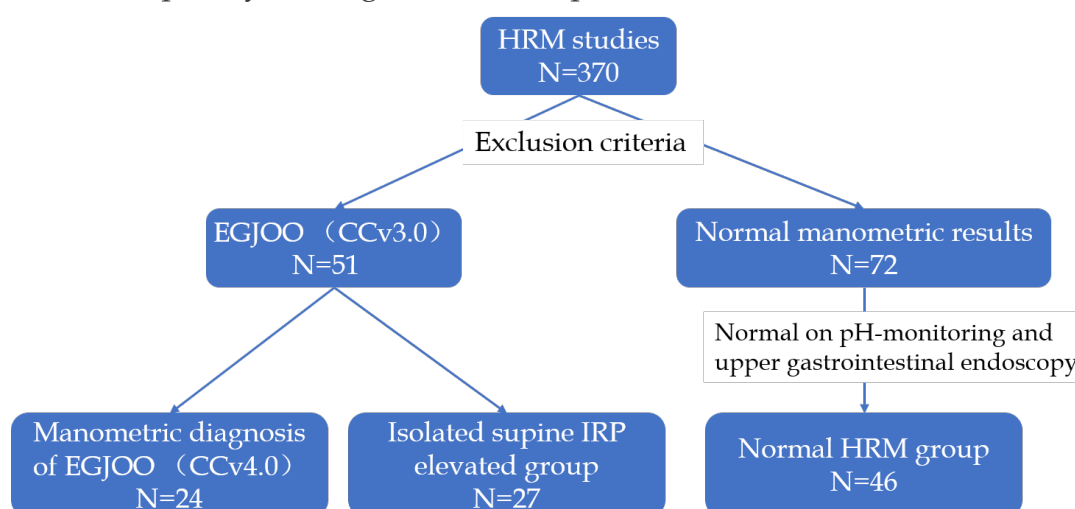


Figure 2. Patient flow. HRM, high-resolution manometry; EGJOO, esophagogastric junction outflow obstruction; CCv3.0, Chicago Classification version 3.0; CCv4.0, Chicago Classification version 4.0; IRP, integrated relaxation pressure.

5. **Comment:** Were these cases all incidental or did patients present with symptoms? What were the symptoms, who had them?

Reply: Among the 24 patients with manometric diagnosis of EGJOO (CCv4.0), there were seven with dysphagia, five with retrosternal pain, and eight with regurgitation, the others were lack of typical symptoms. Symptoms were also counted in isolated supine IRP elevated group and normal HRM group as shown in Table 1 (page 23). Thank you for kindly reminding, we add the description in revised text (line 211-215). Common features of HRM were discovered in patients with the above symptoms (described in the reply to comment 10), while no demographic feature was observed.

6. **Comment:** Were there patients who had median raised IRP when upright but normal when supine? The CC4 does not exclude such patients from being considered EGJOO.

Reply: Thank you for your valuable comment. At the beginning of our study, we hold a similar question and searched for patients who had at least 20% of supine swallows with elevated intrabolus pressurization together

with median raised IRP in the upright position. No eligible case was found among the 370 patients. Therefore, in order to make grouping criteria more concise, we didn't mention the above conditions.

7. **Comment:** With regards to the controls, it needs to be described what symptoms they attended with/why did they have the tests? Did they have dysphagia and were found to have normal HRM? Or did they attend for a completely different reason?

Reply: Thank you for your valuable suggestion. The normal HRM group (control) was obtained from patients who underwent HRM for mild symptoms such as dysphagia, retrosternal pain, regurgitation, or heartburn, with normal HRM results. The patients also fulfilled normal results in pH-monitoring and upper gastrointestinal endoscopy, in order to exclude the possibility of organic diseases. We have added the description in the revised manuscript (line 137-141), and the symptom composition was listed in table 1 (page 23). Heartburn was not listed in table 1 because the symptom was not involved in patients with EGJOO and cannot be used for comparison between groups.

8. **Comment:** It states that among the 24 EGJOO patients there were 5 with spastic features. By definition therefore these are not EGJOO, rather they are Type III achalasia.

Reply: Thank you for your valuable comment. According to CCv4.0 [3], EGJOO with spastic features should fulfill features of type III achalasia (presence of $\geq 20\%$ premature swallows), the five patients with spastic features presented $\geq 20\%$ premature swallows together with evidence of peristalsis that does not match the diagnosis of Type III achalasia. Therefore, these patients might have potential relation with type III achalasia but do not meet the diagnostic criteria of achalasia.

9. **Comment:** In EGJOO there needs to be an attempt at determining if the obstruction is mechanical, mucosal or muscular. There is no data regarding endoscopy findings, exclusion of conditions such as EoE, mechanical stricture/Schatzki rings, narrow caliber esophagus, extrinsic compression nor the use of opiates – all of this data is required before patients are considered to have functional EGJOO and their data used during measurements. CC4 excludes those from being attributed to functional EGJOO from those with mucosal and mechanical pathology.

Reply: Thank you for your kindly reminding. We have neglected to mention that we have performed endoscopy on patients with EGJOO routinely to exclude secondary factors, as recommended by CCv3.0. Therefore, patients in the EGJOO (CCv4.0) group and isolated supine IRP elevated group have excluded the use of opiates, and secondary factors identified by upper gastrointestinal endoscopy or endoscopic

ultrasonography, especially for hiatal hernia, infiltrative disease, mechanical obstruction, and extrinsic compression. More description of the exclusion criteria has been added in the revised manuscript and shown below as well (line 131-137).

Exclusion criteria included (a) patients under 18 years of age, (b) a history of upper gastrointestinal or mediastinal surgery, (c) previous endoscopic treatment for esophageal motor disorders, (d) diseases with abnormal intraabdominal pressure, such as intestinal obstruction or ascites, (e) use of opiates, and (f) secondary factors identified by upper gastrointestinal endoscopy or endoscopic ultrasonography, especially for hiatal hernia, infiltrative disease, mechanical obstruction, and extrinsic compression.

10. **Comment:** In the section on relationship between symptoms and parameters, the parameters measured were based on 97 patients, that means it includes the controls, why? Were any of the abnormalities of parameters and symptoms identified in the controls? If so, these are no longer controls.

Reply: Thank you for your insightful comment. The control group was obtained from patients who underwent HRM for mild symptoms such as dysphagia, retrosternal pain, regurgitation, or heartburn, with normal HRM results. The patients also fulfilled normal results in pH-monitoring and upper gastrointestinal endoscopy, in order to exclude the possibility of organic diseases. It might be more precisely to rename the controls as the normal HRM group, as we have modified this expression throughout the manuscript (e.g. line 214).

Following your suggestion, we eliminated patients with normal HRM and conducted symptom analysis for the 51 patients with elevated IRP, the results showed PD-UESCI, PCI and PEL were significantly lower in patients with dysphagia than patients without dysphagia. PEL was higher in patients with retrosternal pain, compared with patients without the symptom. We also conducted symptom analysis for the 24 patients with manometric diagnosis of EGJOO (CCv4.0), the results were similar but less significant than the 51-patients analysis, probably due to the limited sample size. Therefore, we revised the text according to the symptom analysis for the 51 patients (line 72-74; line 240-245; line 291-295), and changed the figure attached below.

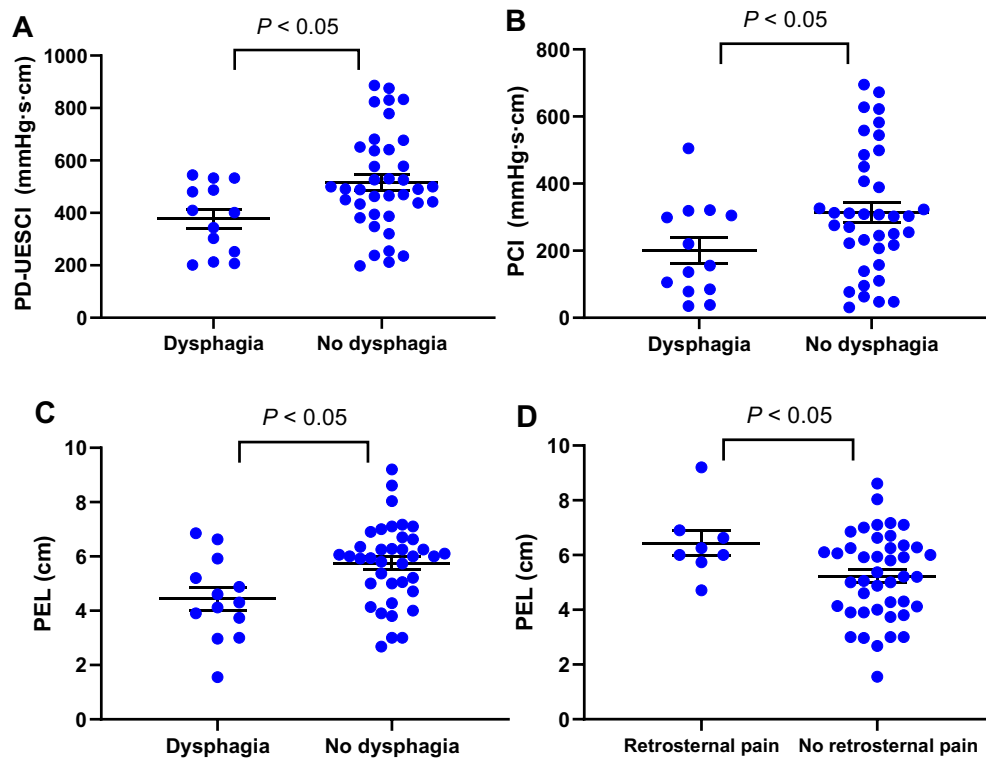


Figure 3 Comparisons of parameters according to symptoms in 51 patients with elevated supine IRP.

11. **Comment:** Both CC4 and the paper by Sanagapalli et al AJG 2021 define the utility of provocative testing with inclusion of solid swallows to better define clinically relevant dysmotility and EGJOO, but there is no mention in this paper anything about using provocative testing.

Reply: Thank you for your insightful comment. Due to the limitation of our study, we are sorry for the lack of provocative testing data such as solid swallows in previous HRM. As the diagnostic value of provocative testing increases, we have performed solid swallows recently. In the future, we will concentrate on clinically relevant dysmotility with the support of provocative tests. In the revised manuscript, we emphasized the value of provocative testing (line113-116) and discussed limitations in the study (line 332-336).

References

[1] Biasutto D, Roman S, Garros A, Mion F. Esophageal shortening after rapid drink test during esophageal high-resolution manometry: A relevant finding? United European Gastroenterol J. 2018;6(9):1323-1330. [PMID: 30386605 DOI: 10.1177/2050640618796752].

[2] Ang D, Hollenstein M, Misselwitz B, et al. Rapid Drink Challenge in high-resolution manometry: an adjunctive test for detection of esophageal motility disorders. *Neurogastroenterol Motil.* 2017;29(1). [PMID: 27420913 DOI: 10.1111/nmo]

[3] Yadlapati R, Kahrilas PJ, Fox MR, et al. Esophageal motility disorders on high-resolution manometry: Chicago classification version 4.0©. *Neurogastroenterol Motil.* 2021;33(1):e14058. [PMID: 33373111 DOI: 10.1111/nmo.14058]