Is the Quantification of Interstitial Cells of Cajal in Gastric Biopsy Samples in Patients With Gastroparesis Ready for Prime Time?

Gastroparesis and functional dyspepsia (FD) are 2 common sensorimotor disorders in the gastroduodenal region, and the distinction between both has been a matter of ongoing debate.[1] The overlap in symptoms between gastroparesis and FD, especially postprandial distress syndrome, is well established.[2] In addition, patients with gastroparesis frequently present with symptoms of avoidant or restrictive food intake disorder,[3] and patients with eating disorders may have objectively delayed gastric emptying.[4] The correlation between retardation of gastric emptying and symptoms is imperfect, either at baseline, during the gastric-emptying measurement, or in response to prokinetic therapy,[2,5–9] and significant intraindividual variation is present in the measured gastric emptying of solids, whether measured using scintigraphy or stable isotope measurements.[10–13] This variation was also documented in 41% of 249 patients with upper gastrointestinal symptoms studied in the National Institutes of Health–National Institute of Diabetes and Digestive and Kidney Diseases gastroparesis consortium follow-up cohort on 2 occasions 48 weeks apart.[14] Seventy-nine of 189 patients with delayed gastric emptying at baseline normalized (<10% retained at 4 hours) at 48 weeks, and 22 of 60 patients with normal emptying at baseline had delayed gastric emptying (>10% retained at 4 hours) at 48 weeks.[14]

In addition, the same cohort a follow-up study presented highly innovative quantification of interstitial cells of Cajal (ICCs) in full-thickness biopsy specimens obtained in the gastric body from 9 patients with idiopathic gastroparesis, 9 nondiabetic patients with FD (similar symptoms but normal gastric-emptying rate) who were undergoing implantation of a gastric electrical stimulator, and 9 control subjects without diabetes or gastroparesis symptoms undergoing obesity surgery.[15] The 3 subgroups each had 8 women and 1 man. Tissue collection followed standardized established protocols for the acquisition and subsequent processing and analysis by a single histology core. Based on that study, it was concluded that the numbers of ICCs per high-power field in the circular muscle layer were no different between FD and gastroparesis and hence that the pathologic basis for these 2 conditions was similar. This observation was used to support the notion proposed by the authors that FD and idiopathic gastroparesis in tertiary care are interchangeable syndromes with common clinical and pathologic features.

We conducted further analysis of the data included in that study[14] and appraised the literature regarding quantitation of ICCs using diverse methodologies and in several different species. This analysis was conducted to address the question of whether quantification of the numbers of ICCs in human gastric biopsy specimens provides conclusive insights regarding the pathogenesis, categorization of diseases presenting with common upper gastrointestinal symptoms, and potentially inform the optimal approach to treatment. Among the 9 patients in each of the gastroparesis and FD groups, there were also differences, such as 2-fold numbers of overweight or obese patients and an average 20% higher body mass indices in the FD compared with the gastroparesis group, and twice as many gastroparesis patients were using narcotics (89% vs 44%) among the gastroparesis compared with FD patients.[14] However, it is unknown whether such potential confounders would impact the histologic findings in the study.

In the cited study,[14] the median ICCs in the circular muscle layer were 5 (interquartile range [IQR], 5–7.75) in obese control subjects, 3.3 (IQR, 3.0–4.1) in the FD group, and 1.5 (IQR, 1.0–4.1) in the idiopathic gastroparesis group. Statistical analysis based on an analysis of variance on ranks showed significant differences ($P < .001$) among the groups, and subsequent pairwise comparisons using the Tukey test showed that each disease group was significantly different from control subjects but not significantly different between the FD and gastroparesis groups, confirming the report in the article.[14] However, the latter comparison was associated with insufficient power (0.184) of the performed test with $\alpha = 0.050$. A similar analysis was performed regarding the numbers of CD206 macrophages in the myenteric plexus, which showed 6.2 (IQR, 5.0–7.75) in control subjects, 4 (IQR, 3.65–4.5) in the FD group, and 4 (IQR, 2.6–5.5) in the gastroparesis group. Similar results were obtained on statistical analysis, but, again, the comparison between FD and gastroparesis was associated with insufficient power (0.05) of the performed test, with $\alpha = 0.050$. Therefore, confirmation of the histopathologic findings in a larger cohort of patients is needed to support the conclusion that there are common pathologic features in gastroparesis and FD.

This report also raises the question of what is known about the density and distribution of ICCs in the stomach. This may be relevant because the precise location of the biopsy samples taken may not be identical, particularly because the different regions of the stomach are quite extensive relative to the size of a high-power field. Thus, for example, is a biopsy sample taken from the greater curvature section of the gastric body identical to the anterior or posterior wall or the lesser curvature of the same region of the stomach? It is impossible to ascertain whether there is potential for such sampling differences in some studies; therefore, we need to address evidence from the literature regarding the density and distribution of ICCs based on animal and human stomachs.

Earlier studies emanating from research conducted at the University of Nevada provided important insights on differences between the corpus, fundus, and antrum of the murine stomach. Thus, Song et al[15] showed that in the corpus intramuscular ICCs (ICC-IM) were found along the greater curvature near the fundus. ICC-IM decreased in density in the circumferential axis toward the lesser curvature and in the longitudinal axis toward the antrum, as was also demonstrated by an earlier study.[16] ICC-IM were absent from the longitudinal layer of the antrum but were found in the circular layer of each region.[17] There were also functional differences, because cholinergic and nitrergic motor neurons formed close contacts with ICC-IM in the corpus but not in the antrum. It is also important to note that prior gastric surgery, such as sleeve gastrectomy in mice, resulted in significant disruption in ICCs in the gastric antrum with a loss of pacemaker activity,[18] suggesting that a prior gastric intervention may introduce confounding in the interpretation of ICCs in gastric biopsy samples.

In the canine stomach antrum,[19] pacemaker capability is observed throughout the circular muscle, usually with a dominant pacemaker near the myenteric plexus that drives slow waves, and the latter actively propagates throughout the circular muscle layer. This pacemaker activity and the active propagation pathway may occur in networks of ICCs that are distributed in the region of the myenteric plexus and throughout the circular muscle layer. Four populations of ICCs were found within the antral muscularis based on anatomic locations: ICCs within the region between the circular and longitudinal muscle layers, ICC-IM within bundles of circular and longitudinal muscle cells, ICCs that lie within septa between bundles of smooth muscle cells, and ICCs at the submucosal surface of the circular muscle layer. All these different classes of ICCs, which have also been identified in the human stomach,[20] are able to generate pacemaker activity. Given this distribution, it is critically important for comparisons to be made in the same layer of muscle taken during gastric biopsy sampling.

More recent studies introduced more automation in the quantitation of ICCs and confirmed the same principles regarding standardization of biopsy site. Mah et al[17] analyzed regional variations of ICCs in the murine distal stomach using confocal imaging and machine learning methods and demonstrated an increase in myenteric plexus ICC volume from the proximal to the distal antrum, but the percentage of ICC volume was similar for longitudinal muscle ICCs and for circular muscle ICCs between the proximal and distal antrum and was much lower than in the myenteric plexus ICCs. This suggests that in addition to the precise anatomic location from which biopsy samples were taken, it is essential that counts of ICCs be performed in a specific muscle layer for comparison. These observations also suggest that if the quantification is performed in a specific layer of muscle, there does not appear to be much difference between the proximal and distal antrum of the murine stomach. The quantitation of ICCs was conducted in the circular muscle layer of the gastric body in the human study comparing FD and gastroparesis.[14] It would be interesting to know whether the ICCs in the myenteric plexus were different in the latter studies.

In a separate study of murine stomach that incorporated supervised machine learning techniques to extract the ICC networks from 3-dimensional confocal microscopy images, Mah et al[21] showed regional variation in ICC network density and thickness along the circumferential and longitudinal axes of the mouse antrum. An inverse relationship was observed in the distal and proximal antrum for density (proximal vs distal: 9.8% $\pm$ 4.0% vs 7.6% $\pm$ 4.6%) and thickness (proximal vs distal: 15 $\pm$ 3 μm vs 24 $\pm$ 10 μm). A more recent study adopted the validated fast random forest classification method using segmentation of the networks of ICC applied to confocal microscopy images of a whole-mount distal antrum of a mouse stomach (583 $\times$ 3376 $\times$ 133 μm^3, parcellated into 24 equal image stacks).[22] The study documented a pronounced decline of up to 80% in longitudinal muscle ICCs (from 3705 μm^3 to 716 μm^3) over 279.3 μm in the distal antrum. These data suggest that quantification of regional ICCs necessitates precise sampling and documentation of the site relative to the circumferential and longitudinal axes of the stomach to facilitate comparisons between disease groups. The data also suggest that the quantitation of ICCs in the circular muscle layer or myenteric plexus may be more informative than the results obtained in the longitudinal muscle layer.

In human gastric biopsy samples obtained in the muscular layer of the junction of the distal antrum and pylorus after submucosal tunneling during a gastric peroral endoscopic myotomy (G-POEM) procedure, Shah et al[23] showed that the numbers of ICCs were higher in those who experienced clinical response to the procedure than in nonresponders as defined by significant improvement in nausea and vomiting after G-POEM. It was also previously shown that in full-thickness biopsy specimens from both the pylorus and the antrum from patients with gastroparesis, there was greater loss of ICCs in the pylorus than in the antrum.[24]

In summary, to avoid equating FD and gastroparesis based on a histopathologic analysis of gastric biopsy samples,[25] it is critically important for standardization of the biopsy location, depth, and analysis in appraising the pathology of the enteric nervous system and pacemaker cells in health and disease. Advances in endoscopic interventions provide an opportunity to standardize biopsy sampling by focusing on the circular muscle layer or myenteric plexus for ICCs by obtaining full-thickness endoscopic biopsy samples at a precise site such as 2 cm proximal to the pylorus or at the incisura of the stomach[26] and the pyloric muscle itself in patients undergoing G-POEM, as shown by Shah et al.[23] If patients are not candidates for G-POEM, an alternative approach is endoscopic full-thickness resection, which is typically used with an over-the-scope clip–assisted system[27] for complete resection of tumors, but it may also be applied for diagnostic purposes using the full-thickness resection device. Such resections are

achieved effectively and safely as documented in 2 recent systematic reviews and meta-analyses.[28,29]

Consensus guidelines for histologic techniques and reporting for the assessment and diagnosis of gastrointestinal neuromuscular disease have been recommended by the Gastro 2009 International Working Group.[30] These recommendations may be further enhanced through the application of standard quantification methods using machine learning methods as recently introduced.[17,21] These approaches are necessary to statistically appraise differences in the numbers of ICCs or CD206 macrophages, given the estimated sample size of 24 patients in each of the 2 groups to demonstrate a 50% difference in the number of ICCs per high-power fields (mean, 1.57) based on the pooled SD (1.913) in the numbers of ICCs observed in the study from the National Institutes of Health gastroparesis consortium.[14] The estimated sample size to detect a 25% difference in the numbers of ICCs per high-power field would be 95 patients in each group. These calculations led to the conclusion that the sample size examined to date[14] was insufficient to conclusively support the hypothesis that there are no differences in the numbers of ICCs in gastric biopsy samples between gastroparesis and FD, in addition to the potential differences in the sites of the full-thickness biopsy samples obtained in the gastric body in light of the published literature showing the variation in numbers of ICCs based on biopsy site in the longitudinal or circumferential axis of the stomach.

MICHAEL CAMILLERI
Division of Gastroenterology and Hepatology
Mayo Clinic
Rochester, Minnesota

JAN TACK
Division of Gastroenterology and Hepatology
Leuven University Hospitals
Leuven, Belgium

References

1. Stanghellini V, Tack J. Gastroparesis: separate entity or just a part of dyspepsia? Gut 2014;63:1972–1978.
2. Parkman HP, Yates K, Hasler WL, et al. National Institute of Diabetes and Digestive and Kidney Diseases Gastroparesis Clinical Research Consortium. Clinical features of idiopathic gastroparesis vary with sex, body mass, symptom onset, delay in gastric emptying, and gastroparesis severity. Gastroenterology 2011;140:101–115.
3. Burton Murray H, Jehangir A, Silvernale CJ, Kuo B, Parkman HP. Avoidant/restrictive food intake disorder symptoms are frequent in patients presenting for symptoms of gastroparesis. Neurogastroenterol Motil 2020;32:e13931.
4. Heruc GA, Little TJ, Kohn MR, et al. Effects of starvation and short-term refeeding on gastric emptying and postprandial blood glucose regulation in adolescent girls with anorexia nervosa. Am J Physiol Endocrinol Metab 2018;315:E565–E573.
5. Sarnelli G, Caenepeel P, Geypens B, Janssens J, Tack J. Symptoms associated with delayed emptying of solids and liquids in functional dyspepsia. Am J Gastroenterol 2003;98:783–788.
6. Janssen P, Harris MS, Jones M, et al. The relation between symptom improvement and gastric emptying in the treatment of diabetic and idiopathic gastroparesis. Am J Gastroenterol 2013;108:1382–1391.
7. Vijayvargiya P, Jameie-Oskooei S, Camilleri M, Chedid V, Erwin PJ, Murad MH. Association between delayed gastric emptying and upper gastrointestinal symptoms: a systematic review and meta-analysis. Gut 2019;68:804–813.
8. Vijayvargiya P, Camilleri M, Chedid V, Mandawat A, Erwin PJ, Murad MH. Effects of promotility agents on gastric emptying and symptoms: a systematic review and meta-analysis. Gastroenterology 2019;156:1650–1660.
9. Chakraborty S, Desai A, Halland M, et al. Relationship between symptoms during a gastric emptying study and intestinal chemosensitivity with daily symptoms. Neurogastroenterol Motil 2019;31:e13686.
10. Camilleri M, Iturrino J, Bharucha AE, et al. Performance characteristics of scintigraphic measurement of gastric emptying of solids in healthy participants. Neurogastroenterol Motil 2012;24:1076-e562.
11. Desai A, O'Connor M, Neja B, et al. Reproducibility of gastric emptying assessed with scintigraphy in patients with upper GI symptoms. Neurogastroenterol Motil 2018;30:e13365.
12. Choi MG, Camilleri M, Burton DD, Zinsmeister AR, Forstrom LA, Nair KS. Reproducibility and simplification of ^{13}C-octanoic acid breath test for gastric emptying of solids. Am J Gastroenterol 1998;93:92–98.
13. Choi MG, Camilleri M, Burton DD, Zinsmeister AR, Forstrom LA, Nair KS. [^{13}C]octanoic acid breath test for gastric emptying of solids: accuracy, reproducibility, and comparison with scintigraphy. Gastroenterology 1997;112:1155–1162.
14. Pasricha PJ, Grover M, Yates KP, et al. Functional dyspepsia and gastroparesis in tertiary care are interchangeable syndromes with common clinical and pathologic features. Gastroenterology 2021;160:2006–2017.
15. Song G, David G, Hirst S, Sanders KM, Ward SM. Regional variation in ICC distribution, pacemaking activity and neural responses in the longitudinal muscle of the murine stomach. J Physiol 2005;564(Pt 2):523–540.
16. Hirst GD, Beckett EA, Sanders KM, Ward SM. Regional variation in contribution of myenteric and intramuscular interstitial cells of Cajal to generation of slow waves in mouse gastric antrum. J Physiol 2002;540(Pt 3):1003–1012.
17. Mah SA, Du P, Avci R, Vanderwinden JM, Cheng LK. Analysis of regional variations of the interstitial cells of Cajal in the murine distal stomach informed by confocal imaging and machine learning methods. Cell Mol Bioeng 2022;15:193–205.
18. Moon SB, Hwang SJ, Baker S, et al. Changes in interstitial cells and gastric excitability in a mouse model of sleeve gastrectomy. PLoS One 2022;17:e0269909.

19. Horiguchi K, Semple GS, Sanders KM, Ward SM. Distribution of pacemaker function through the tunica muscularis of the canine gastric antrum. J Physiol 2001; 537(Pt 1):237–250.
20. Rhee P-L, Lee JY, Son HJ, et al. Analysis of pacemaker activity in the human stomach. J Physiol 2011;589(Pt 24):6105–6118.
21. Mah SA, Avci R, Du P, Vanderwinden JM, Cheng LK. Supervised machine learning segmentation and quantification of gastric pacemaker cells. Annu Int Conf IEEE Eng Med Biol Soc 2020; 2020:1408–1411.
22. Mah SA, Avci R, Du P, Vanderwinden JM, Cheng LK. Antral variation of murine gastric pacemaker cells informed by confocal imaging and machine learning methods. Annu Int Conf IEEE Eng Med Biol Soc 2021;2021:3105–3108.
23. Shah R, Calderon LF, Sanders BE, et al. Quantification of interstitial cells of Cajal in the gastric muscle of patients with gastroparesis at per-oral endoscopic pyloromyotomy: a novel approach for future research in pathogenesis of gastroparesis. Dig Dis Sci 2022; 67:4492–4499.
24. Moraveji S, Bashashati M, Elhanafi S, et al. Depleted interstitial cells of Cajal and fibrosis in the pylorus: novel features of gastroparesis. Neurogastroenterol Motil 2016;28:1048–1054.
25. Tack J, Schol J, Horowitz M. Gastroparesis: a dead-end street after all? Gastroenterology 2021; 160:1931–1933.
26. Rajan E, Gostout CJ, Wong Kee, Song LM, et al. Innovative gastric endoscopic muscle biopsy to identify all cell types, including myenteric neurons and interstitial cells of Cajal in patients with idiopathic gastroparesis: a feasibility study (with video). Gastrointest Endosc 2016;84:512–517.
27. Al-Bawardy B, Rajan E, Wong Kee Song LM. Over-the-scope clip-assisted endoscopic full-thickness resection of epithelial and subepithelial GI lesions Gastrointest. Endosc 2017;85:1087–1092.
28. Bomman S, Klair JS, Canakis A, et al. Safety and efficacy of endoscopic full thickness resection of upper gastrointestinal lesions using a full thickness resection device: a systematic review and meta-analysis [published online ahead of print November 18, 2022]. J Clin Gastroenterol https://doi.org/10.1097/MCG.0000000000001803.
29. Abdallah M, Suryawanshi G, McDonald N, et al. Endoscopic full-thickness resection for upper gastrointestinal tract lesions: a systematic review and meta-analysis [published online ahead of print December 14, 2022]. Surg Endosc https://doi.org/10.1007/s00464-022-09801-x.
30. Knowles CH, De Giorgio R, Kapur RP, et al. Gastrointestinal neuromuscular pathology: guidelines for histological techniques and reporting on behalf of the Gastro 2009 International Working Group. Acta Neuropathol 2009; 118:271–301.

Received January 17, 2023. Accepted March 10, 2023.

Conflicts of interest
The authors disclose no conflicts.

Funding
The study was funded by grants R01-DK12280 and R01-DK125680 from the National Institutes of Health to Michael Camilleri.

Most current article

0016-5085/$36.00
https://doi.org/10.1053/j.gastro.2023.03.204