**SUPPLEMENT**

**Other Tests for Gastroparesis Based on Full-Thickness Biopsies**

This section will briefly review the evidence regarding changes at the level of the stomach as identified in histological and molecular studies performed on biopsies taken from patients with gastroparesis. In addition, the section summarizes 2021 recommendations from the European Society of Neurogastroenterology and Motility and a North American perspective on the same questions pertaining to full-thickness biopsies and pathological diagnosis in patients with gastroparesis

***1. Cellular basis for the development of gastroparesis***

Experimental models of gastroparesis show a reduction in the number of interstitial cells of Cajal (ICC) in the deep muscle plexus with secondary effects in gastric muscles because of the lack of trophic factors (such as stem cell factor) (1-3). Several case reports document the deficiency of ICCs in patients with gastroparesis (4-7). ICC networks may be injured by diverse disease processes. For example, relative insulinopenia and IGF-1 deficiency in diabetes leads to reduced production of stem cell factor by smooth muscle cells, and this is important for the survival of ICCs (3). Secondly, diabetes is associated with evidence of high oxidative stress possibly resulting from upregulation of macrophage heme oxygenase-1 or from the depletion of anti-inflammatory resident M2 macrophages expressing heme oxygenase-1 (HO1) which would protect the pacemaker cells by neutralizing the oxidative mechanisms (8).

Depletion of ICCs may have prognostic significance regarding the efficacy of GES. Thus, full thickness biopsies performed at the time of implantation of GES have shown that symptom response is better in patients with higher ICC counts at baseline (9-11).

1. ***Morphological and transcriptomics evidence from human gastric biopsies***

(i). Neural, pacemaker and muscular elements:

Light microscopy studies of full thickness biopsies of the stomach in patients with idiopathic and diabetic gastroparesis show no significant differences in nerve or smooth muscle or inflammatory cell markers, except for greater reduction in expression of neuronal NO synthase neurons in diabetic compared with idiopathic gastroparesis (12). The loss of ICC in patients with gastroparesis is significantly associated with delayed gastric emptying; overall clinical severity and nausea in idiopathic gastroparesis are associated with the degree of immune cell infiltrate (13).

However, there are conflicting data on a possible decrease of electrically coupled platelet-derived growth factor receptor α-fibroblast-like cells in gastroparesis (14,15).

Despite normal morphology of cells at light microscopy, transmission electron microscopy showed markedly thickened basal lamina, with stroma rich in collagen fibrils and smooth muscle cells distanced from each other in patients with diabetic gastroparesis compared to those with idiopathic gastroparesis and controls (16). In addition, these ultrastructural studies showed that, in comparison to control tissues, ICCs were not in contact with nerve endings and rarely in contact with smooth muscle cells and other ICCs in biopsies from patients with gastroparesis. Moreover, ICC injury was more severe in idiopathic than diabetic gastroparesis.

An additional study assessed full-thickness antral biopsies and showed reduced numbers of ICCs in diabetic and idiopathic patients compared to normal controls, and in fact only 2/43 patients had normal or higher than normal ICC values. Fibrosis was present in 39% and 62% of the idiopathic and diabetic patients with gastroparesis (17).

In a more recent study (18) of full-thickness gastric body biopsy specimens from 9 patients with idiopathic gastroparesis, 9 patients with functional dyspepsia undergoing implantation of a gastric electrical stimulator, and 9 controls without diabetes or gastroparesis symptoms undergoing obesity surgery (albeit not healthy controls), a significant loss of ICC along with a decrease in myenteric plexus CD206-positive staining was seen in both patient subgroups. Similarly, Protein Gene Product 9.5 (a marker for neurons) counts/high-power field were similar in all 3 groups as were a variety of other histologic markers.

(ii). Inflammatory elements

Loss of anti-inflammatory macrophages and increased expression of genes associated with pro-inflammatory macrophages have been reported in full-thickness gastric biopsies from patients with gastroparesis. However, there may be differences in the morphological abnormalities in diabetic and idiopathic gastroparesis in the different studies reported to date (14,19,20). Based on deep RNA sequencing, granulocyte adhesion and diapedesis, as well as a macrophage-based immune dysregulation pathway are the most significantly affected pathways altered in both diabetic and idiopathic gastroparesis. In addition, proteins involved in the complement and prostaglandin synthesis pathway were associated with diabetic gastroparesis. In the same study, immune cell analysis revealed no significant differences in enrichment of genes associated with M1 or M2 macrophages in the biopsies from patients with diabetic gastroparesis and diabetic control samples.

In contrast, genes associated with M1 (pro-inflammatory) macrophages were increased in idiopathic gastroparesis samples compared to their controls. Finally, innate immune mechanisms in diabetic gastroparesis seem to be associated with reduced expression of inflammatory markers on transcriptomics and paradoxically they are associated with M2 macrophage deficiency, which would be expected to be pro-inflammatory in diabetic gastroparesis.

There were higher numbers of mast cells on full-thickness gastric biopsy in idiopathic compared to diabetic gastroparesis (17).

1. ***Section summary:***

Although full thickness biopsies have helped to shed light onto the pathogenesis of gastroparesis, to date, the biopsies have yet to help guide management. Therefore, similar to the European Society of Neurogastroenterology and Motility consensus statement (21), we do not recommend the routine use of full thickness biopsies. Full thickness biopsies should be reserved for research purposes to help better understand the causes of gastroparesis, identify biomarkers, guide therapy, and predict outcomes. Further studies are needed to clarify the role of inflammatory mechanisms in gastroparesis, determination of the impact of concomitant vagal denervation which may exert anti-inflammatory activity and may be lost in patients with vagal dysfunction, (e.g., in patients with diabetes mellitus or post-surgical gastroparesis), and whether loss of ICCs on full-thickness biopsies prior to gastric electrical stimulation impacts outcome to that treatment.

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**THE PICO QUESTIONS**

**RISK FACTORS**

A1. For patients with diabetic gastroparesis (P) does the use of optimal glucose control (I) reduce the future risk of aggravation of the gastroparesis (O) compared with suboptimal glucose control (C)?

A2. For patients with idiopathic gastroparesis (P) does the use of pharmacological treatment for gastroparesis (I) reduce the future risk of aggravation of the gastroparesis (O) compared with lack of use of pharmacological agents (C)?

**DIAGNOSIS**

B1. Should scintigraphic gastric emptying versus radiopaque markers (C) be used to diagnose gastroparesis (O) in patients with upper gastrointestinal symptoms suggestive of gastroparesis (P)?

B2. Should scintigraphic gastric emptying versus wireless motility capsule (C) be used to diagnose gastroparesis (O) in patients with upper gastrointestinal symptoms suggestive of gastroparesis (P)?

B3. Should scintigraphic gastric emptying versus FDA-approved stable isotope breath test (C) be used to diagnose gastroparesis (O) in patients with upper gastrointestinal symptoms suggestive of gastroparesis (P)? In addition, what is the clinical variability of both scintigraphic and breath-based stable isotope gastric emptying tests?

B4. In patients with upper gastrointestinal symptoms suggestive of gastroparesis (P), is scintigraphic gastric emptying (I) more accurate in detecting delayed gastric emptying of solids (O) compared with stable isotope breath test (C)?

B5. Is gastric emptying test conducted for at least 3 hours (I) in patients suspected of having gastroparesis (P) compared with test conducted over 2 hours or less (C) for detecting gastroparesis have a more significant association with upper gastrointestinal symptoms (O)?

B6. Is there a role for EndoFLIP (I) of the pylorus in the selection of patients for peroral pyloromyotomy, or for the overall management (O) of gastroparesis (P)?

**MANAGEMENT**

C1. In gastroparesis (P), what is the effect of small particle diet (I) on patient reported outcomes or symptoms (O) compared with normal diet (non-homogenized) (C)?

C2. In gastroparesis (P), what is the effect of pro-motility agents (I) on patient reported outcomes or symptoms (O) compared with placebo (C)?

C3. In gastroparesis (P), what is the effect of pro-motility agents (I) on gastric emptying (O) compared with placebo (C)?

C4. In gastroparesis (P), what is the effect of anti-emetic agents (I) on patient reported outcomes or symptoms (O) compared with placebo (C)?

C5. In gastroparesis (P), what is the effect of central neuromodulators or behavioral therapies (I) on patient reported outcomes or symptoms (O) compared with placebo (C)?

C6. In gastroparesis (P), what is the effect of pro-motility gastric electrical stimulation or other methods of neuromodulation (high energy GES, Vagal stimulation, spinal stimulation) (I) on patient reported outcomes or symptoms (O) compared with placebo (C)?

C7. In patients with symptoms of gastroparesis with or without baseline gastric emptying delay (P), what is the effect of pyloric botulinum toxin injection, gastric POEM or surgical pyloroplasty (I) on patient reported outcomes or symptoms (O) compared with placebo (C)?

C8. In gastroparesis (P), what is the effect of ghrelin agonists (I) on patient reported outcomes or symptoms (O) compared with placebo (C)?

C9. In gastroparesis, what is the effect of non-traditional prokinetic drugs, such as haloperidol, on patient reported outcomes or symptoms compared with placebo?

C10. What are the effects of CAM therapy such as acupuncture (I) rikkunshito (I), Iberogast (I), peppermint (I) on symptoms (O) and gastric emptying (O) in patients with gastroparesis (P) compared to placebo (C)?