

Clinical-histological associations in gastroparesis: results from the Gastroparesis Clinical Research Consortium

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Abstract

Background Cellular changes associated with diabetic (DG) and idiopathic gastroparesis (IG) have recently been described from patients enrolled in the Gastroparesis Clinical Research Consortium. The association of these cellular changes with gastroparesis symptoms and gastric emptying is unknown. The aim of this study was to relate cellular changes to symptoms and gastric emptying in patients with gastroparesis. **Methods** Earlier, using full thickness gastric body biopsies from 20 DG, 20 IG, and 20 matched controls, we found decreased interstitial cells of Cajal (ICC) and enteric nerves and an increase in immune cells in both DG and IG. Here, demographic, symptoms [gastroparesis cardinal symptom index score (GCSI)], and gastric emptying were related to cellular alterations using Pearson's correlation coefficients. **Key Results** Interstitial cells of Cajal counts inversely correlated with 4 h gastric retention in DG but not in IG ($r = -0.6$, $P = 0.008$, DG, $r = 0.2$,

$P = 0.4$, IG). There was also a significant correlation between loss of ICC and enteric nerves in DG but not in IG ($r = 0.5$, $P = 0.03$ for DG, $r = 0.3$, $P = 0.16$, IG). Idiopathic gastroparesis with a myenteric immune infiltrate scored higher on the average GCSI (3.6 ± 0.7 vs 2.7 ± 0.9 , $P = 0.05$) and nausea score (3.8 ± 0.9 vs 2.6 ± 1.0 , $P = 0.02$) as compared to those without an infiltrate. **Conclusions & Inferences** In DG, loss of ICC is associated with delayed gastric emptying. Interstitial cells of Cajal or enteric nerve loss did not correlate with symptom severity. Overall clinical severity and nausea in IG is associated with a myenteric immune infiltrate. Thus, full thickness gastric biopsies can help define specific cellular abnormalities in gastroparesis, some of which are associated with physiological and clinical characteristics of gastroparesis.

Keywords clinical symptoms, enteric nervous system, gastric emptying, gastroparesis, interstitial cells of Cajal, macrophages.

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INTRODUCTION

Gastroparesis is a gastric motility disorder characterized by delayed gastric emptying (GE) in the absence of mechanical obstruction.¹ The clinical syndrome comprises of nausea, vomiting, bloating, early satiety, and

abdominal pain.² It is an increasingly recognized complication of both Type 1 and Type 2 diabetes.³ Other less common causes are post-surgical and medication related, however, a cause remains unknown in a significant proportion of patients characterized as idiopathic.⁴

Gastroparesis-related morbidity seems to be on the rise. From 1995 to 2004, there has been a 158% increase in hospitalizations with gastroparesis with gastroparetics incurring higher hospitalization costs compared to other upper gastrointestinal (GI) disorders.⁵ The age-adjusted incidence of gastroparesis per 100 000 person-years was 2.4 for men and 9.8 for women for years 1996–2006 from Olmsted County, Minnesota and corresponding prevalence figures were 9.6 for men and 37.8 for women per 100 000 persons. In this study, overall survival was found to be significantly lower than the age- and sex-specific survival of general population.⁶ In spite of increasing recognition and morbidity associated with the condition in last two decades, therapeutic options continue to remain limited at best. A major limiting factor in the development of targeted therapy is the lack of understanding of cellular etiopathogenesis in human gastroparesis.⁷

A few retrospective studies have defined abnormalities in cell types required for a normal gastric function such as loss of interstitial cells of Cajal (ICC) and neuronal nitric oxide synthase (nNOS).^{8–11} Loss of ICC has been associated with gastric dysrhythmias and worse clinical symptoms.^{12,13} Until recently, detailed analysis of various cell types, including the extrinsic innervation to the stomach, enteric nerves, glia, smooth muscle cells, ICC, and immune cells was not available. To meet this need, the National Institutes of Diabetes and Digestive and Kidney Diseases (NIDDK) Gastroparesis Clinical Research Consortium (GpCRC) was established in 2006 to prospectively enroll patients, obtain detailed clinical data and collect full thickness gastric tissue in a standardized approach. We recently described the cellular changes associated with diabetic (DG) and idiopathic (IG) gastroparesis from GpCRC patients.¹⁴ On quantitative comparisons, the most commonly observed findings were loss of ICC and an immune infiltrate characterized by an increase in CD45 and CD68 immunoreactivity in both DG and IG. A 14–17% decrease in the number of enteric nerve fibers as defined by Protein Gene Product 9.5 (PGP9.5) immunoreactivity was also seen. Less common were changes in nNOS, vasoactive intestinal peptide (VIP), substance P (SP), and tyrosine hydroxylase (TH).

The primary aim of this study was to determine associations, in both DG and IG, between specific

histological markers of gastroparesis (ICC loss, nerve fiber loss and immune infiltrate) and specific clinical features (scintigraphically determined impairment in GE and worsening in standardized symptom scores). A secondary aim was to explore associations of less commonly histological features such as nNOS, SP, VIP, and TH expression with GE and clinical symptoms.

METHODS

Subject enrollment

The NIDDK GpCRC consists of a network of seven centers and one data coordinating center collaborating for research on gastroparesis. The Gastroparesis Registry is an observational study to clarify the epidemiology, natural history, clinical course, and other outcomes of gastroparesis (ClinicalTrials.gov identifier: NCT00398801). The Gastroparesis Registry consists of patients >18 years of age with symptoms of at least 12 week duration, delayed GE on scintigraphy (>60% retention at 2 h or >10% retention at 4 h), and no evidence of obstruction. Exclusion criteria included presence of active inflammatory bowel disease, eosinophilic gastroenteritis, neurological conditions, acute liver or renal failure, and history of total or subtotal gastric resection. Registry data at enrollment include detailed history and physical examinations, validated symptom questionnaires, upper endoscopy, 4 h GE, and laboratory tests.

Clinical questionnaires

Patients were questioned about the onset of their symptoms and if they had an initial infectious prodrome. Each patient filled out the 20-item Patient Assessment of Upper Gastrointestinal Disorders Symptoms Severity Index (PAGI-SYM) questionnaire, which assesses symptoms of gastroparesis, dyspepsia, and gastroesophageal reflux disease. It includes the nine symptoms consisting the gastroparesis cardinal symptom index (GCSI), which asks about nausea, retching, vomiting, stomach fullness, inability to finish a meal, excessive fullness, loss of appetite, bloating, and abdominal distention.¹⁵ The GCSI equals the summation of the nausea/vomiting subscore (nausea, retching, and vomiting), postprandial fullness/early satiety subscore (stomach fullness, inability to finish a meal, excessive fullness, and loss of appetite), and bloating subscore (bloating and large stomach). Patients were asked to assess the severity of their symptoms in previous 2 weeks on a 0–5 Likert scale (no symptoms = 0, very mild = 1, mild = 2, moderate = 3, severe = 4, very severe = 5).

Gastric emptying

Gastric emptying scintigraphy was performed using a low-fat egg white meal with imaging at 0, 1, 2, and 4 h after meal ingestion using the standardized protocol to estimate percent gastric retention.¹⁶ This protocol ensures standardized information about delayed GE across all participating sites. With this protocol, delayed GE is characterized by percent gastric retention at any of the time points and can also be classified according to the gastric retention at 4 h as mild ($\leq 20\%$ gastric retention at 4 h), moderate (>20–35%), and severe (>35%).²

Laboratory studies

Laboratory tests obtained on each patient included complete blood cell count with erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and glycosylated hemoglobin (HbA1c).

Histology studies

Tissue was obtained from 20 DG and 20 IG patients undergoing surgery for placement of a gastric stimulator and from 20 years of age and sex matched patients undergoing duodenal switch gastric bypass surgery following Institutional Review Board approved protocols. The full-thickness gastric biopsies were collected in all subjects from the anterior aspect of the stomach, midway between the greater and lesser curvatures where the gastroepiploic vessels meet. Details on tissue acquisition and processing are provided elsewhere.¹⁴ Various components of the enteric nervous system (ENS) were studied using antibodies to PGP9.5, nNOS, VIP (inhibitory innervation), SP (excitatory inhibition), and TH (extrinsic motor innervation). For examining ICC, c-Kit antibody was used and CD45 was used as a general cell marker for immune infiltrate.¹⁴

Statistical analysis

The histological markers for ICC (c-Kit), enteric nerves (PGP9.5), and immune infiltrate (CD45) were used for clinical correlations as outlined in specific aim 1. Patients with marker values $\geq 25\%$ compared to normal were categorized as increased on that marker and those with values $\leq 25\%$ compared to normal were categorized as decreased on that marker. Demographic and other characteristics were compared between those with and without changes in these histological markers in both DG and IG using *t*-test (continuous characteristics) and chi-squared or Fisher's exact test (categorical characteristics). For continuous characteristics, groups with increased and decreased markers were expressed as means \pm standard deviations. Simple Pearson's correlation analysis was done to relate the histological markers (as measured) to the two key clinical variables indicative of symptoms (average GCSI score) and delay in gastric emptying (% gastric retention at 4 h). All statistical analyses were carried out using GraphPad 4 software (GraphPad software Inc., La Jolla, CA, USA). A *P*-value of <0.05 was considered statistically significant. Exact *P*-values were used and these were not corrected for multiple comparisons.

RESULTS

Diabetic gastroparetics

Circular muscle ICC loss There was a 48% decrease in overall quantification of ICC in DG compared to controls (2.8 ± 0.4 vs 5.3 ± 0.2 cells/high power field, $P < 0.0001$). Ten of the 20 patients (50%) with DG were identified as having depleted number of ICC based on a $\geq 25\%$ drop. The group with depleted ICC was older than the group with normal ICC (52.2 ± 13.1 years vs 39.2 ± 11.6 years, $P = 0.03$) but both groups were similar in terms of gender distribution. There were no differences in terms of diabetes type (Type I or II), duration or control (determined by HbA1c). The group with depleted ICC had significantly higher 4 h gastric retention as compared those with normal ICC ($47.6 \pm 25.6\%$ vs $22 \pm 9.4\%$, $P = 0.01$) as shown in Table 1. Interstitial cells of Cajal counts and gastric retention at 4 h were inversely correlated ($r = -0.59$, $P = 0.008$, Fig. 1A). Within the group with depleted ICC, six patients (60%) had severe gastric retention ($>35\%$ at 4 h) whereas four (40%) had mild to moderate gastric retention. Of the 10 patients with normal ICC bodies, only one (10%) had severe gastric retention. There were no significant correlations between the average GCSI score and ICC numbers (Fig. 1D)

Myenteric plexus immune cell infiltrate There was an overall 25% increase in the CD45 expression in DG when compared to controls (25.5 ± 1.5 vs 20.3 ± 1.1 cells/high power field, $P = 0.002$). Nine of the 20 patients (45%) with DG were found to have $\geq 25\%$ increase in CD45 staining cells in the myenteric plexus. The groups with or without immune infiltrate were similar in age, sex, or history of infectious pro-

Table 1 Comparisons of gastric retention and gastric symptoms (GCSI) in diabetic or idiopathic gastroparesis patients with and without cellular alterations: ICC (Kit) loss, enteric nerves (PGP9.5) loss and increased immune infiltrate (CD45)

	ICC low (<i>n</i> = 10)	ICC normal (<i>n</i> = 10)	<i>P</i>	CD45 increased (<i>n</i> = 9)	CD45 normal (<i>n</i> = 11)	<i>P</i>	PGP9.5 low (<i>n</i> = 4)	PGP9.5 normal (<i>n</i> = 16)	<i>P</i>
Diabetic gastroparesis									
% Gastric retention at 4 h (mean \pm SD)	47.6 \pm 25.6	22 \pm 9.4	0.01	39.8 \pm 26.5	31.6 \pm 20.5	0.46	40.2 \pm 20.5	34.2 \pm 24.4	0.66
GCSI average score (mean \pm SD)	2.9 \pm 1.4	2.8 \pm 1.5	0.83	2.8 \pm 1.5	2.9 \pm 1.4	0.83	2.7 \pm 1.6	2.9 \pm 1.4	0.79
Idiopathic gastroparesis									
% Gastric retention at 4 h (mean \pm SD)	17.5 \pm 16.8	28.8 \pm 16.6	0.17	19.8 \pm 15.0	29.6 \pm 22.2	0.29	11.2 \pm 9.1	26.8 \pm 17.8	0.08
GCSI average score (mean \pm SD)	3.7 \pm 1.2	3.2 \pm 1.0	0.39	3.6 \pm 0.7	2.7 \pm 0.9	0.05	3.2 \pm 0.6	3.3 \pm 0.9	0.88
-Nausea	3.6 \pm 1.2	3.2 \pm 1.0	0.38	3.8 \pm 0.9	2.6 \pm 1.0	0.02	3.3 \pm 1.2	3.5 \pm 1.1	0.80
-Fullness	3.6 \pm 1.0	3.6 \pm 1.2	0.93	3.8 \pm 0.9	3.2 \pm 1.4	0.31	3.4 \pm 1.0	3.7 \pm 1.2	0.58
-Bloating	3.3 \pm 1.6	2.2 \pm 1.8	0.18	2.9 \pm 1.6	2.4 \pm 1.9	0.54	3.0 \pm 1.6	2.7 \pm 1.8	0.76

GCSI, gastroparesis cardinal symptom index score; ICC, interstitial cells of Cajal; SD, standard deviation.

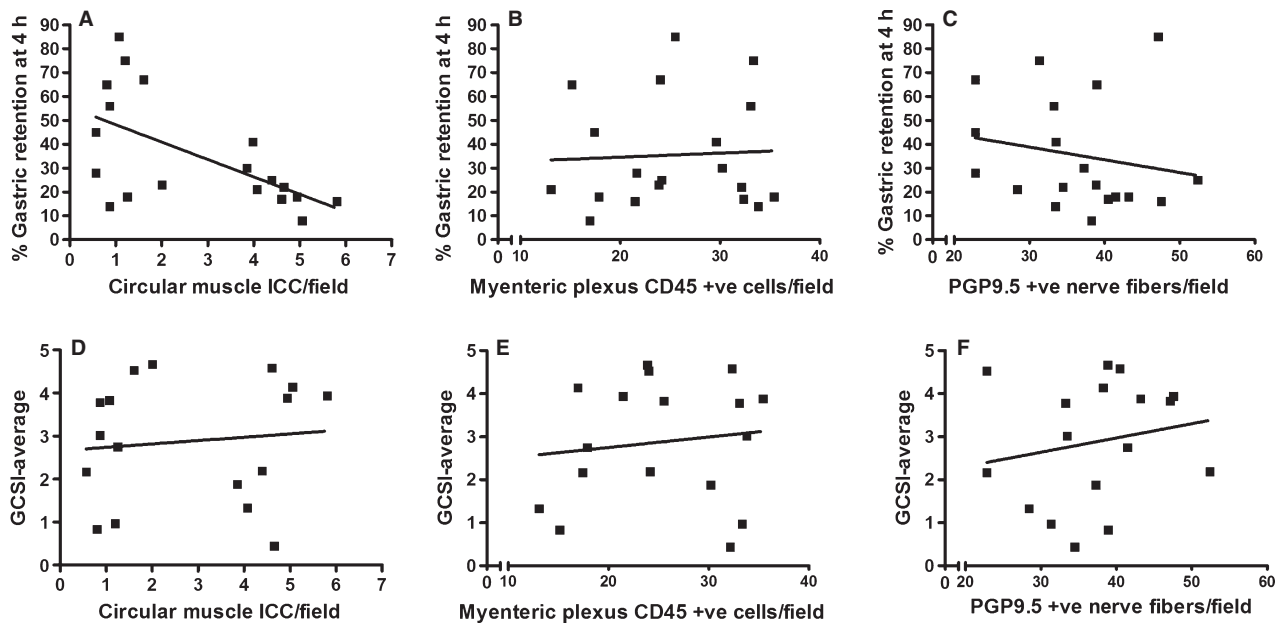


Figure 1 Correlations between % gastric retention at 4 h and average GCSI with ICC, CD45 positive myenteric plexus cells and PGP9.5 positive nerve fibers in diabetic gastroparesis (A) ICC and % gastric retention at 4 h ($r = -0.59$, $P = 0.008^*$, slope = -7.3 , 95% CI = -12.4 to -2.2), (B) CD45 positive cells and % gastric retention at 4 h ($r = 0.05$, $P = 0.83$), (C) PGP9.5 nerve fibers and % gastric retention at 4 h ($r = -0.19$, $P = 0.43$), (D) ICC and GCSI-average ($r = 0.10$, $P = 0.69$), (E) CD45 positive cells and GCSI-average ($r = 0.13$, $P = 0.63$), (F) PGP9.5 nerve fibers and GCSI-average ($r = 0.19$, $P = 0.46$). *Some graphs have less than 20 data points because of missing data on either the counts, GE or GCSI on these patients.

drome. There were no differences in terms of diabetes type, duration or control. Laboratory parameters such as leukocyte count, CRP, and ESR were similar in both the groups. No significant correlations were found between CD45 infiltrate and GE (Fig. 1B) or average GCSI (Fig. 1E). The circular muscle CD45 expression was not different in DG when compared to controls (16.9 ± 0.8 vs 14.3 ± 0.7 cells/high power field, $P = 0.06$).

Circular muscle enteric nerve fiber loss There was a 17% overall decrease in the expression of nerve fiber marker PGP9.5 (36.5 ± 1.8 vs 44.3 ± 2.3 fibers/high power field, $P = 0.01$). Only 4 of the 20 patients (20%) with DG were found to have a PGP9.5 decrease of $\geq 25\%$. There were no differences in age, gender, diabetes type or duration amongst the two groups. Furthermore, no correlations were found between PGP9.5 and GE (Fig. 1C) or between PGP9.5 and average GCSI (Fig. 1F).

Correlation between ICC loss, nerve fiber, and immune infiltrate There was a significant correlation between loss of ICC bodies and PGP9.5 fibers in DG ($r = 0.47$, $P = 0.03$) (Fig. 2A). However, immune infiltrate was not correlated with ICC or nerve fiber loss.

Inhibitory (nNOS, VIP) and excitatory (SP) enteric nervous system and extrinsic innervation (TH) (Table 2) shows correlation coefficients between nNOS positive myenteric neurons, nNOS positive nerve fibers, VIP, SP, TH with GE, and average GCSI. There were no significant correlations between these markers and GE or average GCSI.

Idiopathic gastroparesis

Circular muscle ICC loss There was a 39% decrease in overall quantification of ICC in IG when compared to controls (3.2 ± 0.4 vs 5.3 ± 0.2 cells/high power field, $P < 0.0001$). Ten of the 20 patients with IG were characterized as having ICC loss (50%). There was a similar age and sex distribution amongst those with or without ICC loss. The ICC loss did not correlate with gastric emptying as shown in Fig. 3A or average GCSI (Fig. 3D).

Myenteric plexus immune infiltrate There was an overall 30% increase in the CD45 expression in IG when compared to controls (26.5 ± 1.2 vs 20.3 ± 1.1 cells/high power field, $P = 0.002$). Fourteen of the 20 IG patients (70%) had $\geq 25\%$ increase. The groups with and without immune infiltrate did not differ in terms of age, sex, or history of infection. Leukocyte count,

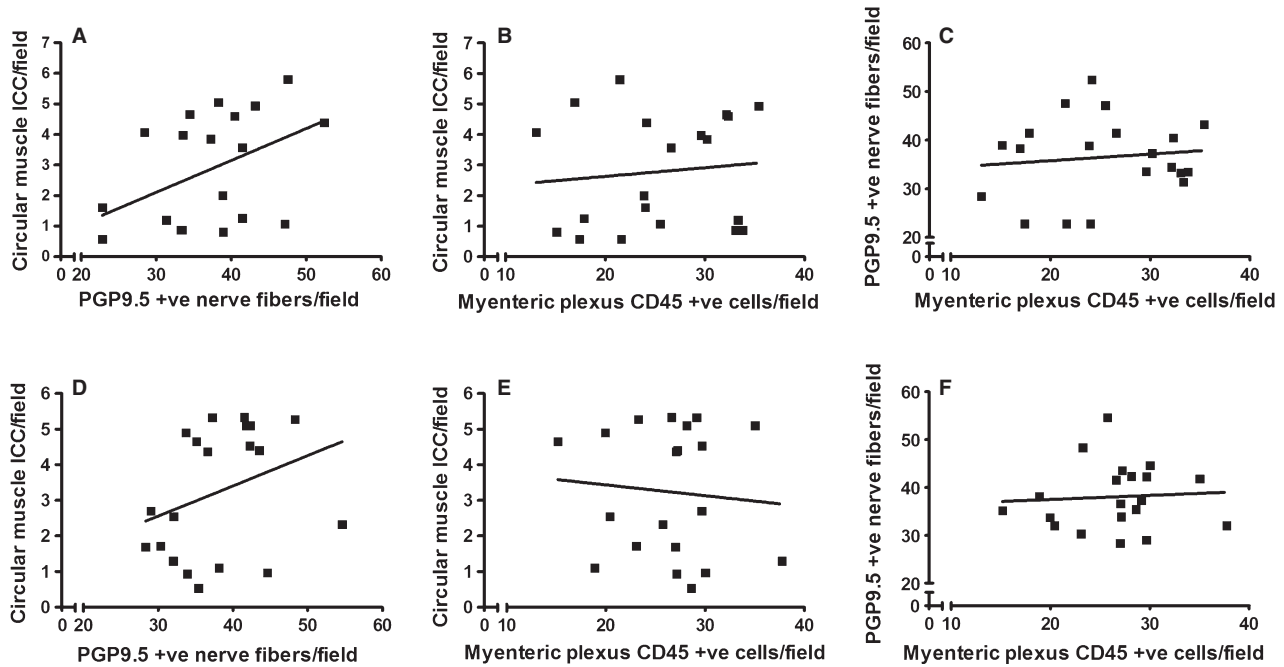


Figure 2 Correlations between ICC, CD45 positive myenteric plexus cells and PGP9.5 positive nerve fibers in diabetic gastroparesis. (A) ICC-PGP9.5 positive nerve fibers ($r = 0.47, P = 0.03^*$, slope=0.11, 95% CI=0.008–0.2), (B) ICC-CD45 positive myenteric cells ($r = 0.1, P = 0.65$), (C) PGP9.5 positive nerve fibers-CD45 positive myenteric cells ($r = 0.11, P = 0.63$). Correlations between ICC, CD45 positive myenteric plexus cells and PGP9.5 positive nerve fibers in idiopathic gastroparesis. (D) ICC-PGP9.5 positive nerve fibers ($r = 0.32, P = 0.16$), (E) ICC-CD45 positive myenteric cells ($r = -0.09, P = 0.71$), (F) PGP9.5 positive nerve fibers-CD45 positive myenteric cells ($r = 0.06, P = 0.78$).

Table 2 Correlations between inhibitory (nNOS, VIP), excitatory (substance P) enteric nervous system and extrinsic innervation (tyrosine hydroxylase) and 4 h gastric retention and GCSI score

	nNOS positive neurons	nNOS positive nerve fibers	VIP positive nerve fibers	SP positive nerve fibers	TH positive labeling
Diabetic gastroparesis					
% Gastric retention at 4 h, correlation coefficient (r)	-0.32 ($P = 0.18$)	-0.03 ($P = 0.89$)	-0.13 ($P = 0.58$)	-0.35 ($P = 0.15$)	-0.12 ($P = 0.61$)
GCSI average score, correlation coefficient (r)	0.17 ($P = 0.48$)	0.21 ($P = 0.45$)	0.22 ($P = 0.37$)	0.20 ($P = 0.47$)	-0.14 ($P = 0.56$)
Idiopathic gastroparesis					
% Gastric retention at 4 h, correlation coefficient (r)	0.13 ($P = 0.61$)	-0.14 ($P = 0.56$)	0.22 ($P = 0.34$)	0.006 ($P = 0.97$)	0.25 ($P = 0.31$)
GCSI average score, correlation coefficient (r)	0.17 ($P = 0.45$)	0.40 ($P = 0.06$)	-0.27 ($P = 0.26$)	0.35 ($P = 0.13$)	-0.14 ($P = 0.56$)

GCSI, gastroparesis cardinal symptom index score; nNOS, neuronal nitric oxide synthase; SP, substance P; TH, tyrosine hydroxylase; VIP, vasoactive intestinal peptide.

CRP, and ESR were similar in both the groups. Overall GCSI was higher in individuals with myenteric plexus CD45 infiltrate (3.6 ± 0.7 vs $2.7 \pm 0.9, P = 0.05$). On comparing the sub-scores, this was found to be predominantly secondary to higher nausea score amongst those with CD45 infiltrate (3.8 ± 0.9 vs $2.6 \pm 1.0, P = 0.02$) as shown in Table 1. However, the correlation between quantitative levels of CD45 and GE (Fig. 3B) or GCSI did not reach statistical significance (Fig. 3E). The circular muscle CD45 expression was not

different in IG when compared to controls (16.5 ± 0.8 vs 14.3 ± 0.7 cells/high power field, $P = 0.06$).

Circular muscle nerve fiber loss There was a 14% overall decrease in the PGP9.5 expression (38 ± 1.5 vs 44.3 ± 2.3 fibers/high power field, $P = 0.01$). Five of the 20 IG had a $\geq 25\%$ dropout. Age and sex were similar between those with and without PGP9.5 loss. Furthermore, no correlations existed between PGP9.5 quantification and GE (Fig. 3C) and GCSI (Fig. 3F).

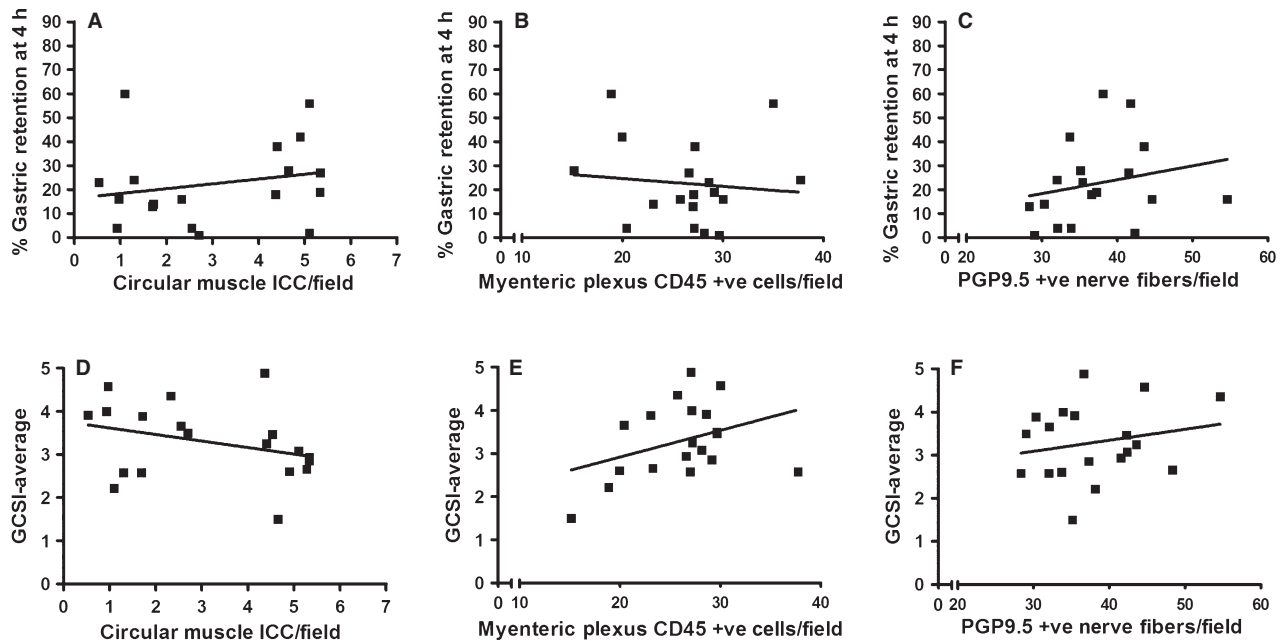


Figure 3 Correlations between % gastric retention at 4 h and average GCSI with ICC, CD45 positive myenteric plexus cells and PGP9.5 positive nerve fibers in idiopathic gastroparesis (A) ICC and % gastric retention at 4 h ($r = 0.21$, $P = 0.38$), (B) CD45 positive cells and % gastric retention at 4 h ($r = -0.10$, $P = 0.68$), (C) PGP9.5 nerve fibers and % gastric retention at 4 h ($r = 0.22$, $P = 0.37$), (D) ICC and GCSI-average ($r = -0.34$, $P = 0.15$), (E) CD45 positive cells and GCSI-average ($r = 0.36$, $P = 0.13$), (F) PGP9.5 nerve fibers and GCSI-average ($r = 0.20$, $P = 0.40$).

Correlation between ICC loss, nerve loss and immune infiltrate Unlike DG, ICC, and nerve fiber loss were not correlated with each other (Fig. 2D). There was no correlation between ICC loss, nerve loss, and immune infiltrate in IG.

Inhibitory (nNOS, VIP) and excitatory (SP) enteric nervous system and extrinsic innervation (TH) Table 2 shows correlation coefficients between nNOS positive myenteric neurons, nNOS positive nerve fibers, VIP, SP, TH with GE, and average GCSI. There were no significant correlations between these markers and GE or average GCSI.

DISCUSSION

Gastroparesis continues to be a clinically challenging syndrome with limited insights into its pathophysiology and no major breakthroughs in treatment options.¹⁷ The cellular defects in human gastroparesis had not been comprehensively studied until recently and no substantive data is available on correlations between various cellular defects and gastric function or clinical symptoms. Interstitial cells of Cajal is a key component of the gut control mechanisms.¹⁸ Loss of ICC is the most common and most consistently reported cellular defect in animal models of DG and in human gastroparesis. Significant loss of ICC at

12 weeks was observed in gastric antrum of streptozotocin induced diabetic rats,¹⁹ and similar observations have been made in the non obese diabetic (NOD) mice.^{20,21} Amongst human studies, profound loss of ICC in the antrum in a third of patients with refractory gastroparesis was seen.¹³ In another study, intramuscular ICC were lost in eight patients with severe diabetes, along with loss of nNOS-neurons.²² ICC loss correlated with development of delayed GE in NOD mice.²⁰ In human DG, ICC loss is associated with disruption in the generation and propagation of electrical slow waves, resulting in gastric dysrhythmias^{12,13} associated with abnormal GE.^{23,24} Our study, within the limitations of the relatively small sample size, is the first to describe a positive correlation between ICC loss and severity of the delay in GE in human DG. The group with ICC loss had more than twice gastric retention compared to those with normal ICC.

Coordinated gastric motor function relies on intact intrinsic and extrinsic sensorimotor innervation. It requires acetylcholine and purinergic mediated excitatory stimuli²⁵ but also VIP, carbon monoxide and nitric oxide (NO) mediated inhibitory stimuli.²⁶ Abnormalities in both of these ENS components have been described in animal models of DG and humans. Most common being loss of expression of nNOS.⁷ Both nNOS knockout²⁷ and pharmacological inhibition²⁸

have been associated with delayed GE in animal models. Human data on abnormalities in nNOS and other components of ENS is sparse and is mostly in form of case reports of diabetics with or without gastroparesis^{8,10} with nearly no data on whether these changes associate with gastric function and clinical symptoms. Even less is known about ENS changes in IG^{11,29}. The current study provides the first comprehensive description of associations between ENS defects and gastric function and symptoms in both DG and IG. The decrease in pan-neuronal marker PGP9.5¹⁴ did not correlate with either GE or gastroparesis severity. This was perhaps not surprising considering relatively minor nerve dropout in DG (17%) and IG (14%) when compared with controls. A study comparing appendiceal tissue of six diabetic patients with controls found no differences in PGP9.5 expression.³⁰ Overall nerve loss as measured by PGP9.5 immunoreactivity may be less common in gastroparesis than previously thought and it does not correlate with GE or clinical symptoms. However, even minor neuronal losses when combined with other abnormalities may result in physiological changes. Also it is possible that while the sample size in the current study is the largest reported sample size may not have been sufficient to achieve adequate power to detect differences for nerves and other markers. In accordance with previous animal literature^{7,9,31,32} and case reports,^{8,10,30} myenteric and muscle nNOS expression trended to decrease in our DG and IG compared to controls, but this did not reach significance. In addition, these changes did not correlate with GE or clinical severity. It remains to be determined if measurement of other forms of nNOS such as dimerized nNOS correlates better with clinical features.³³ Likewise, substance P immunostain trended towards a decrease in gastroparetics compared to controls ($P = 0.06$) but was also not associated with clinical features or GE. Quantitative immunostaining for VIP or extrinsic innervation (TH) were not different in gastroparetics as compared to controls and were not associated with GE or symptom severity.

Recent work has highlighted a potential role for immune cells in the pathophysiology of gastroparesis. In an animal study, development of diabetes was associated with activation of a population of heme oxygenase-1 (HO1) positive M2 macrophages (alternatively activated, antiinflammatory).³⁴ Mice that developed delayed GE showed selective loss of these macrophages and activation of HO1 negative M1 macrophages (proinflammatory). In addition to our report on myenteric CD45 immune cell infiltrate in both DG and IG,¹⁴ Parkman *et al.* have also shown

presence of immune infiltrate in a separate cohort of DG³⁵ and Zarate *et al.* reported lymphocytic immune infiltrate in muscle layer of a single patient with IG.¹¹ In this study, we find that IG with an infiltrate scored higher on average GCSI, especially the nausea subscale suggesting that these cells might be important in clinical symptomatology of patients with IG. On visual grading, gastroparetics differed from controls on staining for macrophages (CD68) and not T (CD3, CD4, CD8) or B lymphocytes (CD79) suggesting that this infiltrate is from macrophages. A limitation is that CD68, the most commonly used marker for human macrophages^{36–38} is not entirely specific. Future studies are required to explore subpopulations of human macrophages and secreted cytokines to determine if selective loss or gain of a subtype of these macrophages is more commonly associated with gastroparesis.

This study highlights the difficulties in correlating symptoms with physiological or pathophysiological end points. The association between symptoms and GE has been an area of debate with several studies³⁹ finding no correlation while others correlating fullness, upper abdominal pain and reduced hunger with delayed GE.^{40,41} More recently data from the GpCRC suggests an association, albeit not very strong, between the severity of GE and symptoms.⁴ Given the central influence on symptom perception, the complex physiological basis for GE and the presence of other factors such as visceral hypersensitivity,⁴² this is not too surprising. Future studies will need to compare gastric segmental function and severity of the GE defect as well as take into account central input on symptom generation to decipher the relative contribution of each factor to symptoms. Also, the region of the human stomach that is most appropriate to biopsy and the optimal size of the biopsy to account for patchiness²¹ remains to be determined. This study also highlights potential differences in the pathophysiology of DG and IG such as correlation between GE and ICC numbers in DG but not IG and the stronger association between immune cells and symptoms in IG.

In conclusion, this study describes correlations between clinical, physiological and cellular changes in a relatively large sample of prospectively enrolled gastroparetics. The main findings of the paper are that loss of ICC is associated with development of delayed gastric emptying in DG and that non-lymphocytic myenteric infiltrate correlates with overall clinical severity and nausea in IG. Full thickness gastric biopsies may help define specific cellular abnormalities in gastroparesis some of which are associated with physiological and clinical characteristics of gastroparesis.

APPENDIX

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There were no financial, professional, or personal disclosures from any of the authors.

AUTHORS CONTRIBUTIONS

HP, TA, WS, PJP, MG, GF performed the acquisition of tissue and drafting of manuscript, critical review of the manuscript for important intellectual content, and obtained funding; HP, TA, WS, PJP, MG, GF, ML, CB, MSF-P, TS contributed to study concept and design; All authors contributed to analysis and interpretation of data, critical revision of the data for important intellectual content.

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