Diabetic Complications Consortium

Application Title: Autonomic Dysregulation and Enteric Nerve Changes in the Pathophysiology of Diabetic Gastroparesis

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1. Project Accomplishments:

This Project Accomplishments section is divided into Introduction, Executive Summary (including Study Aims), Demographics, Statistical Methods, and Glossary

Introduction: This study concerned the pathophysiology of patients with the symptoms of diabetic gastroparesis and involved the use of three areas of measurement: electrophysiology, anatomy and autonomic nervous measures. The study used both an older and newer method for each of the three areas: for electrophysiology these were 1. Serosal electrograms (sEG) vs 2. Mucosal electrograms (mEG), respectively; for anatomy, these were 3. Full thickness gastric biopsies (FTBx) vs 4. Mucosal neuronal density (MND) measures; and for autonomic measure these were 5. Traditional autonomic testing (named ACEM, which includes ANS and Electrogastrogam=EGG) versus 6. Heart rate variability, by power spectrum analysis (HRV by PSA; named ANSAR). These 6 measures were used to investigate the three aims, and their related hypotheses, as described below. These measures were reported at baseline as well as after temporary gastric stimulation, and eventually will be reported after permanent electrical stimulation. Since not all patients enrolled completed the study and since some patients have not yet had permanent GES devices and/or reached the 6-month mark for long-term data, the numbers of observations reported vary.

Executive Summary: This protocol, named Autonomic Dysregulation and Enteric Nerve Changes in the Pathophysiology of Diabetic Gastroparesis, was designed to explore the mechanisms involved in diabetic gastroparesis and had three main aims:

1. Study any associations between gastric mucosal electrograms (mEG) and gastric mucosal neuronal density (MND) in clinical DM Gp patients.
2. Investigate mucosal electrograms and gastric mucosal neuronal density associations and compare them to measures of systemic autonomic function by heart rate variability (HRV) in consecutive DM Gp patients.
3. Compare mucosal electrograms, gastric mucosal neuronal density, and heart rate variability to established measures of serosal electrograms (sEG), gastric full thickness biopsies (FTB) and traditional autonomic function testing (ANS) in patients with symptoms of DM Gp.

Study Flow

The study was conducted from April 2014 to April 2015 and enrolled 44 patients; 38 of those patients finished the protocol. The demographics of the patients enrolled is listed below. The decision to add a control group of patients with idiopathic gastroparesis was made with the purpose of comparing the two groups, whose pathophysiology may or may not be similar. Patients were enrolled with the symptoms of gastroparesis and this was independent of their gastric emptying test results much like the NIH GPCRC has done in their registry studies. The three main aims were analyzed by the following statistical methods which are detailed in the text below.

Study Aims:

Aim 1: The data supported Aim 1 in mucosal EG amplitude and mucosal neuronal volume and, although not statistically significant, the results were similar for both DM and ID patients.

Aim 2: The data analyzed supported Aim 2 was much greater (reported as initial and then further associations) than for Aim 2 and included subsets of:

A. Initial Mucosal Neuronal Density via Length and Density as Predictors vs. outcome variables and there were 6 associations found.

B. Initial Mucosal Frequency, Amplitude and Ratio as Predictors vs. outcome variables and there were 8 associations found.

C. Further mucosal nerve density via length and volume as predictors vs. outcome variables, with 7 associations found.

D. Further mucosal frequency, mucosal amplitude, or mucosal FAR, with a number of outcome measures, with 10 associations found.

Aim 3: The data supporting Aim 3 was also greater than for Aim 1 and is divided into sub-and then further sub-areas for Serosal EG measures:

A. Serosal Electrogram (EG) measures: (A-1. frequency, A-2. amplitude and A-3. ratio) as a predictor vs. outcome variables, which had a total of 9 associations found.

B. ICC (B-1) and S100 (B-2) as predictors vs. outcome variables, with 6 associations found.

C. Mast Cells as predictor vs. outcome variables, with 18 associations found.

The text of this report discusses the Progress in Aims for each aim separately.
Briefly, there was some support for Aim 1, but much greater support for Aims 2 and 3.

Demographics: The study enrolled a total of 44 patients starting 3/18/14 until 4/17/15. Six subjects were withdrawn before completion for a variety of reasons that did not allow them to finish the protocol. The remaining 38 individuals were divided by etiology: 19 diabetic and 19 idiopathic patients. The diabetic population consisted of 14 females (10 White, 4 African-American=AA) and 5 males (3 white, 2 AA). The mean age was 45.6 yrs. (range 25-69) and the mean Body Mass Index (BMI) was 31.27 (range 19.02-44.1). The idiopathic population consisted of 12 females and 7 males. All were white except for 1 female African American. The mean age was 42.3 years (range 31-58) and the mean BMI was 32.04 (range 21.34-53.3)

Statistical Methods of Analysis: Generalized linear models were used to quantify differences between cell types in morphological characteristics of enteric cells, such as counts and cell size, as well as electrophysiological measures. Other outcomes such as symptom severity scores, inflammation, histologic quantification, autonomic testing and gastric emptying times were analyzed in a similar manner. Effects were adjusted for confounding variables of subject age, gender, race, and BMI and stratified models were estimated, to examine effect modification by diabetic group. The overall approach taken was hypothesis generating and p-values are not adjusted for multiple testing. Results were reported as slope, 95% CI and p values for relationships. Baseline data was labeled as 0 and data after temporary stimulation was labeled as 1 in the data reported.

Glossary of terminology and abbreviations (if applicable)

Electrogram	EG
Electrogastrogram	EGG
Mucosal	m
Serosal	s
Mucosal Neuronal Density	MND
Mucosal Neuronal Length	MNL
Mucosal Neuronal Volume	MNV
Full Thickness GI Biopsy	FTB
Interstitial Cells of Cajal	ICC
S100 protein immunostain	S-100
Mast Cells	Mast Cells
Autonomic Nervous System	ANS
Adrenergic	Adrenergic
Cholinergic	Cholinergic
Enteric Nervous System	ENS
Metabolic	Metabolic
ACEM system	ACEM
Heart Rate Variability	HRV
Power Spectrum Analysis	PSA
ANSAR system	ANSAR
Diabetes Mellitus	DM
Idiopathic	ID
Body Mass Index	BMI
Gastroparesis	Gp

Specific Aims:

AIM ONE

Specific Aim 1. Study any associations between gastric mucosal electrograms (mEG) and gastric mucosal neuronal density (MND) in clinical DM Gp patients.

Hypothesis for Aim 1: Abnormalities of gastric mucosal electrograms are related to abnormal findings of gastric mucosal neuronal density.

0. Data Values Summarized and used for statistical comparisons:

Outcomes

Variable	Obs	Mean	Std. Dev.	Min	Max
mucfreq0	43	4.695349	1.410581	2.75	7.5
mucamp0	43	1.340698	0.9373605	0.07	4
mucfar0	43	8.17998	13.27952	0.9375	75

Predictors

Variable	Obs	Mean	Std. Dev.	Min	Max
mnl0	42	1.155974	0.6393467	0.0825	3.0476
mnv0	42	3.836388	2.03039	0.4673	8.6452

Obs=number of observations
Note: mnl0 & mnv0 were multiplied by 1000 for better unit translation

Comparisons for Aim 1:

Mucosal electrophysiology and mucosal neuronal density measures

1. Mucosal amplitude vs. mucosal neuronal density at baseline. **Relationship**-- Mucosal Amplitude- and idiopathic Gp had relationship with Mucosal Nerve Volume, as follows:

   Slope 0.160, CI (-0.087,0.407), p=0.185.

Figure 0— (see middle bottom row panel for this relationship):

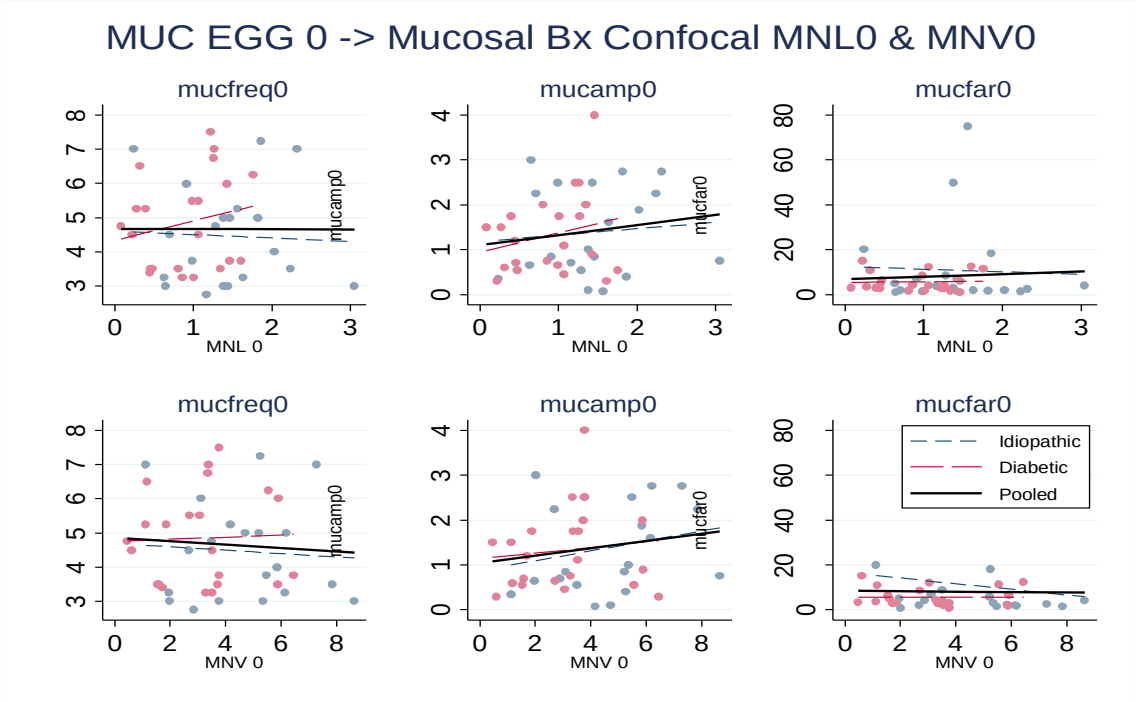


Figure 0: for mucosal amplitude and mucosal neuronal volume there is an assocation that is not present for frequency.

Progress in Aim One--This suggests that with increased Mucosal Neuronal Density, the measure of MNV (mucosal neuronal volume) at baseline, EG Mucosal Amplitude increases, but this was not true for frequency. Although this was not statistically significant, the relationship was similar for DM Gp and for the whole pooled groups as is noted in the lower mid panel of figure 0 above which is reproduced in figure 1 below.

AIM TWO

Specific Aim 2: Investigate mucosal electrograms and gastric mucosal neuronal density associations and compare them to measures of systemic autonomic function by heart rate variability (HRV) in consecutive DM Gp patients.

Hypothesis for Aim 2: Abnormalities of gastric mucosal electrograms and gastric mucosal density are related to abnormalities of autonomic function measured by heart rate variability.

0. Data values summarized and used for statistical comparisons:

Predictors

Section	Variable	Obs	Mean	Std. Dev.	Min	Max
1	mnl0	42	1.155974	0.6393467	0.0825	3.0476
1	mnv0	42	3.836388	2.03039	0.4673	8.6452
2	mucfreq0	43	4.695349	1.410581	2.75	7.5
2	mucamp0	43	1.340698	0.9373605	0.07	4
2	mucfar0	43	8.17998	13.27952	0.9375	75
3	mucfreq1	38	3.965	0.8354696	3	6.5
3	mucamp1	38	1.162895	0.892394	0.05	3.5
3	mucfar1	38	15.2736	23.25164	1	86

Outcomes

Section	Variable	Obs	Mean	Std. Dev.	Min	Max
1	maxhr0	43	87.83721	12.55641	60	126
1	minhr0	43	71.74419	11.69064	46	103
1	rrchg0	43	15.86047	5.688675	6	28
1	br	43	25.02628	15.83371	7.79	108
1	valsalvara~0	43	1.353721	0.1389934	1.11	1.75
1	ra3015tio0	43	1.31807	1.37332	0.9	10.1
1	autparest0	43	119676.3	120456.1	6480	552000
1	autpaarmdn0	43	30572.67	32148.51	450	168000
1	autpaarmup0	43	71420.93	87693.2	900	386000
1	autpastdg0	42	25371.67	28261.13	2100	159600
1	aupar0	43	6.927907	7.472404	2	46.7
1	autpacold0	43	38700.28	53103.84	312	285600
1	aupervc0	43	67.79535	19.23132	19.6	96
1	eggsupine0	43	4.211744	0.8367681	2.7	5.9
1	eggstdg0	42	4.429762	1.023863	2.82	7.56
1	eggcold0	43	4.563488	1.101996	2.7	7
1	bmeanhr0	43	81.4186	13.07894	44	106
1	brangehr0	41	20.04878	11.82149	4	56
1	bsympmod0	43	3.078605	3.315498	0.02	11.73
1	bparasmod0	43	1.707674	2.73063	0.06	12.39
1	bsympvagal~0	43	2.883488	2.420657	0.2	13.09
1	bsbp0	42	129.381	18.88857	90	178
1	bdbp0	42	78.45238	14.77176	43	112
1	dbpara0	42	18.39262	36.8819	0.31	235.82
1	dbrangehr0	42	17.42857	12.87301	3	66
1	dbsbp0	42	125.6667	19.45685	90	172
1	dbdbp0	42	74.69048	13.30595	45	110

1	vsymp0	43	31.26209	37.17827	0.08	172.81
1	vpara0	43	3.796744	6.016214	0.04	26.76
1	vrangehr0	43	25.27907	15.91544	4	63
1	vsbp0	41	125.4878	22.12817	77	169
1	vdbp0	41	78.60976	14.22125	47	115
1	smeanhr0	41	89.26829	13.70588	54	127
1	srangehr0	41	33.43902	43.57927	7	238
1	ssymp0	41	14.05122	64.37029	0.01	413.71
1	spra0	41	19.66388	109.5431	0.019	701.7
1	ssbp0	41	127.4878	21.13306	83	185
1	sdbp0	41	79.2439	13.99604	48	118
2	maxhr1	41	88.7561	12.96877	60	115
2	minhr1	41	73.07317	12.84988	44	107
2	rrchg1	41	15.4878	6.173014	6	28
2	ew	41	21.79683	9.761236	6.5	40.58
2	valsalvara~1	41	1.308537	0.1548961	1.08	1.8
2	ra3015tio1	41	1.747683	3.990641	0.92	26.67
2	autparest1	41	93740.49	77195.33	19800	388800
2	autpaarmdn1	41	21052.44	16865.13	2400	66000
2	autpaarmup1	40	49789	55665.48	960	237600
2	autpastdg1	41	18247.07	14726.04	1800	76800
2	aupar1	41	7.121951	6.596193	2	39
2	autpacold1	41	20776.83	17985.58	110	93600
2	aupervc1	41	72.50317	20.19671	22.2	99
2	eggsupine1	41	4.715244	1.136052	3	8.4
2	eggstdg1	41	4.887561	1.054758	2.8	7.5
2	eggcold1	41	4.885366	1.203277	1	7
2	bmeanhr1	41	83.41463	13.11102	52	118
2	brangehr1	41	31.58537	43.33819	3	211
2	bsympmod1	40	4.24675	5.785235	0.01	27.69
2	bparasmod1	41	6.077073	21.36854	0.03	132.89
2	bsympvagal~1	41	3.04122	2.488333	0.39	12.69
2	bsbp1	41	132.439	19.61383	93	193
2	bdbp1	41	81.70732	12.16603	48	104
2	dbpara1	41	1840.15	11663.3	1.4	74700
2	dbrangehr1	41	24.21951	31.5567	3	196
2	dbsbp1	37	130.8378	22.36877	96	193
2	dbdbp1	37	77.21622	12.09485	54	99
2	vsymp1	41	30.10561	38.24004	0.39	152.01
2	vpara1	40	11.3804	55.07188	0.016	349.94
2	vrangehr1	41	27.58537	19.7838	2	104
2	vsbp1	39	136.3333	21.18382	88	209
2	vdbp1	39	81.84615	10.67841	56	102
2	smeanhr1	41	92.7561	14.40101	62	128
2	srangehr1	41	35.87805	40.42165	8	214
2	ssymp1	41	6.088659	13.17151	0.01	79.45

2	spra1	41	8.45	36.80931	0.02	228.39
2	ssbp1	41	130.5122	22.27905	74	192
2	sdbp1	41	81.90244	16.18457	50	137

Comparisons for Aim 2:

A. Initial Mucosal Nerve Length and Volume Data as predictors vs. outcome variables

(Example of how this data is reported in the rest of the text)

For ACEM minimal heart rate versus mucosal nerve length:

1. Mucosal neuronal length vs. minimal heart rate: **Relationship**--
   There is a relationship between minimal heart rate and mucosal nerve length for the pooled values of both groups as noted here by slope, 95% CI and p value:
   Slope -5.947, CI (-11.349,-0.545), p=0.032.

(Subsequent data is reported in the same format: slope, CI and p value.)

Progress in Aim 2--This suggests that the autonomic parameter, by traditional autonomic testing, of heart rate correlates with the mucosal neuronal nerve length. Thus, heart rate variability might correlate with ANS measures. (*See end of Aim 2 for further discussion of Progress in Aim 2.)*

2. Mucosal Nerve Volume vs. Valsalva ratio at baseline: **Relationship—**MND reported as volume related to ANS Valsalva in ID (but not DM or pooled) Gp patient groups.

   Mucosal Nerve Volume:
   Slope -0.030 CI (-0.060, 0.001) p value p=0.055

3. Mucosal Neuronal Length and Volume vs ACEM Baseline-TPA REST: **Relationship**--
   MND related to adrenergic PAR in ID and pooled groups.

   Mucosal Nerve Length:
   Slope-50184.884 CI (-109123.453, 8753.685) p value p=0.093 for pooled group

   Mucosal Nerve Volume:
   Slope -15828.061 CI (-34139.035, 2482.913) p value p=0.088 for pooled group

4. Mucosal Neuronal Length and Volume vs. ACEM Baseline-TPA COLD: **Relationship**--
   MND related to pooled (both patient groups) for adrenergic PAR.

Mucosal Nerve Length:
Slope-23875.799 CI (-50971.482, 3219.884) p value p=0.082 for pooled group

Mucosal Nerve Volume:
Slope -7764.134 CI (-16157.188,628.919) p value p=0.069 for pooled group

5. Mucosal Neuronal Length and Volume vs. ACEM Baseline-EGG Standing: **Relationship**--MND related to enteric EGG for DM Gp patients. MNV vs EGG baseline

   Mucosal Nerve Length:
   Slope -0.916 CI (-1.798, -0.033) and p value: p=0.043 for DM patients

   Mucosal Nerve Volume:
   Slope-0.237 CI (-0.486,0.011) p value p=0.060 for DM patients

6. Mucosal Neuronal Length and Volume vs. ACEM Baseline-EGG COLD: **Relationship**-- MND related to enteric EGG for DM Gp patients at baseline.

   Mucosal Nerve Length:
   Slope -0.942 CI (-1.939, 0.055) p value p=0.062 for DM group

   Mucosal Nerve Volume:
   Slope -0.248 CI (-0.528, 0.032) p value p=0.079 for DM patients

B. Initial Mucosal Electrogram (EG) Frequency, Amplitude and Ratio as Predictors vs outcome variables

1. Mucosal EG frequency vs. ACEM-Max HR: **Relationship**--
   mEG frequency related to Maximum heart rated for ID and pooled patient groups.

   Slope 2.369 CI (-0.311, 5.048) p value p=0.081 for pooled patients

   Slope 6.358 CI (3.017, 9.700) p value p=0.001 for ID patients

2. Mucosal EG frequency vs. ACEM-RR Change : **Relationship--** MEG frequency related to EKG r to r interval for ID and pooled patient groups

   Slope 1.245 CI (0.012, 2.478 p value p=0.048 for pooled patients

   Slope 2.385 CI (0.940, 3.830) p value p=0.003 for ID group

3. Mucosal EG frequencies vs. ACEM Baseline- Vasoconstriction: **Relationship—**MEG frequency related to vasoconstriction for pooled patient groups at baseline

Slope 4.534 CI (0.320, 8.747) p value p=0.036

4. Mucosal EG Amplitude vs. mucosal neuronal volume: **Relationship--**
   MEG amplitude related to MND as volume for DM and for pooled patient groups

   Slope 5.617 CI (0.127, 11.108) p=0.045 for pooled group

   Slope 14.141 CI (4.365, 23.916) p=0.007 for DM group

5. Mucosal Frequency vs parasympathetic modulation:
   Relationship--Between Mucosal EG Frequency, and baseline parasympathetic modulation by HRV, for pooled patients.

   Slope 1.097 CI (-0.045, 2.240) p value p=0.059 for pooled group

6. Mucosal Frequency vs. ANSAR-Deep Breathing-Parasympathetic:
   Relationship--Between Mucosal EG Frequency and parasympathetic modulation by HRV for ID and pooled patients

   Slope 19.881 CI (5.532, 34.230) p value p=0.008 for pooled group

7. Mucosal EG Amplitude vs. ANSAR-Baseline Parasympathetic Modulation

   Relationship-- Between mEG amplitude and parasympathetic modulation for DM group at baseline

   Slope -1.518 CI (-3.234, 0.197) p value p=0.079

8. Mucosal EG Ratio vs. ANSAR-Baseline Parasympathetic Modulation:
   Relationship--Between mEG FAR and parasympathetic modulation for DM and pooled groups at baseline

   Slope 0.050 CI (0.011, 0.089) p=0.013 for pooled patients

   Slope 0.078 CI (0.037, 0.119) p=0.001 for DM patients

C. Further Mucosal nerve density via length and volume as predictors vs. outcome variables for autonomic and enteric tests

1. Mucosal Neuronal Length and Volume vs. ACEM Temporary Postural Adjustment Ratio (PAR): **Relationship--** Between MND and PAR after temporary stimulation for DM group

Mucosal Nerve Length:
Slope 3.309 CI (0.149, 6.469) p=0.041 for DM patient group

Mucosal Nerve Volume:
Slope 0.835 CI (-0.043, 1.714) p=0.061 for DM patients

2. Mucosal Neuronal Length and Volume vs. ACEM Temp-EGG Supine: **Relationship--** Between MND (length and volume) and EGG, after temporary stimulation, for DM group.

Mucosal Nerve Length:
Slope -0.880 CI (-1.884, 0.125) p=0.081 for DM patient group

Mucosal Nerve Density:
Slope -0.229 CI (-0.504, 0.047) p=0.097 for DM patients

3. Mucosal Neuronal Length and Volume vs. ANSAR-Baseline Range HR: **Relationship--** Between MND and heart rate range after temporary stimulation for ID group.

Mucosal Nerve Length:
Slope -35.370 CI (-68.489, -2.251) p=0.038 for ID patient group

Mucosal Nerve Density:
Slope -9.318 CI (-20.634, 1.998) p=0.099 for ID patients

4. Mucosal Neuronal Length and Volume vs. ANSAR-Baseline Parasympathetic Modulation: **Relationship**--Between MND and parasympathetic modulation for ID group.

Mucosal Nerve Length:
Slope -23.408 CI (-45.578,-1.237) p=0.040 for ID group

Mucosal Nerve Density:
Slope -6.236 CI (-13.783,1.311) p=0.098 for ID group

5. Mucosal Neuronal Length and Volume vs. ANSAR-Deep Breathing Range HR: **Relationship--**Between MND (length and volume) and deep breathing for DM group by HRV

Mucosal Nerve Length:
Slope 10.506 CI (0.990, 20.023) p=0.033 for diabetic (DM) patient group

Mucosal Nerve Density:
Slope 2.727 CI (0.099,5.355 p=0.043 for DM patients

6. Mucosal Neuronal Length and Volume vs. ANSAR-Valsalva Range HR:
   Relationship--Between MND (length and volume) and Valsalva range heart rate after stimulation, for the ID group.

   Mucosal nerve length:
   Slope -19.651 CI (-33.793,-5.509) p=0.010 for ID group

   Mucosal nerve volume:
   Slope -6.162 CI (-10.795,-1.529) p=0.013 for ID group

7. Mucosal Neuronal Length and Volume vs. ANSAR-Valsalva Diastolic B/P:
   Relationship-- Between MND and Valsalva after stimulation for ID group.

   Slope 3.153 CI (0.142, 6.163) p=0.041 for ID group

D. Further Comparisons of the predictors: mucosal frequency, mucosal amplitude, or mucosal FAR with a number of outcome measures:

1. Mucosal Frequency and MND vs. Mucosal Nerve Volume for ID patients after temporary stimulation:
   Relationship--between mEG frequency and MND via Mucosal Nerve volume for ID patient group after temporary stimulation for the ID group.

   Slope -3.848 CI (-6.894, -0.803) p value p=0.017

2. Mucosal EG Frequency vs. ACEM-total pulse amplitude after temporary stimulation:
   Relationship--mEG frequency and Total Pulse Amplitude Arm Down after temp stimulation for pooled patients

   Slope -5632.540 CI (-9585.775,-1679.304) p value p=0.007 for pooled group

3. (Mucosal EG Frequency vs. ACEM Baseline-TPA Standing: **Relationship--**
   Between mEG frequency and total pulse amplitude after temp stimulation for ID and DM subgroups but with different slope directions for each group.

   ID group
   Slope -4395.532 CI (-9211.526, 420.462) p value p=0.071

   DM group
   Slope 4405.919 CI (-756.310, 9568.148) p value p=0.089

4. Mucosal EG Frequency vs. ACEM Baseline-TPA Cold: **Relationship--**

Between mEG Frequency and total pulse amplitude with cold stress after stimulation for pooled groups.

Slope -4835.406 CI (-9450.327, -220.486) p value p=0.041 for pooled group

5. Mucosal EG Frequency vs. ANSAR-Baseline Mean HR: **Relationship--**
Between mEG Freqency and mean heart rate after temporary stimulation for ID and pooled groups.

Slope 3.343 CI (0.247, 6.438) p value p=0.035 for pooled group

6. Mucosal EG Frequency vs. ANSAR-Valsalva Range HR after temporary stimulation: **Relationship--**Between mEG frequency and Valsalva after temporary stimulation for ID group.

Slope 8.486 CI (0.077, 16.895) p value p=0.048 for ID patients

7. Mucosal EG Frequency vs. ANSAR-Standing Systolic Blood Pressure after temporary stimulation: **Relationship--**Between mEG frequency and standing systolic blood pressure after stimulation for ID group.

Slope -6.663 CI (-13.154, -0.173) p value p=0.045 for ID group

8. Mucosal EG amplitude and ratio vs. ACEM-RR Change:
Relationship--Between mEG Mucosal Amplitude and for FAR with RRI change with respiration after stimulation for ID group.

Slope 2.427 CI (0.296, 5.150) p value p=0.076 for ID patients

Mucosal Ratio
Slope-0.146 CI (-0.291,-0.000) p value p=0.050 for ID patient group

9. Mucosal EG ratio vs. ANSAR-Valsalva Sympathetic Response:
Relationship--Between mEG FAR and Valsalva after stimulation for pooled groups.

Slope 0.980 CI (0.084,1.876) p value p=0.033 for pooled groups

10. Mucosal EG Ratio vs. ANSAR-Standing Sympathetic Response: **Relationship--**Between mEG FAR with standing sympathetic response after stimulation for DM group.

Slope 0.152 CI 0.050, 0.253) p value p=0.007

Progress in Aim Two: Multiple relationships were found for Mucosal Neuronal Density (MND) via Length and Volume and a variety of outcome measures, largely those involving the ANS and the ENS. The primary findings were with the traditional ANS and ENS measures via the ACEM

system. All of these support the hypothesis related to Aim 2, regarding MDN and EG measures and systemic autonomic measures, whether by HRV or more traditional techniques
Multiple relationships were also found between Mucosal Electrogram (mEG) measures and a variety of outcome measures also involving ANS measures. These relationships were present for both the traditional ANS/ENS system of ACEM but also with HRV by PSA via the ANSAR system.
Additional analysis of MND and mEG with outcome variables added to the above observations and suggest that both traditional and newer system of measure ANS might be helpful in Diabetic, and non-diabetic/idiopathic Gp patients.
Figure 2 is an example of one of the findings from Specific Aim 2.

AIM THREE

Specific Aim 3. Compare mucosal electrograms, gastric mucosal neuronal density, and heart rate variability to established measures of serosal electrograms (sEG), gastric full thickness biopsies (FTB) and traditional autonomic function testing (ANS) in patients with symptoms of DM Gp.

Hypothesis for Aim 3: That the newer measures of gastric mucosal electrograms, gastric mucosal neuronal density and heart rate variability correspond to the traditional measures of gastric serosal electrograms, gastric full thickness biopsies and traditional autonomic function testing.

0. Data Values summarized and used for statistical comparisons:

Predictors

Variable	Obs	Mean	Std. Dev.	Min	Max
serfreq0	37	4.889189	1.349492	3.25	8.5
seramp0	37	0.8202703	0.592722	0.02	2.5
serfar0	37	21.00949	43.04905	1	225
fibrosis0	39	0.5128205	0.5063697	0	1
iccin0	36	2.494444	1.932126	0	7
s100in0	38	10.67632	5.481982	2.2	23.5
mastin0	31	2.267742	1.192585	0.5	5
iccout0	36	1.911111	1.590468	0	6.3
s100out0	38	7.894737	3.113263	2.3	16
mastout0	31	1.880645	1.040647	0.1	4.1
cd4hpfmy0	36	1.527778	1.547276	0	8.1
cd8hpfmy0	36	2.922222	2.311352	0.3	9.3
cd68hpfmy0	36	2.319444	3.403512	0.1	17.4
mastmy0	31	0.1677419	0.3091786	0	1

Outcomes

Variable	Obs	Mean	Std. Dev.	Min	Max
maxhr0	43	87.83721	12.55641	60	126
minhr0	43	71.74419	11.69064	46	103
rrchg0	43	15.86047	5.688675	6	28
br	43	25.02628	15.83371	7.79	108
valsalvara~0	43	1.353721	0.1389934	1.11	1.75
ra3015tio0	43	1.31807	1.37332	0.9	10.1
autparest0	43	119676.3	120456.1	6480	552000
autpaarmdn0	43	30572.67	32148.51	450	168000
autpaarmup0	43	71420.93	87693.2	900	386000
autpastdg0	42	25371.67	28261.13	2100	159600
aupar0	43	6.927907	7.472404	2	46.7
autpacold0	43	38700.28	53103.84	312	285600
aupervc0	43	67.79535	19.23132	19.6	96
eggsupine0	43	4.211744	0.8367681	2.7	5.9
eggstdg0	42	4.429762	1.023863	2.82	7.56
eggcold0	43	4.563488	1.101996	2.7	7
bmeanhr0	43	81.4186	13.07894	44	106
brangehr0	41	20.04878	11.82149	4	56
bsympmod0	43	3.078605	3.315498	0.02	11.73
bparasmod0	43	1.707674	2.73063	0.06	12.39
bsympvagal~0	43	2.883488	2.420657	0.2	13.09
bsbp0	42	129.381	18.88857	90	178
bdbp0	42	78.45238	14.77176	43	112
dbpara0	42	18.39262	36.8819	0.31	235.82
dbrangehr0	42	17.42857	12.87301	3	66
dbsbp0	42	125.6667	19.45685	90	172
dbdbp0	42	74.69048	13.30595	45	110
vsymp0	43	31.26209	37.17827	0.08	172.81
vpara0	43	3.796744	6.016214	0.04	26.76
vrangehr0	43	25.27907	15.91544	4	63
vsbp0	41	125.4878	22.12817	77	169
vdbp0	41	78.60976	14.22125	47	115
smeanhr0	41	89.26829	13.70588	54	127
srangehr0	41	33.43902	43.57927	7	238
ssymp0	41	14.05122	64.37029	0.01	413.71
spra0	41	19.66388	109.5431	0.019	701.7
ssbp0	41	127.4878	21.13306	83	185
sdbp0	41	79.2439	13.99604	48	118
maxhr1	41	88.7561	12.96877	60	115

minhr1	41	73.07317	12.84988	44	107
rrchg1	41	15.4878	6.173014	6	28
ew	41	21.79683	9.761236	6.5	40.58
valsalvara~1	41	1.308537	0.1548961	1.08	1.8
ra3015tio1	41	1.747683	3.990641	0.92	26.67
autparest1	41	93740.49	77195.33	19800	388800
autpaarmdn1	41	21052.44	16865.13	2400	66000
autpaarmup1	40	49789	55665.48	960	237600
autpastdg1	41	18247.07	14726.04	1800	76800
aupar1	41	7.121951	6.596193	2	39
autpacold1	41	20776.83	17985.58	110	93600
aupervc1	41	72.50317	20.19671	22.2	99
eggsupine1	41	4.715244	1.136052	3	8.4
eggstdg1	41	4.887561	1.054758	2.8	7.5
eggcold1	41	4.885366	1.203277	1	7
bmeanhr1	41	83.41463	13.11102	52	118
brangehr1	41	31.58537	43.33819	3	211
bsympmod1	40	4.24675	5.785235	0.01	27.69
bparasmod1	41	6.077073	21.36854	0.03	132.89
bsympvagal~1	41	3.04122	2.488333	0.39	12.69
bsbp1	41	132.439	19.61383	93	193
bdbp1	41	81.70732	12.16603	48	104
dbpara1	41	1840.15	11663.3	1.4	74700
dbrangehr1	41	24.21951	31.5567	3	196
dbsbp1	37	130.8378	22.36877	96	193
dbdbp1	37	77.21622	12.09485	54	99
vsymp1	41	30.10561	38.24004	0.39	152.01
vpara1	40	11.3804	55.07188	0.016	349.94
vrangehr1	41	27.58537	19.7838	2	104
vsbp1	39	136.3333	21.18382	88	209
vdbp1	39	81.84615	10.67841	56	102
smeanhr1	41	92.7561	14.40101	62	128
srangehr1	41	35.87805	40.42165	8	214
ssymp1	41	6.088659	13.17151	0.01	79.45
spra1	41	8.45	36.80931	0.02	228.39
ssbp1	41	130.5122	22.27905	74	192
sdbp1	41	81.90244	16.18457	50	137

A. Serosal Electrograms vs. Outcome variables

1. Serosal EG frequency vs. vasoconstriction: **Relationship--**Between serosal frequency and adrenegric vasoconstriction at baseline for pooled groups.

   Slope 4.203 CI (-0.511,8.916) p value p=0.079

2. Serosal EG Frequency Vs.
   ACEM-EGG Supine:
   Relationship--Between serosal frequency and Cutaneous EGG at baseline for ID and pooled groups, for supine and for ID group standing.

   Supine
   Slope -0.179 CI (-0.375, 0.018) p value p=0.073 for pooled

   Standing
   Slope -0.422 CI (-0.811, -0.033) p value p=0.036 for ID patient group

3. Serosal EG Frequency Vs.
   ANSAR-Baseline Mean HR:
   Relationship--Between serosal frequency and ANS range of heart rate at baseline, for DM group.

   Slope -4.683 CI (-8.510,-0.855) p value p=0.021

4. Serosal EG Frequency vs. ANSAR-Baseline Sympathetic Modulation: **Relationship--**
   Between serosal frequency and sympathetic modulation at baseline for DM group.

   Slope -0.583 CI (-1.169, 0.003) p value p=0.051 for DM group

5. Serosal EG Amplitude vs. ANSAR-Valsalva Sympathetic Response: **Relationship--**
   Between serosal amplitude and both sympathetic and parasympathetic response at baseline for DM groups, and for parasympathetic for pooled group

   Sympathetic:
   Slope 24.598 CI (2.808, 46.388) p value p=0.030 for DM patient group

   ParaSympathetic:
   Slope 3.796 CI (0.395, 7.197) p value p=0.030 for pooled group

6. Serosal EG Amplitude vs. ACEM-Valsalva Ratio: **Relationship--**
   Between serosal amplitude and valsalva ratio after temp stimulation for ID group.

   Slope 0.097 CI (-0.013, 0.207) p value p=0.078

7. Serosal EG Amplitude vs. ANSAR-Valsalva Range HR:

Relationship--Between serosal amplitude and valsalva range of heart rate after temp stimulation for DM group

Slope 25.236 CI (12.321,38.150) p value p=0.001 for DM patient group

8. Serosal EG Amplitude vs. ANSAR B Baseline Mean HR: **Relationship--** Between serosal amplitude and HRV mean heart rate at baseline for ID group

Slope -8.466 CI (-11.111,-5.821) p value p=0.000 for ID patient group

9. Serosal EG Ratio vs. ANSAR-Baseline diastolic blood pressure: **Relationship--**Between serosal FAR and diastolic bp after temp stimulation for pooled groups

Slope -0.109 CI (-0.199,-0.018) p value p=0.020 for ID patients

B. ICC and S-100 as predictors vs. outcome variables.

1. ICC inner at baseline vs. ACEM-Max HR:
Relationship--Between ICC inner and max heart rate at baseline on ANS testing for ID patients

Slope-5.396 CI (-9.121, -1.671) p value p=0.010 for ID patient group

2. ICC outer at baseline vs. Max HR:
Relationship--Between ICC outer and max heart rate at baseline on ANS testing for ID patients

Slope-6.117 CI (-9.527, -2.706) p value p=0.003 for ID patient group

3. ICC outer at baseline vs.
ACEM-Min HR:
Relationship--Between ICC outer and min heart rate at baseline on ANS testing for ID patients

Slope -4.809 CI (-8.829, -0.789) p value p=0.025 for ID patient group

4. ICC outer at baseline vs. ANSAR-Baseline Mean HR:
Relationship--Between ICC outer at baseline and baseline mean HR on ANS testing by HRV for ID patients

Slope -8.466 CI (-11.111, -5.821) p value p=0.000 for ID patient group

5. ICC outer at baseline vs.
ANSAR-Standing Mean HR:
Relationship-- Between ICC outer at baseline and baseline standing mean HR on ANS testing by HRV.

Slope -6.445 CI (-11.958, -0.931) p=0.027 for ID patients

6. S100 Outer vs. EKG R to R interval change after temporary stimulation: **Relationship**--Between S100 on full thickness biopsy vs. EKG R to R interval change after temp stimulation for ID patient group.

Slope 0.744 CI (0.037,1.452) p=0.041 for ID patient group

C. Mast Cells as a predictor vs outcome variables.

1. Mast cell in outer layer vs total pulse amplitude arm down:
   Relationship-- Between mast cells in outer layer at baseline and baseline total pulse amplitude arm down

   Slope 14365.706 CI (1991.325, 26740.087) p=0.025 for pooled patients

2. Mast cell in outer layer vs. ACEM Baseline-TPA Arm Up: **Relationship--**Between mast cells in outer layer at baseline and baseline ANS testing.

   Slope 39356.984 CI (3397.833,75316.135) p value p=0.033 for pooled

3. Mast cell in outer layer vs ACEM Baseline-TPA Cold:
   Relationship--Between mast cells in outer layer at baseline and baseline Total pulse amplitude for ID and pooled groups.

   Slope 22149.239 CI (153.624, 44144.854) p value p=0.049 for pooled

4. Mast cell in outer layer vs. ANSAR-Standing Sympathetic Response:

   Relationship--Between mast cells in outer layer at baseline and standing sympathetic response for ID and pooled groups.

   Slope 4.217 CI (1.580,6.853) p value p=0.003 for pooled

5. Mast cell in outer layer vs. ACEM-Valsalva Ratio: **Relationship--**
   Between mast cells in outer layer and baseline Valsalva ratio after temporary GES for ID group.

   Slope -0.127 CI (-0.178, -0.076) p value p=0.002 for ID patient group

6. Mast cell in outer layer vs. ANSAR-Valsalva Diastolic BP:

Relationship--Between mast cells in outer layer at baseline and baseline valsalva diastolic blood pressure for ID, DM and pooled groups.

Slope -4.555 CI (-8.051, -1.058) p value p=0.013 for pooled patient data

7. Mast cell in myenteric plexus vs. Maximum heart rate:

   Relationship--Between mast cells in myenteric plexus vs. maximum heart rate at baseline for ID patient and pooled patient groups.

   Slope 14.087 CI (-1.479, 29.653) p value p=0.074 for pooled

8. Mast cell in myenteric plexus vs. Minimum HR
   Relationship--Between mast cells in myenteric plexus vs minimum heart rate at baseline for ID patient pooled groups.

   Slope 14.765 CI (0.225, 29.305 p=0.047

9. Mast cell in myenteric plexus vs. ACEM-TPA at Rest: **Relationship**--
   Between mast cells in myenteric plexus and total pulse amplitude at baseline for ID patient pooled groups.

   Slope 158273.563 CI (4828.524, 311718.601) p=0.044

10. Mast cell in myenteric plexus vs. ACEM Baseline-TPA Arm Down: **Relationship**--
    Between mast cells in myenteric plexus vs. total pulse amplitude at baseline for ID patient and pooled patient groups.

    Slope 48818.513 CI 6405.695, 91231.331) p value p=0.026 for pooled

11. Mast cell in myenteric plexus vs. ACEM Baseline-TPA Arm Up: **Relationship**--
    Between mast cells in myenteric plexus vs. total pulse amplitude with arms up at baseline for ID patient pooled groups.

    Slope 115683.378 CI (-10607.370,241974.127) p=0.071 for pooled

12. Mast cell in myenteric plexus vs.
    ACEM-EGG Standing: **Relationship**--Between mast cells in myenteric plexus vs Cutaneous EGG at baseline for DM and pooled groups.

    Slope -1.262CI (-2.637, 0.114) p=0.070 for pooled

13. Mast cell in myenteric plexus vs. ANSAR-Standing Sympathetic: **Relationship**--
    Between mast cells in myenteric plexus vs. standing sympathetic at baseline for ID and pooled groups.

Slope 16.723 CI (6.931, 26.514) p=0.002

14. Mast cell in myenteric plexus vs. egg-cold stress after temporary stimulation: **Relationship**--Between mast cells in myenteric plexus vs Cutaneous EGG with cold stress after temp for DM and ID groups

    Slope -3.849 CI (-6.057, -1.642) p=0.002 for DM patients

15. Mast cell in myenteric plexus vs. ANSAR Temp baseline range of heart rate **Relationship**--Between mast cells, in myenteric plexus vs. ANSAR range of heart rate for ID, and pooled, groups, after temporary stimulation.

    Slope 66.169 CI (5.591, 126.748) p=0.034 for pooled

16. Mast cell in myenteric plexus vs: ANSAR-Valsalva Parasympathetic Response): **Relationship**--Between mast cells in myenteric plexus vs ANSAR Valsalva parasympathetic response for ID and pooled groups.

    Slope 131.588 CI (54.050, 209.126) p value p=0.002 for pooled

17. Mast cell in myenteric plexus vs. ANSAR-Valsalva Systolic BP: **Relationship**--Between mast cells in myenteric plexus vs ANSAR Valsalva Systolic B/P for ID and pooled groups.

    Slope -20.784 CI (-41.838, 0.271) p=0.053 for pooled

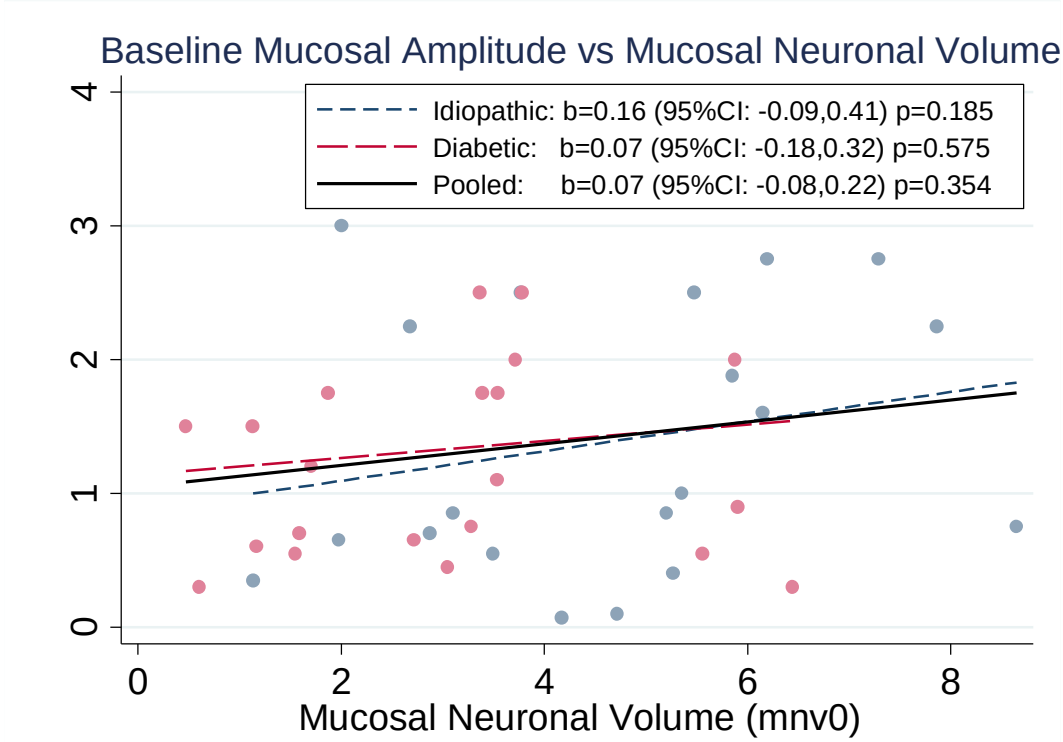
18. Mast cell in myenteric plexus vs. ANSAR-Standing Parasympathetic measure: **Relationship**--Between mast cells in myenteric plexus vs ANSAR standing sympathetic after temporary stimulation for ID and pooled groups.

    Slope 24.920 CI (9.860, 39.979) p=0.002 for pooled

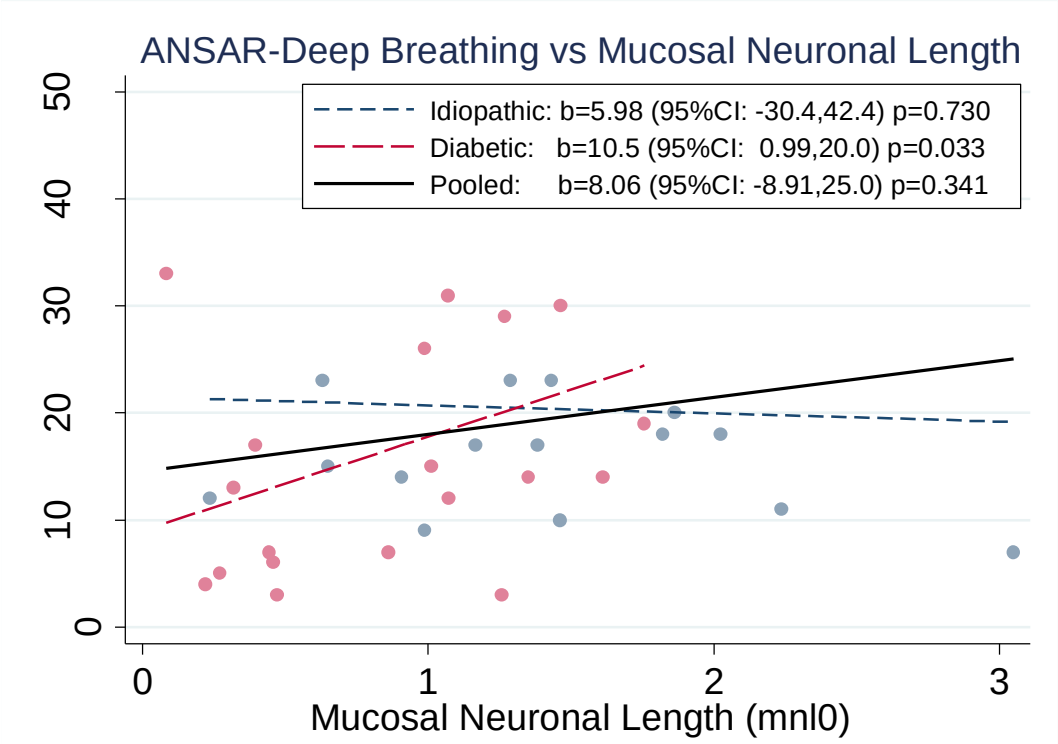
Progress in Aim Three: As in Aim 2, multiple relationships were found that support the hypothesis related to Aim 3, regarding comparisons between traditional and newer diagnostic methods. Serosal EGG measure had relationships with ANS measures, whether traditional or by HRV.
In addition, the full thickness biopsy measure of cells such as ICC and S100 showed relationships with ANS measures, whether traditional or newer approaches.
Lastly, there exist a striking number of relationships with mast cells on full thickness GI biopsy and a number of ANS measures by either technique.
The large number of relationships involving mast cells is certainly intriguing as mast cell dysfunction has long been speculated as being part of the pathophysiology of gastric motor disorders.
Figure 3 is a representative example of relationships noted when investigating the hypothesis related to Specific Aim 3.

Representative Figures from Specific Aims (SA) 1, 2, and 3.

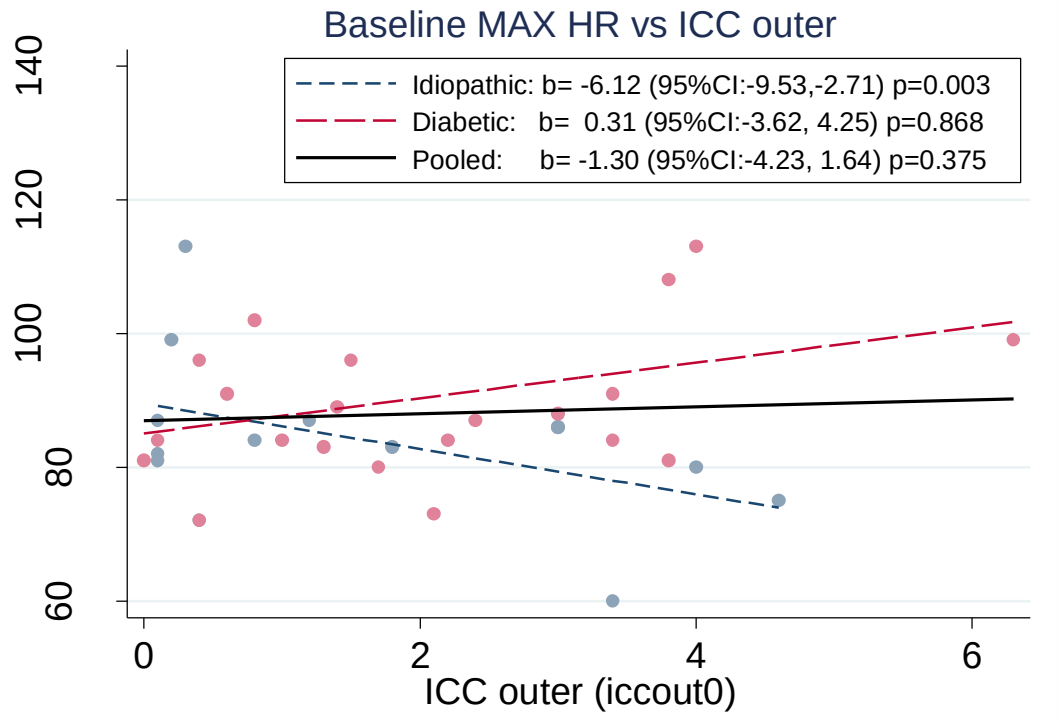
(SA1: coded as MNV0-x axis vs. mucamp0-y axis)



(SA2: Coded as mnl0—x axis vs. dbrangehr-y axis)



(SA3: Coded as iccout0- x axis vs. maxhr0-y axis)



Legends for figures 1, 2 and 3:

Figure 1: For increased Mucosal Neuronal Density at baseline, as the measure of MNV (mucosal neuronal volume) increases; EG Mucosal Amplitude increases for both groups, but this was not statistically significant. See Specific Aim 1.

Figure 2: As Mucosal Neuronal Length increases at baseline, the autonomic measure of deep breathing by HRV increases for the diabetic, but not the idiopathic subgroup. See Specific Aim 2.

Figure 3: At baseline, as the FTB measure of ICC Outer increases the ACEM ANS measure of maximum heart rate decreases significantly for the idiopathic but this relationship does not exist in the diabetic subgroup. See Specific Aim 3.

Future analysis from the data collected from this study:
Some of the long-term patient data was not finished by Sept 2015 and can be reported in an addendum once the data is collected and analyzed.
Although the study was designed with three aims, the large amount of data collected may allow some additional investigation in the future, using either the data collected so far or as part of additional studies.

Three additional aims and related hypothesis are listed below:

Aim 4— Examine symptoms and gastric emptying at baseline, at temporary and then permanent GES in patients with DM Gp and control ID Gp patients.
Hypothesis for Aim 4: That (GI) symptoms and gastric emptying at baseline, at temporary and then permanent GES will differ between DM Gp and control ID Gp patients.

Aim 5--Look at Neurohormonal Changes at baseline, at temporary and then permanent GES in patients with DM Gp and control ID Gp patients.
Hypothesis for Aim 5: That neurohomonal changes at baseline, at temporary and then permanent GES in patients will differ between DM Gp and control ID Gp patients.

Aim 6—Examine energy of stimulation with temporary and then permanent GES in patients with DM Gp and control ID Gp patients and related this to symptoms response when accounting for the Cajal (CD117) density of the patients.
Hypothesis for Aim 6: That energy of stimulation with temporary and then permanent GES in patients will differ between DM Gp and control ID Gp patients and are related to symptom response when accounting for the Cajal (CD117) density of the DM and ID patients.

Additional Future Work that this Data May Influence:
The findings that mucosal neuronal density (MND), which is a less invasive test that full thickness biopsy (FTBx), may provide useful information may lead to further investigation with the mucosal approach.
Likewise the finding that mucosal electrograms (EG), which is also less invasive that serosal EG, may provide useful data, may allow further investigation with this mucosal approach.
Both mucosal approaches can be undertaken endoscopically, making them much less invasive.

An additional area is the data from FTBx regarding the ICC and the degree of Mast Cell activity, which may direct further work with both Diabetic and Idiopathic Gastroparesis patients. Full thickness biopsy work is already focused on ICC measures, but little work has been done of the presence of Mast cells.

Summary and Conclusions based on analysis of this data as of September 2015

The data from this study, Autonomic Dysregulation and Enteric Nerve Changes in the Pathophysiology of Diabetic Gastroparesis, has allowed investigation of 3 Aims and their related hypotheses. The study has allowed for comparisons between patients with the symptoms of Gastroparesis, whether diabetic gastroparesis, as the main focus of the grant, or idiopathic gastroparesis as a contrasting group. The data has showed relationships not fully appreciated before regarding Enteric Anatomy and Physiology as well as Systemic Autonomic Function. Several unexpected findings, especially regarding the possible role of mast cells in the pathophysiology of gastroparetic syndromes, need further investigation.

Publications:

Abstracts DDW 2015:

1. Chirag Patel, Kaartik Soota, Xiu Yang, Siraj M. El Jamal, Mostafa M. Fraig, Karen Beatty, Ed Miller, Archana Kedar, Michael G. Hughes, Lindsay McElmurray, Abigail M. Stocker, Thomas L. Abell. Su1864 Clinical Correspondence of Interstitial Cells of Cajal Values From Full Thickness Gastric Biopsy in Patients Undergoing Gastric Stimulation for Diabetic Gastroparesis. Gastroenterology 04/2015; 148(4):S-537. DOI:10.1016/S0016-5085(15)31800-X

2. Samir Vermani, Aniruddh A. Patel, Ed Miller, Karen Beatty, Abigail M. Stocker, Michael G. Hughes, Hani Rashed, Archana Kedar, Thomas L. Abell. Su1422 Autonomic and Enteric Responses to Temporary Gastric Electrical Stimulation. Gastroenterology 04/2015; 148(4):S-505. DOI:10.1016/S0016-5085(15)31693-0

3. Chirag Patel, Xiu Yang, Gwen Wendelschafer-Crabb, William R. Kennedy, Karen Beatty, Ed Miller, Archana Kedar, Michael G. Hughes, Lindsay McElmurray, Mostafa M. Fraig, Abigail M. Stocker, Thomas L. Abell. Su1420 Evaluating Gastric Mucosal Nerve Density in Relation to Full Thickness Biopsy, Gastric Emptying, Symptoms and Response to Temporary Gastric Stimulation. Gastroenterology 04/2015; 148(4):S-504. DOI:10.1016/S0016-5085(15)31691-7

4. Chirag Patel, Thomas L. Abell, Kaartik Soota, Xiu Yang, Mostafa M. Fraig, Karen Beatty, Ed Miller, Archana Kedar, Michael G. Hughes, Lindsay McElmurray, Abigail M. Stocker, William R. Kennedy, Gwen Wendelschafer-Crabb**.** Su1851 Clinical Correspondence of S100 Levels on Full Thickness Gastric Biopsy. Gastroenterology 04/2015; 148(4):S-533. DOI:10.1016/S0016-5085(15)31787-X