Initial Assessment of the Percutaneous Electrical Phrenic Nerve Stimulation (PEPNS) System in Patients on Mechanical Ventilation

SUPPLEMENTAL DIGITAL CONTENT

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1. PEPNS Console Description and Use: Technical Aspects

The custom PEPNS Console outputs independent balanced current biphasic pulses bilaterally at user specified current levels. The resultant compliance voltages generated is a function of the resistance and impedance of the tissue between the electrodes. The compliance voltage was limited in hardware to 12.5 volts. The PEPNS Console flow and pressure sensors were zeroed before connection to the ventilator circuit to eliminate flow and pressure offsets before use. The current ventilator settings for gas composition (FiO_2), type of humidifier (Heat & Moisture Exchanger (HME) or Heated Humidifier (HH)), site barometric pressure and patient's static compliance and resistance were entered into the PEPNS user interface to compensate for the density effects on measured gas flow and work performed by the patient.

A bidirectional SpiroQuant H flow differential pressure sensor (EnviteC, Wismar, Germany) was connected to the patient circuit wye measuring the flow and pressure in and out of the patient, oriented to ensure wye flow was positive during inspiration. The mechanical ventilation (MV) inspired tidal volume, exhaled tidal volume, PEEP and respiratory rate were compared with that measured by the PEPNS Console to confirm proper connection and function of the flow sensor and pressure transducer with the attached ventilator. WOB measurements were displayed both graphically and numerically at the end of each inspiration independent of whether electrical stimulation occurred or not and displayed in real time to the clinician. Alarm settings on the PEPNS Console were set for the maximum allowable inspiratory period for electrical stimulation (typically set a 5 sec.), maximum allowable apnea period (typically set at 15 seconds), WOB high and low limits (typically set at -0.5 and 2 J/L) and maximum allowable

respiratory rate (typically set at 30 bpm). Upon detection of an alarm when electrical stimulation was active, stimulation was ceased, and an audible and visual alarm annunciated. Stimulation settings were adjusted to prevent volutrauma when patients were ventilated using pressure control and support modes.

Real time data was logged during the study during each stimulation session as shown in Supplemental Figure 1. The wye flow (Qwye), wye pressure (Pwye), Stim Signal (denoting frequency of stimulation) and Trig Signal (denoting beginning and end of inspiration) using analog output signals from the PEPNS Console. ADInstruments Labchart and PowerLab data acquisition system, was used to log data at a 1kHz sample rate for later analysis on WOB and synchrony of electrical stimulation. Independent calibrations for input signals to the LabChart system (separate from the calibrations performed on the PEPNS Console) were performed for PEPNS flow, pressure and WOB analog out signals used as input to the LabChart data acquisition system.

The Stim Signal logged by the data acquisition system was the pulse command rate sent to the waveform generator (WG) within the PEPNS system and was read directly from hardware. The Trigg Signal was output by software and logged. Data was logged from the initiation of the PEPNS setup and for each 2-hour electrical stimulation session for all six stimulation sessions, equaling 12 hours of total stimulation sessions logged for each patient.

The PEPNS Console also displayed on its graphical user interface (GUI) Supplemental Figure 2, four graph windows display one or more parameters to the clinician in real time. This

enabled the operator to visually determine the timing and effects of electrical stimulation on wye flow (Qwye), wye pressure (Pwye), WOB and diaphragm pressure effort (P_{mus}).

Stimulation parameters ranged in terms of pulse width (150, 200 and 300µs), current (0.5 to 25mA) and pulse frequency (5 to 25Hz) were set on the custom PEPNS Console based upon achieving a desired range of work of breathing (WOB 0.2 to 2) in J/L. Platinum iridium electrodes were used on the pdSTIM lead for maximal biocompatibility. Both the left and right phrenic nerve channels were setup independently after lead insertion before combining the electrical stimulation of both channels. Charge density of the electrodes was limited to 25 µC/cm^2/phase and compliance voltage to <12.5volts in hardware. Stimulation was initiated once every four breaths based upon the inspiratory trigger flow exceeding a user specified trigger sensitivity value and ceased once flow dropping below the expiratory trigger flow value. Inspiratory flows were set to prevent auto-triggering and slightly higher that the inspiratory flow sensitivity of the ventilator.

2. Measurement of Wye Pressure and Differential Wye Flow Pressure

Flow was calibrated in terms of standard temperature and pressure dry (STPD) and converted to body temperature and pressure saturated (BTPS) during use to account for gas composition, humidification type and site barometric pressure. Wye pressure and differential wye flow pressure were measured using Honeywell pressure sensors HSCDRRN002NDAA3 ± 2 inH_2O for Qwye, SSCMRRN160MDAA3 ±160 mbar for Pwye and connected to the flow sensor differential pressure lines. Patient WOB was calculated in real time during the inspiratory period

of each breath using the equation of motion and the ventilator calculated static compliance (C_{STAT}) and resistance (R_{STAT}).

$$PwyePredicted = (1/C_{STAT} * \int_0^t Qwye\ dt) + (R_{STAT} * Qwye) + PEEP \quad \text{(Equation 1)}$$

$$P_{mus} = PwyePredicted - Pwye \quad \text{(Equation 2)}$$

Where PEEP = Positive end expiratory pressure, PwyePredicted = predicted wye pressure, P_{mus} = diaphragm muscle effort exerted by patient all in cmH_2O. If C_{STAT} and R_{STAT} values were unavailable dynamic compliance and resistance values were used as initial estimates and adjusted manually such that PywyePredicted equaled the measured Pwye on breaths where no patient effort (P_{mus}) was present.

3. Measurement of Work of Breathing (WOB)

WOB was normalized to J/L and displayed to user at the end of each breath.

$$W = \int_{t0}^{t1} Pmus * Qwye\ dt \quad \text{(Equation 3)}$$

$$WOB = W / \int_0^{Ti} Qwye\ dt \quad \text{(Equation 4)}$$

Where W = work in J/L

In pressure regulated modes no discernable difference in the Pwye pressure waveforms were visible between stimulated and unstimulated pressure waveforms. Flow and volume curves were affected and show an increase in peak flow and inspired volume. The effect of electrical stimulation on each side after lead insertion was initially assessed by examining a low stimulation

pulse frequency of 5Hz on the pressure waveform (Supplemental Figure 3). Optimum pairing and polarity of electrodes was determined in all patients by examining the effect on the magnitude of WOB and the presence of a 5Hz pressure perturbation on the wye pressure waveform. In order to minimize the potential for collateral stimulation, the lowest possible stim parameters were used while achieving the desired WOB requirements. Patients were observed for signs of pain, neck, arm and shoulder twitching and leads were withdrawn in 2 to 3 mm increments if suitable electrode pairs could not be found before electrode stimulation setup recommenced. It was found that lead insertion using the lateral border of the IJ as a maximum insertion depth generally required the retraction of the lead to gain electrical stimulation.

4. Other Measurements Performed

a. Electrocardiogram (ECG)

A 12-lead ECG as a baseline before electrical stimulation during one of the 6 electrical stimulation sessions, after the completion of electrical stimulation therapy, and during any period of time dictated by standard hospital procedure. The primary purpose of recording these ECGs was to rule out cardiac, vagal and carotid sinus pacing which could precipitate an arrythmia or an asystolic event.

b. Chest X-rays

Routine chest x-rays were taken prior to initiation of electrical stimulation and at ±7 days of the 30-day follow-up visit, to determine that there were no obvious changes in lung volume, degree of atelectasis, nor the presence of an elevated hemidiaphragm as a result of

electrical stimulation. A comparison of the left, stimulated side lung and the right, non-stimulated side lung for the initial two patients was also performed.

c. Impact of Neck Movement and Positional Changes during Electrical Stimulation

The ability of the pdSTIM lead to accommodate neck movement and patient positional changes while retaining capture was also assessed and logged during electrical stimulation. Each instance of patient repositioning during the 48-hour study period was logged as part of standard medical practice at each site. The effect positional changes had if any on electrical stimulation was noted.

d. Non-Diaphragmatic Impact of Electrical Stimulation

Patients were observed for muscle twitching, if any, during stimulation to determine if any collateral stimulation or unintended nerve or muscle stimulation was occurring. Any instances were noted for their location, duration, and time.

The Critical Care Pain Observation Tool (CPOT) and Richmond Agitation & Sedation Scale (RASS) were assessed at baseline and approximately every 6 hours during the 48-hour study period. These assessments were used to objectively determine if any discomfort was caused by electrical stimulation therapy.

The effect of electrical stimulation on blood gas parameters, such as arterial oxygen (PO_2), arterial carbon dioxide (PCO_2), and other relevant parameters, was recorded. Data was

recorded from blood gases taken in accordance with hospital protocol for the 24 hours before, during, and after the 48-hour study period.

Blood gas analysis, $ETCO_2$, pulse oximetry (SpO_2), and vital signs, including heart rate, respiratory rate, temperature, and blood pressure, were monitored and recorded in a manner consistent with care under normal and phrenic nerve stimulation conditions.

5. Statistical Analysis

a. Hypothesis

*H*o: The proportion of capture is less than or equal to the performance goal (*PG*) of 80%.

Note: This hypothesis used a 90% performance goal in the protocol for the purposes of sample size calculation to ensure that there would be sufficient data to achieve the 80% performance goal when taking into account lost to follow up due to death, etc.

*H*o: $p\hat{p}1 \leq PG$

*H*A: The proportion of capture is greater than the performance goal of 80%.

*H*A: $p\hat{p}1 > PG,$

where $p\hat{p}1$ is the estimated proportion of capture from the random effects model above accounting for repeated measurements within a subject.

b. Sample Size

The following assumptions were the basis for the sample size calculation for the performance endpoint evaluating stimulation capture of the diaphragm:

- Power: 80%
- 1-sided α: 0.025
- Performance goal: 90%
- Expected success rate: 97.5%

With an anticipated success rate of 97.5% and a performance goal of 90%, 80 observations would yield ≥80% power. Note that, for the purposes of sample size calculations, the observations are assumed to be independent. However, the primary analyses will account for within patient correlation, as described below. Increasing the number of observations will increase the statistical power.

6. Other Study Results

a. Lead Adhesion and Infection

Leads were implanted for a total of 48 hours and were very easy to remove. Sutures, steri-strips or glue were not required to close the insertion wound. There were no signs of tissue adhesion visible on any of the leads removed. There were no signs of infection at the wound site throughout procedures and after lead removal.

b. Pain Level - CPOT

A total of 109 CPOT assessments were made on the 10 patients undergoing single and bilateral lead placement during the study period. The CPOT is based on four pain-related domains including the patient's facial expressions, body movements, compliance with ventilator (or voice use for non-intubated patients), and muscle tension. Each domain has

a possible score of 0 to 2. The total score can vary between 0 and 8, where 0 indicates no pain behavior and 8 indicates clear signs of pain behavior. There was no sign of pain during electrical stimulation. The mean CPOT during electrical stimulation was 0.51±0.44 versus 0.91±0.86 without electrical stimulation (Supplemental Table 4).

c. Sedation Scale - RASS

A total of 115 RASS score assessments were made on 10 patients undergoing single and bilateral lead placement for the study. The RASS measures agitation and sedation level, the score ranging from +4 to –5, where a score of 0 indicates an awake and adequate patient. Scores from –1 to –5 indicate an increasingly sedated patient, and scores from +1 to +4 indicate an increasingly irritable and agitated patient. On average, most patients were moderately to highly sedated during stim and non-stim periods. The exception being P12S01, who was only sedated during the lead insertion procedure. (Supplemental Table 5).

d. Patient Repositioning

Patients were repositioned approximately every 2 to 4 hours per hospital protocol and disease management requirements (Supplemental Table 6). Many of the patients treated in the PEPNS Study had some form of trauma, with some requiring active intracranial pressure monitoring and control. Leads were disconnected from the electrical stimulator using the extension lead when not stimming. Reestablishing stimulation after

reconnection typically took 5 to 10 minutes. One patient (P12S01) was able to sit in a chair and adjust body position autonomously while on CPAP and maintain electrical stimulation.

e. Blood Gasses

Blood gases were analyzed to determine effects, if any, were caused by PEPNS therapy. Recorded parameters included pH, pCO_2, pO_2, HCO_3, SBE, SBC, SaO_2, HB, Na^+, K^+, Cl^-, Ca^{+2}, glucose, and lactate. Electrical stimulation did not result in any observable effects either positive or negative on blood gas analysis. Changes in oxygen or carbon dioxide levels were not identified at the time of the study design as an endpoint within the study and therefore blood gas analysis was not timed to determine whether stimulation would have significantly affected these parameters.

f. Vital Signs

There was no observed effect of electrical stimulation on vital signs, including heart rate, respiratory rate, blood pressure, and temperature.

g. Weaning Time

Supplemental Table 7 reviews the time to wean from mechanical ventilation for the 10 patients with bilateral lead placement enrolled in the study. Four subjects died during the study without weaning for reasons unrelated to the study. One patient remained on ventilation beyond the 30-day follow up. For the 5 other patients, the mean time to wean

from the start of electrical stimulation was 12.6±8.3 days and the cumulative mean time to mean from the start of MV was 18.4±7.4 days.

7. Additional Diaphragm Thickness Data

a. Diaphragm Thickness Measurement Technique

DT was measured at the zone of apposition between the eighth or ninth intercostal spaces on the right side in the midaxillary line (Supplemental Figure 5). One study site used an Ultrasonix machine with a "L14-5" Linear Array probe (14 MHz - 5 MHz range) to measure diaphragm thickness. The other site used a SonoSite HFL38X transducer linear array 13-6MHz range probe to measure diaphragm thickness. See Supplemental Figure 5 below.

Diaphragm thickness was measured once daily at the end of expiration on three separate breaths with 3 thickness measurements attempted on each breath where possible, at end expiration, 24±4 hours, and at 48±4 hours.

b. Detailed Patient Diaphragm Thickness Measurement Data

Supplemental Table 8 statistically compares the fractional change of diaphragm thickness from baseline at 24 and 48 hours respectively. Patients 1 and 2 were excluded as pilot patients, and patient 6 was excluded from these analyses due to difficulties with diaphragmatic measurement resulting from the presence of pleural effusions and a high body mass.

Supplemental Table 9 lists patient specific data for the average diaphragm thickness (in cm) at baseline, 24 and 48 hours as well as the fractional change in diaphragm thickness between the baseline and 24-hour and 48-hour measurements for patients with bilateral lead placement. Patient 6 was excluded from these analyses due to difficulties with diaphragmatic measurement resulting from the presence of pleural effusions and a high body mass. Patient 5 was stimulated on the left side only most likely due to an incorrect lead position on the right side.

c. Stimulated vs. Non-Stimulated Sides

Supplemental Table 10 shows the fractional changes in diaphragm thickness for the electrically stimulated and unstimulated diaphragm sides for the same patient population described above. The number of diaphragm sides (left or right or both sides) noted in the 2nd column. The data is broken out as the whole population, the right side only and the left side only. A previous report from a sheep study concluded that electrical stimulation of the diaphragm preserves diaphragmatic architecture and minimizes or even prevents fiber atrophy on the stimulated side (1).

Supplemental Tables 11 and 12 compares the fractional relative change in diaphragm thickness between stimulated and non-stimulated patient hemidiaphragms with bilateral lead placement, except patient 6 who had a pleural effusion. While there was a trend towards increased diaphragm thickness in the stimulated patient hemidiaphragms with this trend being greater at 48 hours, this difference was not significant, likely due to the

low number of unstimulated hemidiaphragms (n=3). There was a 13% increase in diaphragm thickness in the stimulated group vs. 2.4% increase in the unstimulated group at 48hrs (P=0.1113).

d. Assist/Spontaneous Breaths vs. Mandatory Breaths

Supplemental Table 13 shows the fractional changes in diaphragm thickness for patients with assist/ spontaneous breaths vs. mandatory breaths per hemidiaphragm for patients with bilateral lead placement. The number of hemidiaphragms included in the analysis is shown in the 2nd column. The data is broken out as the whole population, the right side only and the left side only. This analysis includes stimulated sides only.

Supplemental Tables 14 and 15 compare the fractional relative change in diaphragm thickness between patients on assisted breathing versus those on mandatory breathing for patients with bilateral lead placement. The fractional relative change at 24 hours in the mandatory breathing group was significantly greater than in the assisted breathing group (p=0.0452) with diaphragm thickness increasing 4.5% in the assisted breathing group vs. 18.7% in the mandatory breathing group. At 48 hours the difference between groups became marginal again (p=0.0831) with the assisted breathing group having a 12.6% increase and the mandatory breathing group having a 23.2% increase in diaphragm thickness.

e. COPD vs. non-COPD

Supplemental Table 16 shows the fractional changes in diaphragm thickness for patients with COPD vs. those without COPD per hemidiaphragm side for patients with bilateral lead placement. The number of diaphragm sides included in the analysis is shown in the 2nd column. The data is broken out as the whole population, the right side only and the left side only. This analysis includes stimulated sides only.

Supplemental Tables 17 and 18 compare the fractional relative change in diaphragm thickness between patients with COPD vs. those without COPD for patients with bilateral lead placement, except Patient 6 who had a pleural effusion. There was no significant difference in the fractional relative change in diaphragm thickness between the two groups at either 24 or 48 hours. The fractional relative change at 24 hours in the COPD group was 11.7% vs. 5.7% in the non-COPD group. At 48 hours, patients with COPD had 16.3% increase in diaphragm thickness compared to a 14.4% increase in the non-COPD group.

8. Additional Detail on Lead Insertion

The initial step associated with lead insertion is to use ultrasound imaging to assess if the patient is suitable for therapy. This is performed to ensure the anatomical landmarks associated with the PN in the patient's neck can be visualized and identified before insertion

is attempted. If the anatomical landmarks could not be visualized, the patient was not enrolled in the study.

In humans the phrenic nerve is normally located 2 to 10 mm subdermal in the supraclavicular fossa region. The PN exits C3, C4 and C5 and runs medially across the anterior scalene muscle underneath the sternocleidomastoid muscle. Supplemental Figure 6 shows an ultrasound picture with the important anatomical landmarks beside the PN highlighted and identified. Use of ultrasound imaging enabled the user to safely guide the needle to the correct location, avoiding the brachial plexus and major blood vessels and arteries. Choosing this position isolates the phrenic nerve and provides easy landmarks for locating the nerve. Kessler et al. (2) previously reported the ability to identify the phrenic nerve in 93.5% of 23 volunteers whose neck regions were scanned with high resolution ultrasonography (2).

The second step for lead deployment was actual lead insertion. After the landmarks had been successfully identified, the sterile field was prepared with a preoperative skin prep (ChloraPrep™ or similar) and a sterile drape was placed over the access site. Using a sterile ultrasound probe and sterile technique, the previously marked area of the Phrenic Nerve was identified, and 1% lidocaine was applied to the skin only at level of lower thyroid cartilage. Using a number 11 scalpel, a small 1 to 2mm skin incision was made at the designated insertion site. Supplemental Figure 7 shows a close-up of the Tuohy tipped needle used for lead insertion with the echogenic indentations on the needle shaft to aid in ultrasound visualization. The needle was primed with saline (Supplemental Figure 8) before

insertion. The syringe was left attached to aid with hydrodissection as the needle was advanced. The needle was then advanced beyond the skin incision site as shown in Supplemental Figure 9 and guided between the fascia of the ASM and SCM using ultrasound imaging. Saline hydrodissection was used to increase separation between the muscles and minimize the potential for the needle to contact the PN. The needle was advanced past the ASM and just short of the internal jugular vein. The saline filled syringe and extension tube were removed once the needle was in place. The stimulation lead was inserted into the needle lumen until it reached the tip of the needle. The needle was then retracted (Supplemental Figures 10 and 11). Supplemental Figure 10 shows the ultrasound image of the needle being retracted with the lead remaining in place of the retracted needle. Once the needle was retracted over the lead, tests for ideal pair of electrodes began as described above. Once the ideal lead pair was identified, the lead was secured in place using a with a GRIP-LOK securement device and a strain relief loop.

References

1. Masmoudi H, Coirault C, Demoule A, et al: Can phrenic stimulation protect the diaphragm from mechanical ventilation-induced damage? Eur Respir J. 2013 Jul;42(1):280-3. doi: 10.1183/09031936.00045613. PubMed PMID: 23813311.
2. Kessler J, Schafhalter-Zoppoth I, Gray AT. An ultrasound study of the phrenic nerve in the posterior cervical triangle: Implications for the interscalene brachial plexus block. Reg Anesth Pain Med. 2008 Nov-Dec;33(6):545-50.

9. Supplemental Tables

Supplemental Table 1. Study Inclusion and Exclusion Criteria

Candidates for this study must meet all of the following inclusion criteria
18 years or older (Adult).
Male or Female.
Able and willing to give informed consent or whose legally authorized representative is able and willing to give informed consent.
Patient who in the opinion of the admitting consultant/intensivist is likely to be ventilated for > 48 hours from time of recruitment.
Candidates for this study must not have any of the following exclusion criteria
Subject has a left ventricular ejection fraction (LVEF) < 20%.
Subject unlikely to survive 72 hours due to coexisting medical conditions.
Subject has an implanted pulse generator or implanted electronic device: Examples: Cardiac pacemaker, Defibrillator, ICD, Watchman, Vagus nerve stimulator, Spinal cord stimulator, Gastric stimulator or Diaphragmatic stimulator.
Subject has experienced an Acute Myocardial Infarction (AMI) within 72 hours prior to this screening or patient is on high dose inotropic support or patient is deemed to be in cardiogenic shock.
Subject has significant bleeding diathesis, or is at risk of significant hemorrhage, patient is receiving full dose systemic anticoagulation
Subject has a known or suspected phrenic nerve paralysis or neuromuscular or inflammatory muscle diseases where the diaphragm itself may not be functional.
Subject has an active systemic infection or local infection at or around the insertion site. Patient is neutropenic or has signs of significant immunocompromise.
Subject is known or suspected to be pregnant or is lactating.
Subject will be unavailable for, or is unwilling to comply with, follow up requirements of the protocol.
Subject is currently enrolled or is expected to be enrolled in any other study of an investigational drug or device who has received treatment under that protocol with the investigational product during the 30 days prior to screening.
Subject has undergone a surgery or interventional procedure within the neck region aside from placement of an internal jugular (IJ) vein catheter.
Subject has been diagnosed and has been treated for neck cancer within the past 5 years.
Subject is known to have a demonstrated intra cardiac thrombus on echocardiography.
Subject has uncontrolled hyperthyroidism, hypertension.
Subject has had any cerebral ischemic event (Stroke or Transient Ischemic Attack TIA) in the 6-month interval preceding the screening date.
Subject has degenerative nerve disorders such as amyotrophic laterals sclerosis (ALS).
Subject has an elevated hemidiaphragm on chest x-ray.
Subject written informed consent not obtained.

Supplemental Table 2. Demographic and Study Data Collected

Test / Evaluation	Screening (within 2 days pre-procedure)	At index procedure	At 30-day follow up
Signed Written Informed Consent/Relative Assent	X		
Subject Demographics	X		
Medical History	X		
Height/Weight/BMI	X		
Blood Pressure	X		
Chest x-ray	X [1]		X
12 lead ECG		X	
Lead Insertion assessment		X	
1 hr. cessation of electrical stimulation		X	
Patient movement / repositioning assessment		X	
CMAP [2]		X	X
CPOT/RASS assessments		X	
Muscle twitching observation		X	
Ultrasound screen shots (optional)		X	
Ventilation settings and end-tidal CO_2 required		X	
Stimulation Data		X	
Vital Signs / pulse oximetry		X	
Standard Blood gases: pH, paO_2, $PaCO_2$ etc.		X	
Diaphragm thickness (optional)		X	
Lead Removal assessment		X	
Adverse Events		X	X
Device Malfunctions		X	

[1] It was acceptable if this had been taken up to 30 days prior to the screening visit.

[2] Only for the first 2 patients enrolled.

Supplemental Table 3. Phrenic Nerve Compound Motor Action Potentials

		Height (cm)	Before Therapy		After Therapy (30 days)	
			Latency (ms)	Amplitude (mV)	Latency (ms)	Amplitude (mV)
Patient #1	Right	168	6.78	1.02	6.94	0.81
			6.99	0.95	7.59	0.72
	Left		6.97	0.91	6.84	1.21
			7.1	0.78	7.01	1.07
Patient #2	Right	171	7.48	0.24	6.88	0.33
			8.39	0.23	8.6	0.39
	Left		7.93	0.32	6.53	0.50
			7.58	0.27	7.74	0.59

Supplemental Table 4. CPOT Assessment Results

Patient ID	Mean CPOT during electrical stimulation	Mean CPOT during without electrical stimulation
P03S01	0.5	2.2
P04S01	0.8	1.4
P05S02	0.8	0.3
P06S02	0.0	0.0
P07S02	0.0	0.0
P08S01	1.2	2.2
P09S02	1.0	1.2
P10S02	0.0	0.0
P11S01	0.8	1.6
P12S01	0.0	0.3
Mean	**0.51**	**0.92**
St. Dev.	**0.45**	**0.86**

Supplemental Table 5. RASS Score Assessment Results

Patient ID	Mean RASS Scores electrical stimulation	Mean RASS score without electrical stimulation
P03S01	-3.5	-3.4
P04S01	-3.3	-2.7
P05S02	-5.0	-5.0
P06S02	-2.0	-2.5
P07S02	NA	-4.0
P08S01	-2.8	-1.2
P09S02	-2.0	-2.0
P10S02	-5.0	-4.6
P11S01	-4.5	-3.0
P12S01	-0.8	-0.4
Mean	**-3.21**	**-2.88**
St. Dev.	**1.56**	**1.46**

Supplemental Table 6. Patient Repositioning Analysis

Reason for patient repositioning	Per hospital protocol	To regain stim at start of a new session	In preparation of electrical stimulation	Patient moved themselves	Total
P03S01	11	0	4	0	**15**
P04S01	2	0	2	0	**4**
P05S02	9	1	0	0	**10**
P06S02	12	0	1	0	**13**
P07S02	8	0	2	0	**10**
P08S01	5	0	3	0	**8**
P09S02	8	0	5	0	**13**
P10S02	9	0	0	0	**9**
P11S01	13	0	0	0	**13**
P12S01	7	0	0	3	**10**

Supplemental Table 7. Time to Weaning

Patient ID	Time on vent before initial stim #1 (days)	Time to wean from start of MV (days)	Time to wean from initiation of stim (days)
P03S01†	5.7	11.8	6.0
P04S01	5.5	17.3	11.9
P05S02	6	31.5	25.5
P06S02	9.1	11.5	2.4
P07S02†	7.2	19.7	12.5
P08S01†,††	5.9	14.4	8.5
P09S02†	11.2	43	31.8
P10S02	4.2	16.6	12.4
P11S01	4.3	14.9	10.6
P12S01♣	12.8	44.4	31.5

† Died within 30 days of enrollment
†† Was alive 14.9 days after weaning prior to death
♣ Did not wean within 30 days of enrollment

Supplemental Table 8. Diaphragm Thickness Change Versus Baseline

Time Comparison	N	Fractional change from baseline	Lower 95% Bound	P value
0 vs. 24 hours	17	0.0783	0.0161	0.0216
0 vs. 48 hours	17	0.1507	0.0947	0.0001

* Per protocol, Patient 1 and 2 were excluded.

Supplemental Table 9. Diaphragm Thickness Measurements

Patient ID	Side	Mean Thickness (cm)			Fractional Change	
		Baseline	24 Hours	48 Hours	0 to 24 hours	0 to 48 hours
P01S02	Left	0.2333	0.2200	0.2133	-0.0571	-0.0857
	Right	0.2100	0.2133	0.2233	0.0159	0.0635
P02S02	Left	0.2567	0.2767	0.2567	0.0779	0.0000
	Right	0.2567	0.2633	0.2800	0.0260	0.0909
P03S01	Left	0.2530	0.2410	0.2917	-0.0474	0.1528
	Right	0.1770	0.1867	0.1943	0.0546	0.0979
P04S01	Left	0.2167	0.2010	0.2157	-0.0723	-0.0046
	Right	0.2170	0.2110	0.2270	-0.0276	0.0461
P05S02	Left	0.2933	0.2844	0.3044	-0.0303	0.0379
	Right	0.2867	0.2700	0.2633	-0.0581	-0.0814
P07S02	Left	0.1422	0.1689	0.1667	0.1875	0.1719
	Right	0.1622	0.1556	0.2022	-0.0411	0.2466
P08S01	Left	0.1768	0.1803	0.1902	0.0201	0.0761
	Right	0.1784	0.1753	0.2233	-0.0174	0.2516
P09S02	Left	0.2978	0.2511	0.2811	-0.1567	-0.0560
	Right	0.1867	0.2367	0.2578	0.2679	0.3810
P10S02	Left	0.1144	0.1456	0.1456	0.2718	0.2718
	Right	0.1311	0.1744	0.1622	0.3305	0.2373
P11S01	Left	0.1730	0.2203	0.2343	0.2736	0.3545
	Right	0.2263	0.2370	0.2408	0.0471	0.0638
P12S01	Left	0.1567	0.1873	0.1952	0.1957	0.2461
	Right	0.1790	0.1926	0.1766	0.0757	-0.0137

Supplemental Table 10. Fractional Change in Diaphragm Thickness – Stimulation vs. Non-Stimulated

	N	Side	Fractional Change 0 to 24 hours	Std. Dev 0 to 24 hours	Fractional Change 0 to 48 hours	Std. Dev 0 to 48 hours
Stimulated + Non-Stimulated	22	Total	0.0607	0.1350	0.1158	0.1373
	11	Right	0.0612	0.1256	0.1258	0.1365
	11	Left	0.0603	0.1500	0.1059	0.1440
Stim Only	19	Total	0.0712	0.1421	0.1303	0.1394
	8	Right	0.0862	0.1390	0.1638	0.1346
	11	Left	0.0603	0.1500	0.1059	0.1440
non-Stim Only	3	Total	-0.0054	0.0459	0.0243	0.0926
	3	Right	-0.0054	0.0459	0.0243	0.0926
	0	Left	NA	NA	NA	NA

Supplemental Table 11. Fractional Change in Diaphragm Thickness – Stimulated vs. Non-Stimulated (0 to 24 hours)

	N	Fractional Change	Lower 95% Bound	Upper 95% Bound	P-value
Stimulated	19	0.0712	0.00271	0.1397	0.1869
Non-Stimulated	3	-0.00543	-0.1195	0.1087	-
Difference		0.0766	-0.0686	∞	-

∞ = Infinity

Supplemental Table 12. Fractional Change in Diaphragm Thickness – Stimulated vs. Non-Stimulated (0 to 48 hours)

	N	Fractional Change	Lower 95% Bound	Upper 95% Bound	P-value
Stimulated	19	0.1303	0.0631	0.1975	0.1113
Non-Stimulated	3	0.0243	-0.2057	0.2543	-
Difference		0.1059	-0.0392	∞	-

∞ = Infinity

Supplemental Table 13. Fractional Comparison of Diaphragm Thickness – Assisted vs. Mandatory Breaths

	N	Side	Fractional Change 0 to 24 hours	Std. Dev 0 to 24 hours	Fractional Change 0 to 48 hours	Std. Dev 0 to 48 hours
Stimulated with Assisted + Mandatory	19	Total	0.0712	0.1421	0.1303	0.1394
	8	Right	0.0862	0.1390	0.1638	0.1346
	11	Left	0.0603	0.1500	0.1059	0.1440
Stimulated with Assisted	15	Total	0.0402	0.1240	0.1032	0.1444
	6	Right	0.0667	0.1068	0.1378	0.1486
	9	Left	0.0226	0.1375	0.0801	0.1456
Stimulated with Mandatory	4	Total	0.1872	0.1631	0.2319	0.0426
	2	Right	0.1447	0.2628	0.2419	0.0066
	2	Left	0.2297	0.0596	0.2219	0.0707

Supplemental Table 14. Fractional Change in Diaphragm Thickness - Assisted vs. Mandatory Breaths (0 to 24 hours)

	N	Fractional Change	Lower 95% Bound	Upper 95% Bound	P-value
Assist	15	0.0402	-0.0284	0.1089	0.0320
Mandatory	4	0.1872	-0.0724	0.4467	-
Difference		-0.1469	-∞	-0.0180	-

-∞ = Negative Infinity

Supplemental Table 15. Fractional Change in Diaphragm Thickness - Assisted vs. Mandatory Breaths (0 to 48 hours)

	N	Fractional Change	Lower 95% Bound	Upper 95% Bound	P-value
Assist	15	0.1032	0.0232	0.1832	0.0509
Mandatory	4	0.2319	0.1641	0.2997	-
Difference		-0.1287	-∞	0.000778	-

-∞ = Negative Infinity

Supplemental Table 16. Fractional Comparison of Diaphragm Thickness – COPD vs. non-COPD

	N	Side	Fractional Change 0 to 24 hours	Std. Dev 0 to 24 hours	Fractional Change 0 to 48 hours	Std. Dev 0 to 48 hours
Stimulated with and without COPD	19	Total	0.0712	0.1421	0.1303	0.1394
	8	Right	0.0862	0.1390	0.1638	0.1346
	11	Left	0.0603	0.1500	0.1059	0.1440
Stimulated with COPD	7	Total	0.0923	0.1638	0.1272	0.1970
	3	Right	0.1302	0.1200	0.1437	0.2091
	4	Left	0.0639	0.2039	0.1147	0.2191
Stimulated without COPD	12	Total	0.0589	0.1339	0.1321	0.1030
	5	Right	0.0598	0.1558	0.1759	0.0967
	7	Left	0.0582	0.1292	0.1008	0.1024

Supplemental Table 17. Fractional Change in Diaphragm Thickness - COPD vs. non-COPD (0 to 24 hours)

	N	Fractional Change	Lower 95% Bound	Upper 95% Bound	P-value
COPD	7	0.0923	-0.0592	0.2438	0.3171
Non-COPD	12	0.0589	-0.0262	0.1440	-
Difference		0.0335	-0.0867	∞	-

∞ = Infinity

Supplemental Table 18. Fractional Change in Diaphragm Thickness - COPD vs. non-COPD (0 to 48 hours)

	N	Fractional Change	Lower 95% Bound	Upper 95% Bound	P-value
COPD	7	0.1272	-0.0551	0.3094	0.5285
Non-COPD	12	0.1321	0.0667	0.1976	-
Difference	.	-0.00496	-0.1236	∞	-

∞ = Infinity

Supplemental Table 19. Patient Data: Stimulated vs. Non-Stimulated Breaths

Patient	Non-Stimulated (weighted mean) Volume (mL)	Stimulated (weighted mean) Volume (mL)	% Increase in Volume	# Stimulated Breaths	# Not Stimulated Breaths	Σ Breaths	% Stimulated	ΔDT (cm) 0- to 48 -Hours	WOB (all breaths) Increase Stim vs. Non-Stim (J/L)	WOB (stimulated breaths) Increase vs. Preceding Breath (J/L)
P03	472.03	639.87	35.56%	3110	10104	13214	23.536%	0.125	0.073	0.096
P04	499.87	717.25	43.49%	3133	10061	13194	23.746%	0.021	0.342	0.347
P05	435.15	490.82	12.79%	5833	18124	23957	24.348%	-0.022	-0.014	0.015
P06	495.55	627.80	26.69%	4182	12879	17061	24.512%	Excluded	0.384	0.388
P07†,††	448.98	675.17	50.38%	3557	10726	14283	24.904%	0.209	0.513	0.518
P08	514.13	802.19	56.03%	2407	7532	9939	24.218%	0.164	0.143	0.205
P09	601.02	705.86	17.44%	3605	10999	14604	24.685%	0.163	0.102	0.097
P10††	500.52	662.31	32.32%	3587	10686	14273	25.131%	0.255	0.406	0.420
P11	520.27	833.58	60.22%	3125	10058	13183	23.705%	0.209	0.328	0.377
P12	429.45	475.34	10.69%	3630	11810	15440	23.510%	0.116	0.111	0.170
Mean	**491.7**	**663.0**	**34.6%**	**3616.9**	**11297.9**	**14914.8**	**24.2%**	**0.138**	**0.239**	**0.263**
StdDev	47.7	109.6	16.9%	862.1	2623.4	3480.8	0.6%	0.085	0.167	0.159

† Died within 30 days of enrollment
†† Ventilated entirely in mandatory mode

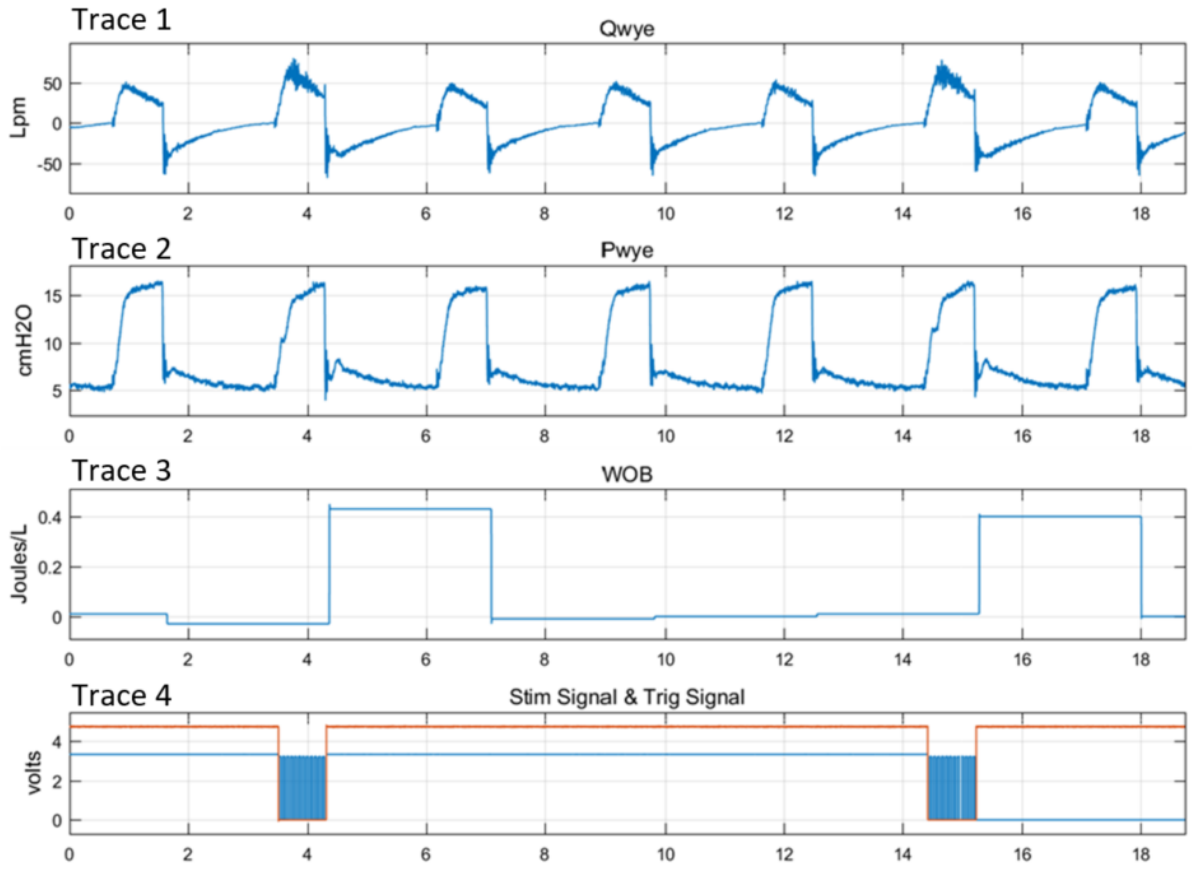
Definitions for Supplemental Table 19

Non-Stimulated Weighted Mean = Mean inspired tidal volume (in mL) of non-stimmed breaths
Weighted Mean = Mean inspired tidal volume (in mL) of stimmed breaths
% Increase = Percent increase relative to the non-stimulated weighted mean
# Stimulated = total stimulated breaths for a patient during the 6 stimulation sessions
# Not Stimulated = total non-stimulated breaths for a patient during the 6 stimulation sessions
Σ Breaths = sum of stimulated + non-stimulated breaths during the 6 stimulation sessions
% Stimulated = % of total breaths that were stimulated during the 6 stimulation sessions
ΔDT (cm) 0 to 48 Hours = Diaphragm thickness change in cm. To determine if there was a correlation with % increase (R^2 of 0.18)
WOB, All Breaths Increase Stim vs. Non-Stim (J/L) - weighted mean WOB values for all non-stimulated breaths subtracted from weighted mean WOB values for all stimulated breaths (difference)
WOB, Stim Breath Increase vs. Preceding Breath (J/L) - Weighted mean WOB values of the non-stimulated breath immediately prior to a stimulated breath subtracted from the weighted mean WOB values of the respective stimulated breath.

10. Supplemental Figures

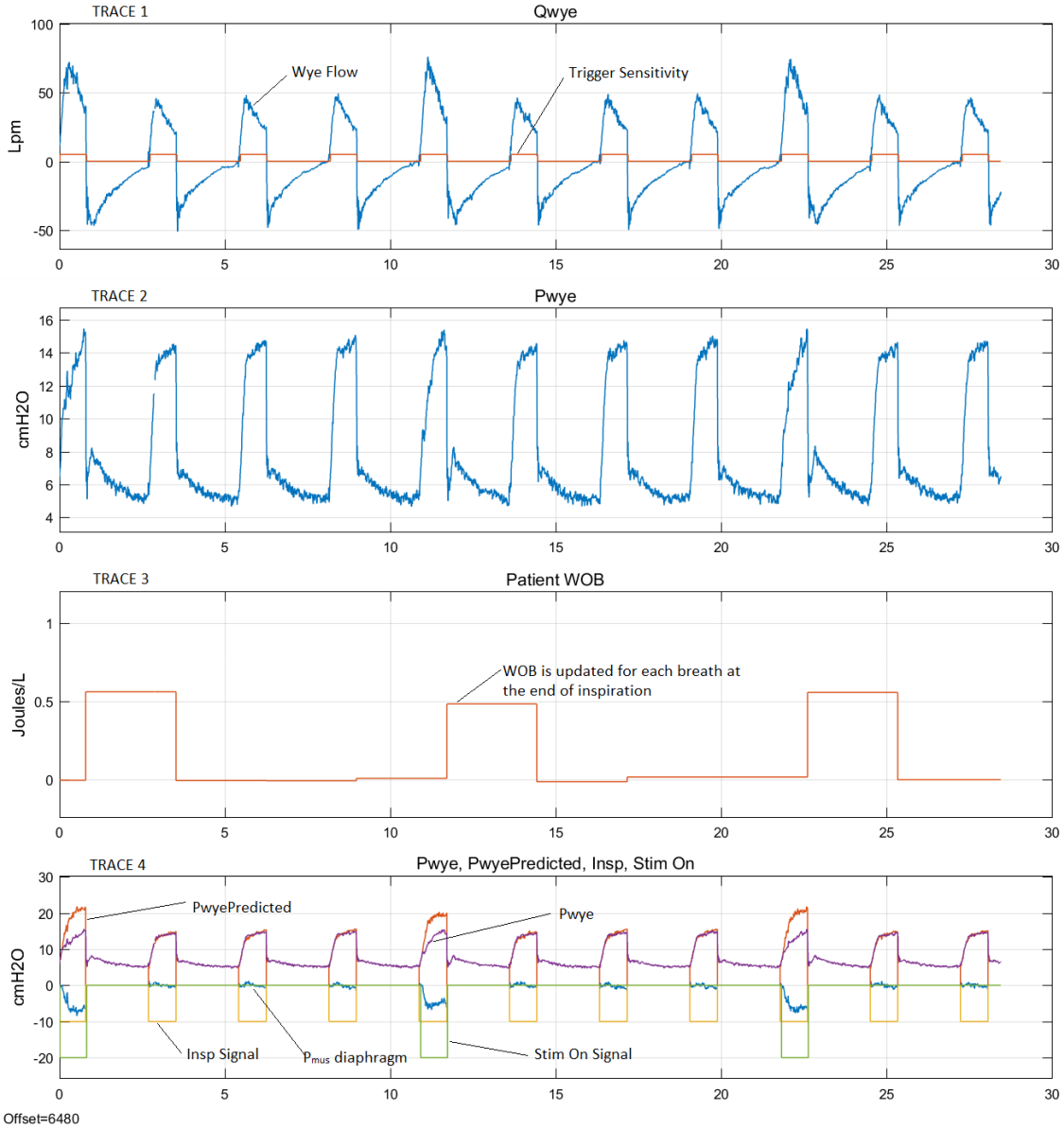
Supplemental Figure 1. Example of wye flow (Qwye), wye pressure (Pwye), Stim Signal and Trig Signal data collected

Data acquired by ADInstruments Labchart at 1kHz sample rate. Trace 1 shows Qwye in BTPS Lpm, Trace 2 Pwye in cmH_2O, Trace 3 WOB in J/L updated at the end of each inspiration and Trace 4 the Stim Signal showing electrical stimulation pulse rate frequency and Trig Signal showing when the PEPNS software detected beginning and end of inspiration in volts (0Volts = inspiration, 5V = exhalation). This data is extracted from P0702 in PRVC SIMV.



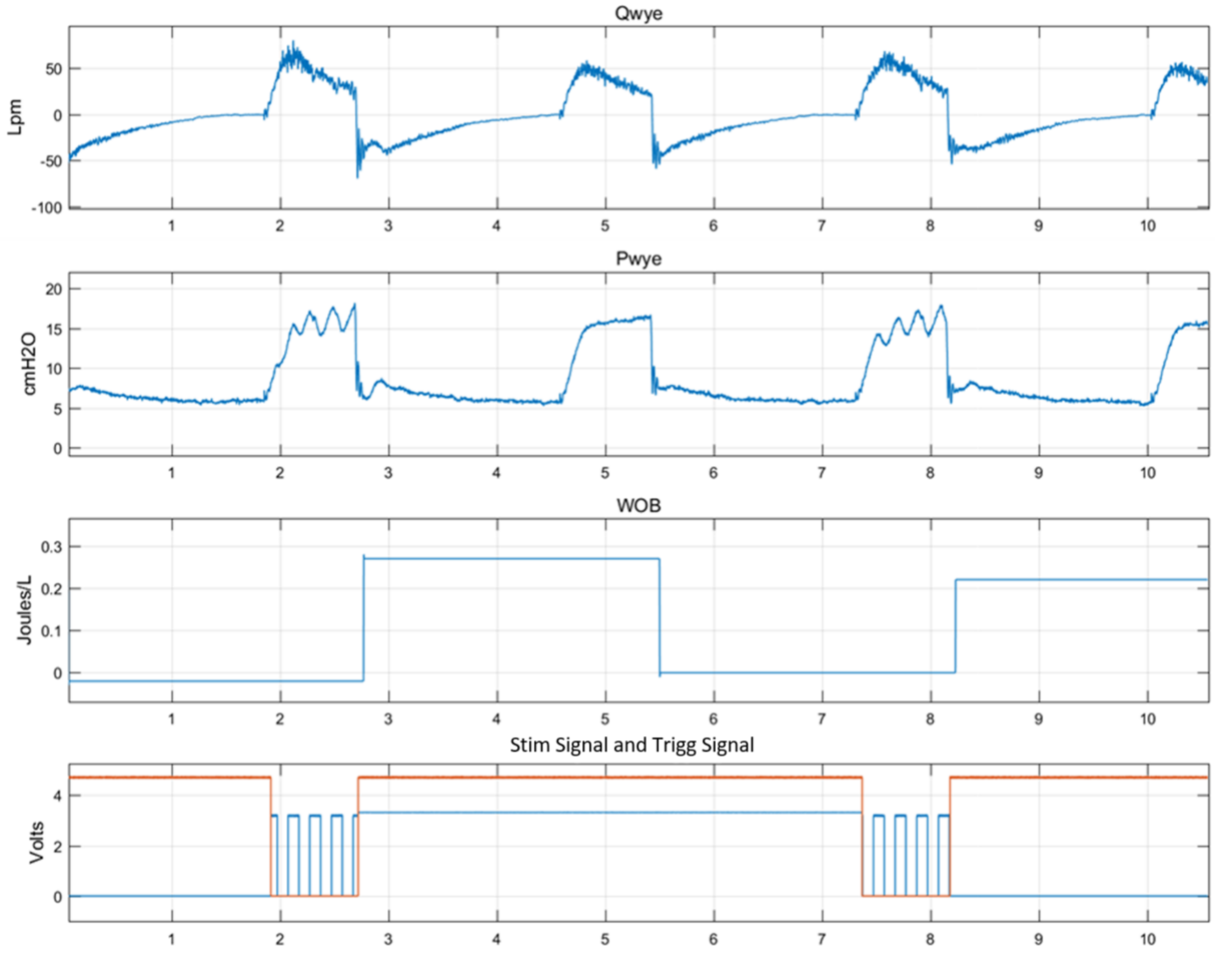
Supplemental Figure 2. Example of PEPNS Console Real Time Graph Display

PEPNS Console real time graph display for P0702 Stim session 2. Trace 1: shows Qwye, wye flow (blue) and inspiratory trigger (red) setting in BTPS Lpm. Trace 2: Shows Pwye, wye pressure (blue) in cmH_2O. Trace 3: shows WOB (red) updated at the end of each inspiration cycle for both stimulated an unstimulated breath in Jules/L. Trace 4: Shows measured wye pressure Pwye (purple), the predicted wye pressure based upon the equation of motion PwyePredicted (red), the diaphragm pressure (Pmus Diaphragm), all in cmH_2O, as a result of the electrical stimulation and / or patient effort (blue), inspiratory signal (yellow) where -10 = inspiration and 0 = exhalation, electrical stimulation signal on Stim On (green) where -20 = stimulation on and 0 = stimulation off.



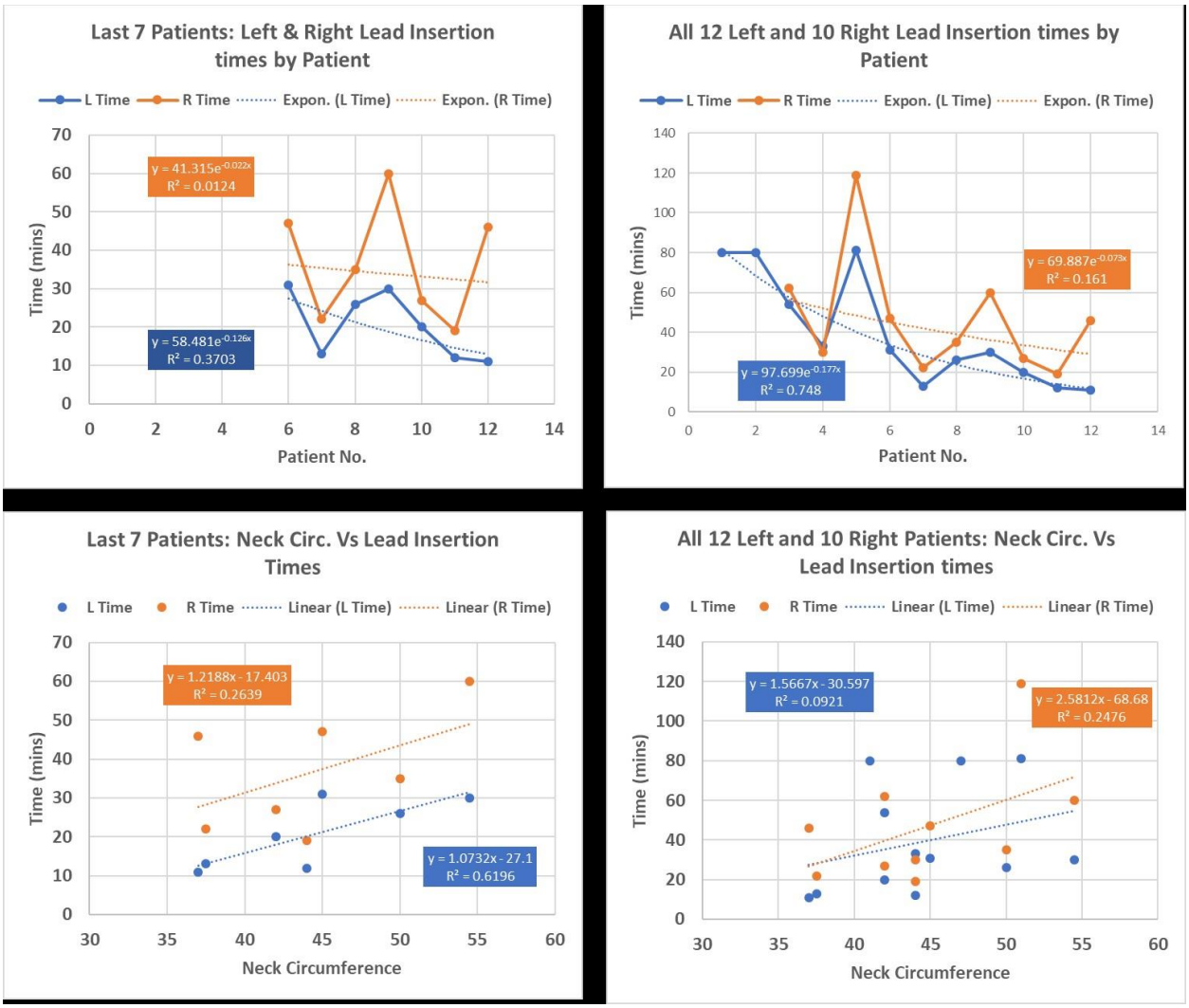
Supplemental Figure 3. Example of the effect of electrical stimulation at 5Hz

Example of the effect of electrical stimulation at 5Hz during setup on a pressure support breath with PEEP of 5 cmH_2O and the pressure support level at 10 cmH_2O. During setup in this patient, every second breath was electrically stimulated. WOB increase from approximately 0 to 0.25 J/L on stimulated breaths while the resultant pressure was maintained at the target level. Inspired volume and peak flow increased on stimulated breaths during pressure-controlled breaths.

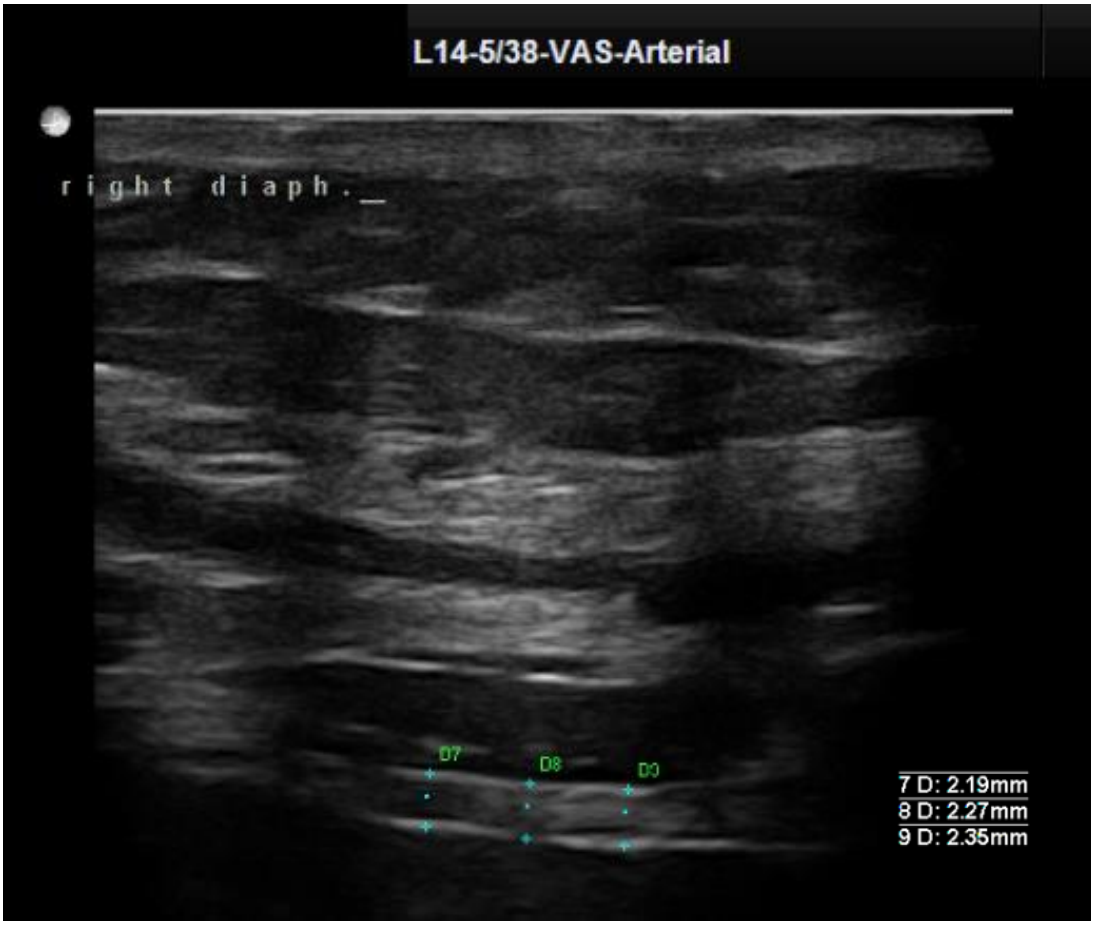


Supplemental Figure 4. Lead Insertion Times by Patient and Neck Circumference

The lead insertion times were examined for all 12 patients for both left and right lead insertion times, when applicable, and for the last 7 patients after experience with placing leads was gained.

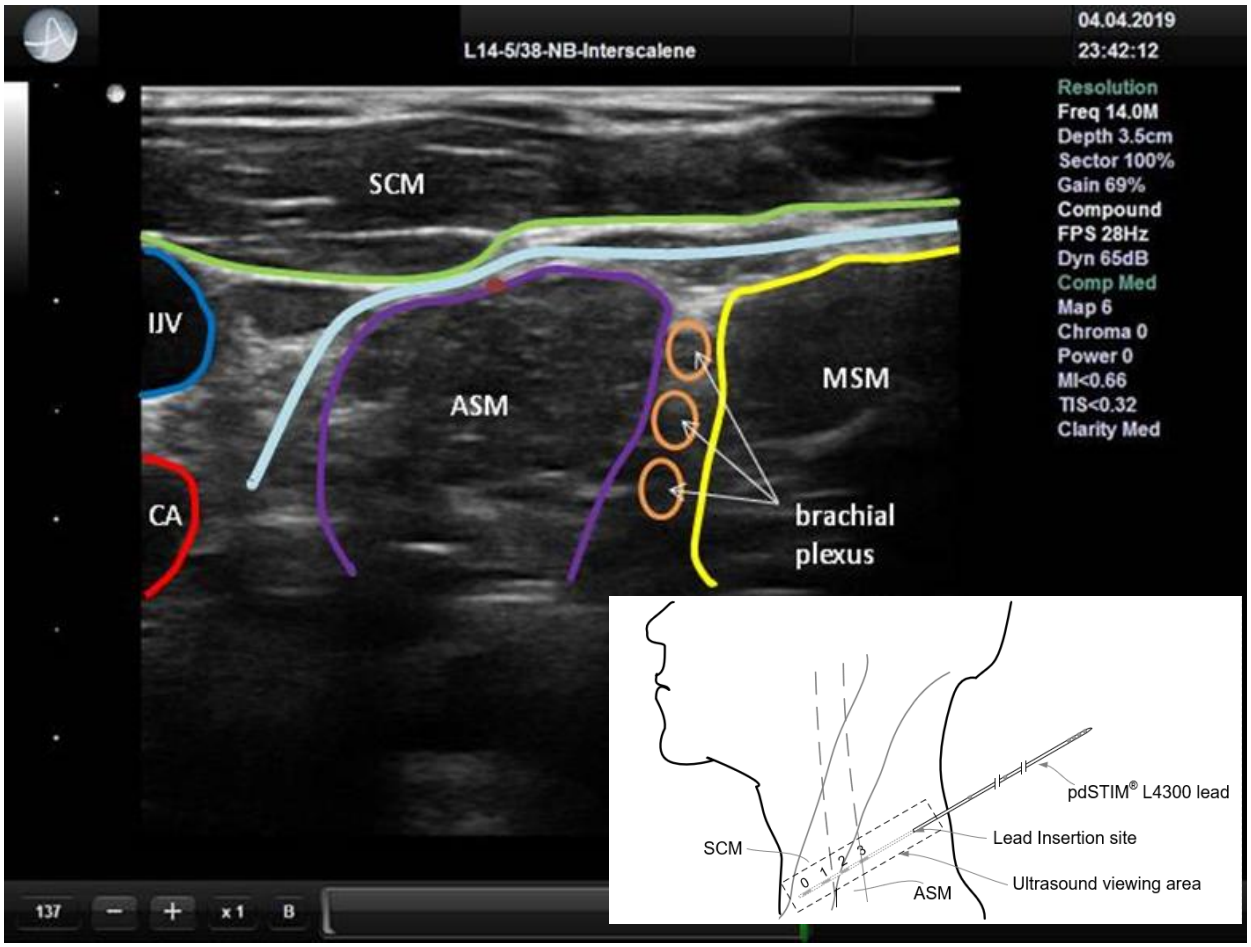


Supplemental Figure 5. Example of Ultrasound Measurement of Diaphragm Thickness.
This image reflects the right-sided measurement of diaphragm thickness.

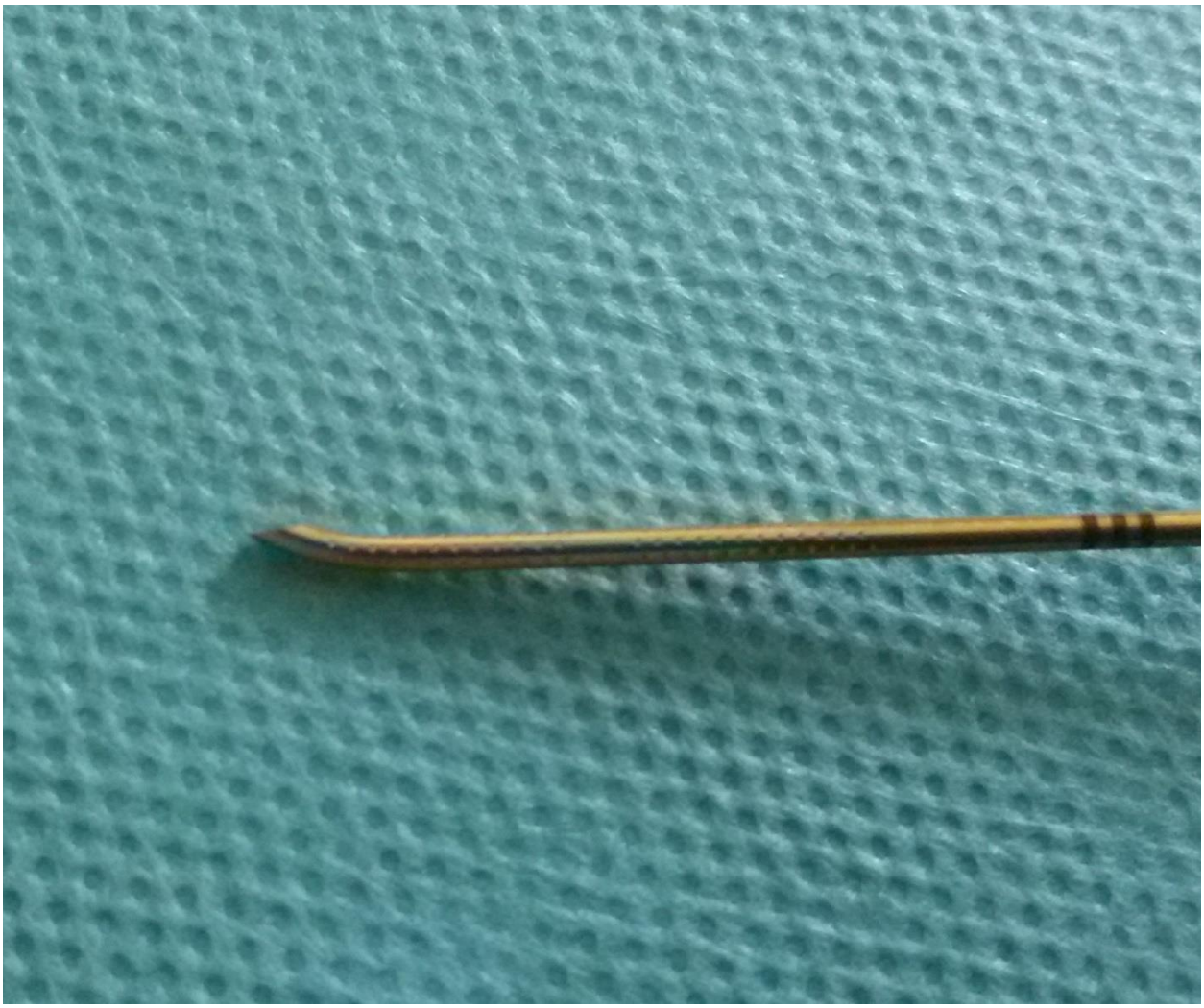


Supplemental Figure 6. Ultrasound Image Showing Neck Anatomy and Lead Insertion Path

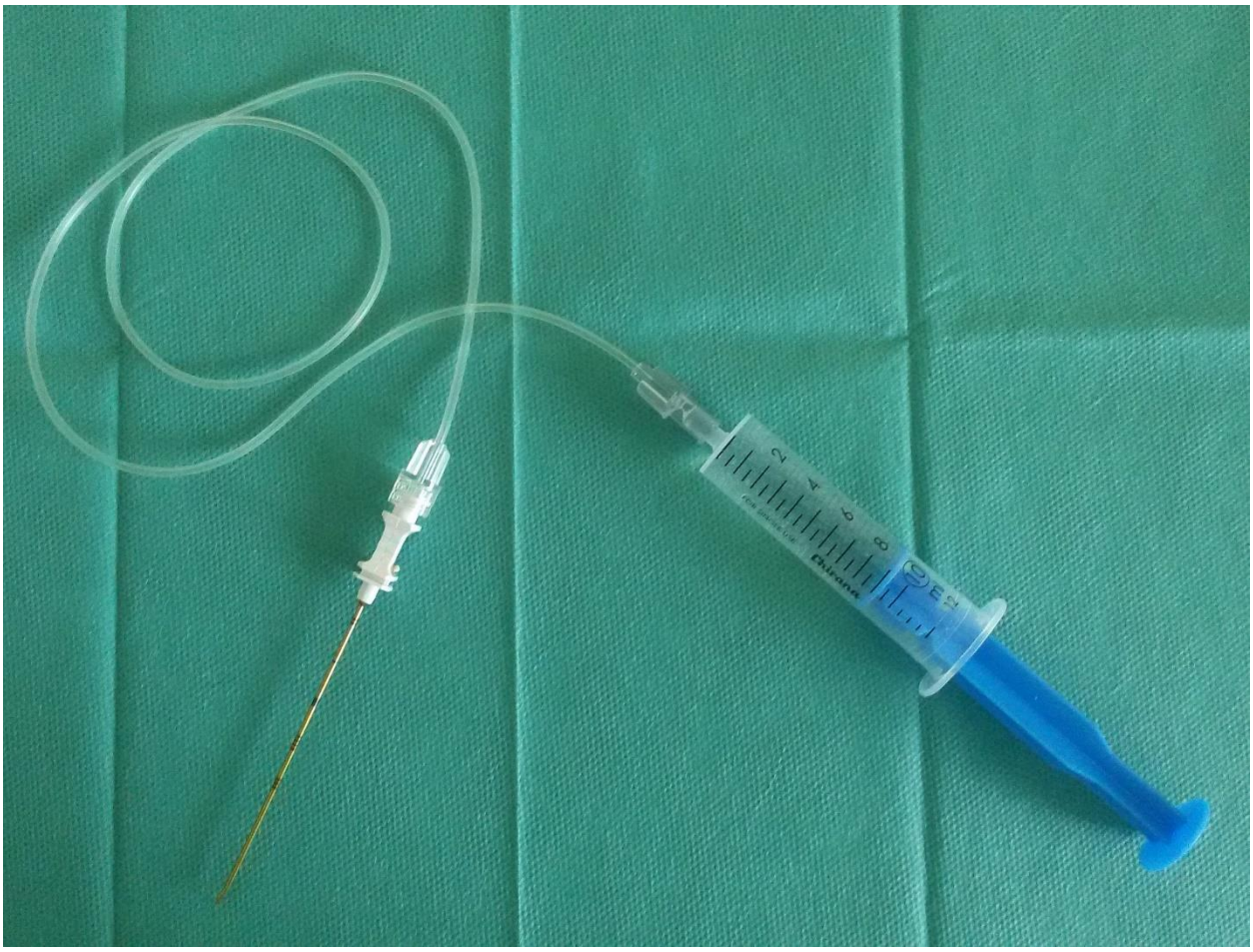
The Phrenic Nerve (PN), identified by the dark red dot, is located medially between the Sternocleidomastoid (SCM) and Anterior Scalene Muscle (ASM), medial to the brachial plexus and superficial to the anterior scalene muscle (ASM). The borders of the SCM, ASM and the Middle Scalene Muscle (MSM) are shown in green, purple and yellow, respectively. The Internal Juglar Vein (IJV) and Carotid Artery (CA) are also identified in the image. The light blue line shows the lead path. The large tick marks on the left in the ultrasound image are spaced 5 mm apart. The boxed area outlined with a dotted line in the image at the bottom right shows the ultrasound viewing area along the lead insertion path. It is necessary to move the ultrasound probe to maintain visualization as the needle is inserted.



Supplemental Figure 7. Blunt Tuohy-Tipped Needle with Echogenic Indentations

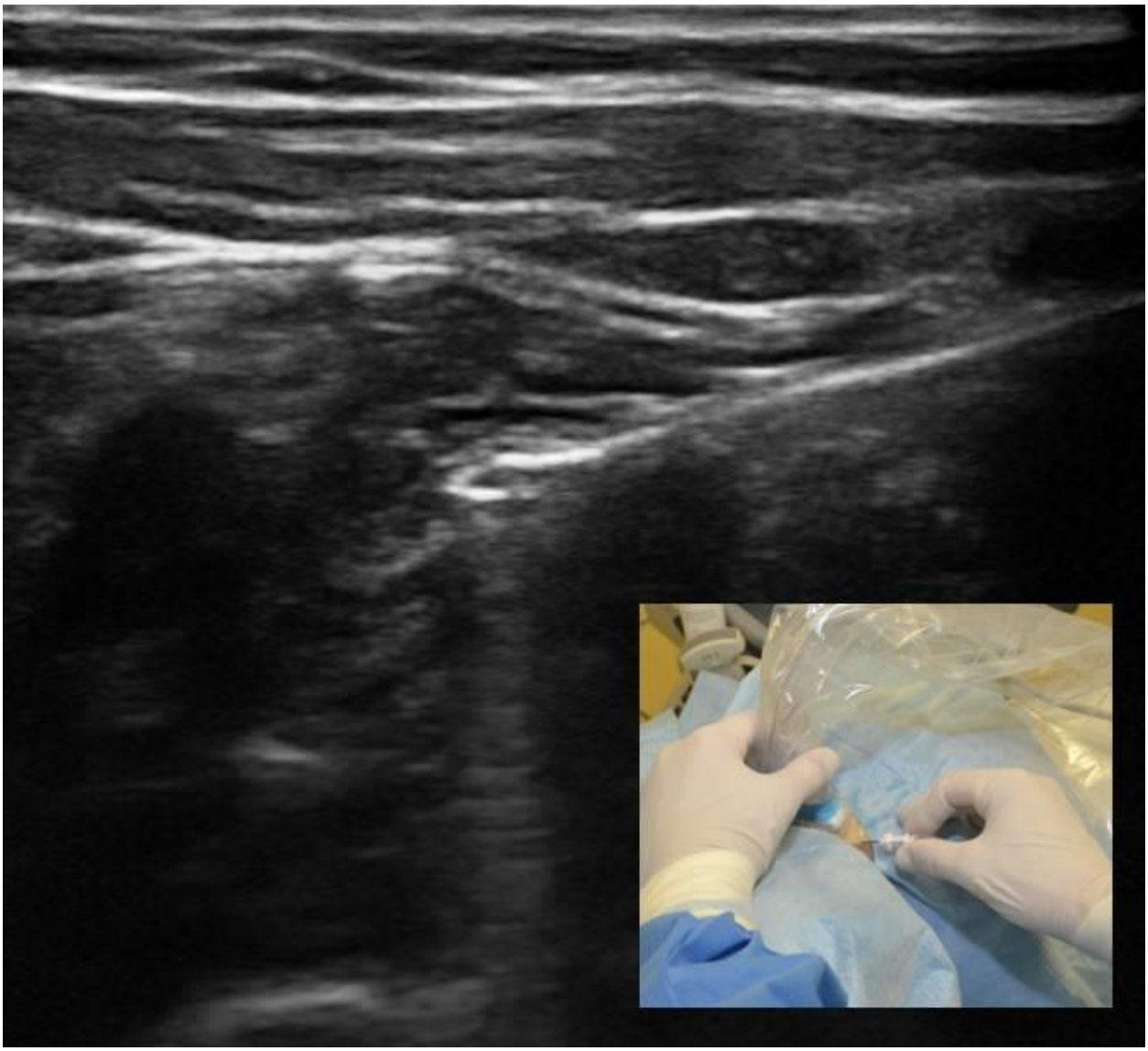


Supplemental Figure 8. Primed Needle Connected to Syringe used for Hydrodissection

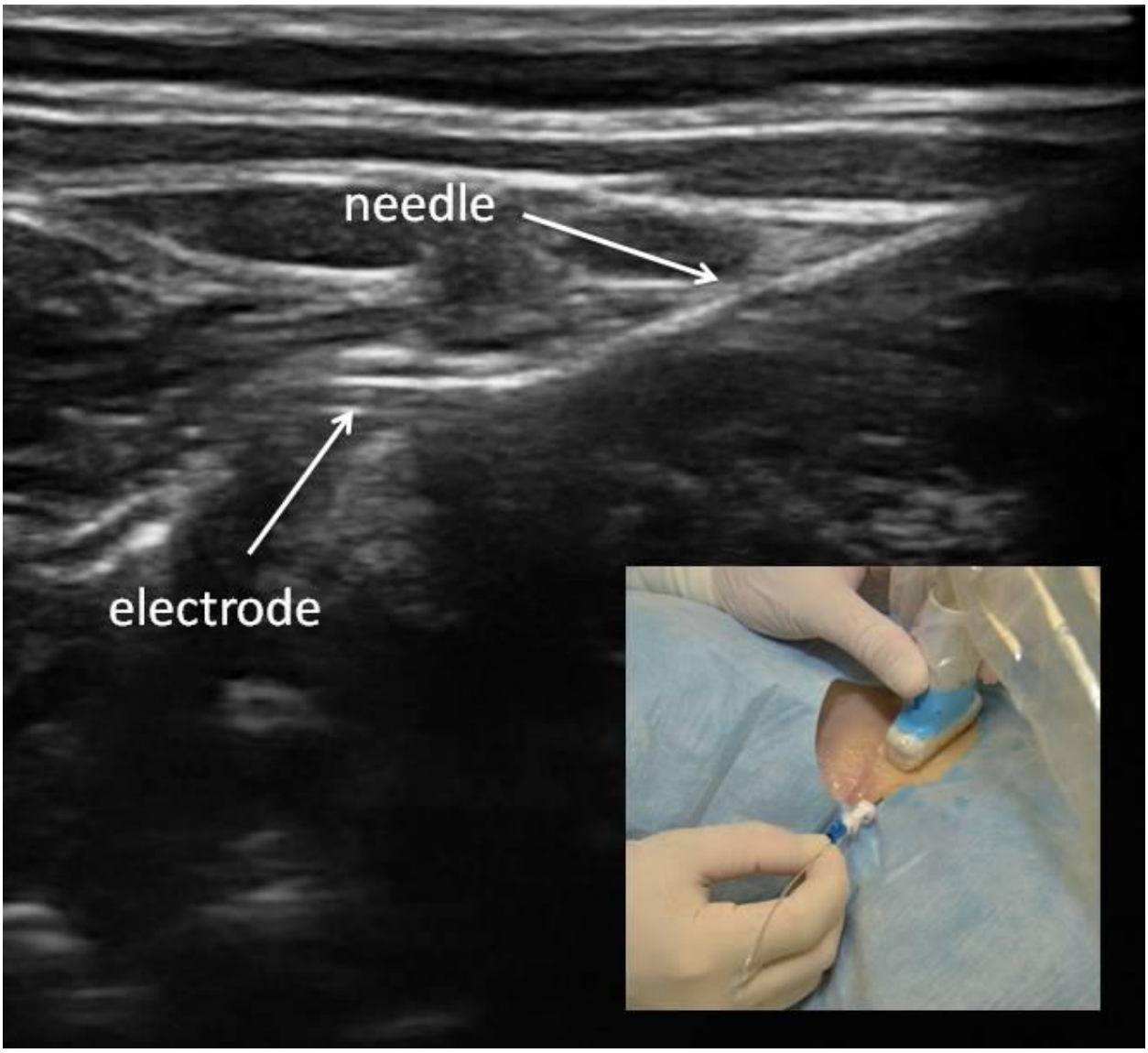


Supplemental Figure 9. Needle Being Inserted Under Ultrasound Imaging

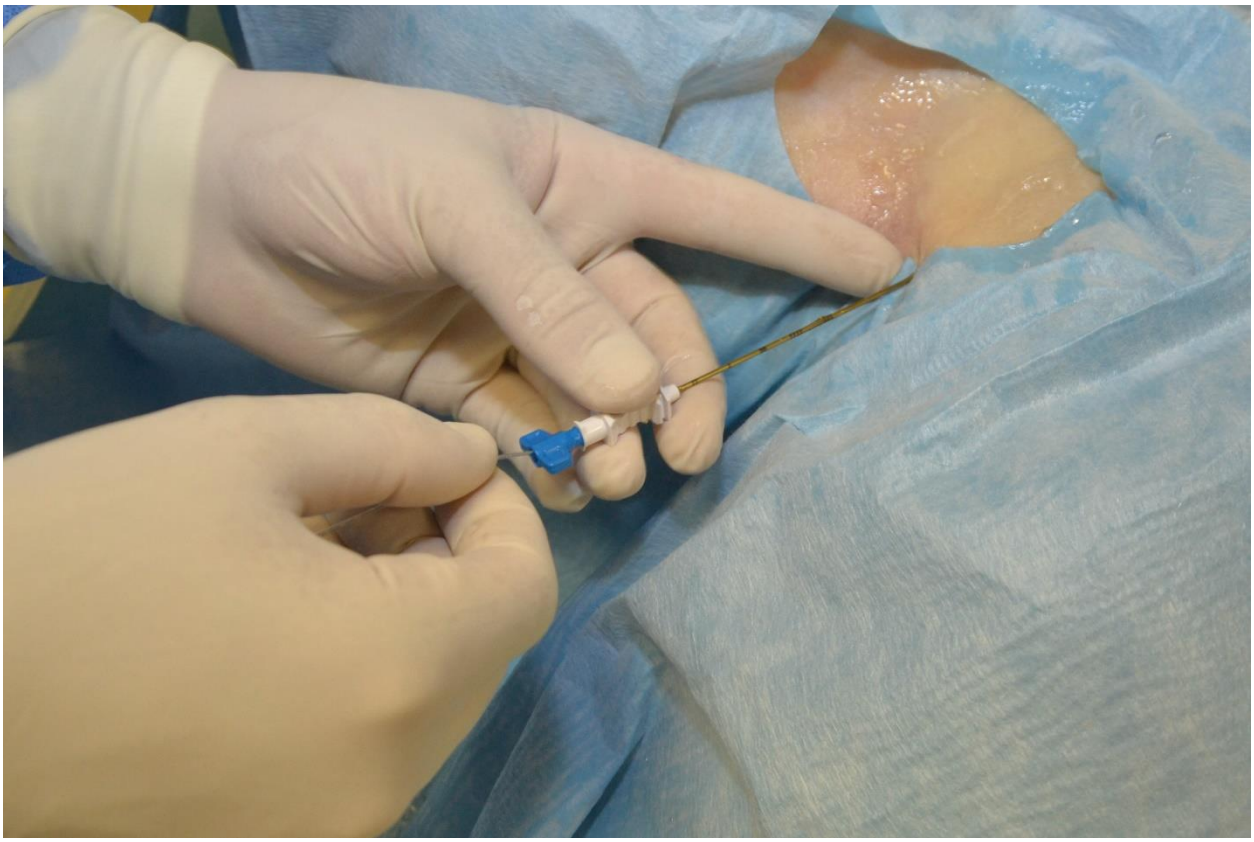
The Tuohy-Tipped needle is clearly visible in the middle of the ultrasound image with the needle tip bend at the center.



Supplemental Figure 10. Needle Being Withdrawn Over Lead with Electrode Shown Exiting Needle Tip



Supplemental Figure 11. Needle Being Retracted as Lead is Held in Place



Supplemental Figure 12. Mean ΔWOB Vs Mean Overall Change in DT (cm) 0 to 48 hrs
Mean ΔWOB = the mean difference in WOB between stimulated and unstimulated breaths for each patient over 6 stimulation sessions. Mean Change in DT = the mean difference in diaphragm thickness between stimulated and unstimulated breaths for each patient over 6 stimulation sessions for both left and right diaphragm side. WOB is the sum of the effort on the left and right side of the diaphragm.