EGS RESEARCH & CONSULTING

OBSERVATIONAL STUDY:

PERIPHERAL NEUROPATHY AND ULTRAFINE BUBBLES

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I. BACKGROUND, OBSERVATIONAL STUDY DESIGN AND IMPLEMENTATION

1. Peripheral Neuropathy – Overview

In the United States more than 40 million people are affected by Peripheral Neuropathy. Overall, 33 percent are labeled as Diabetic, 33 percent as Idiopathic, and 34 percent with other causes including hypothyroidism, auto-immune disease and chemotherapy.

There are about 20 million people in the U.S. who are diabetic; between 70 and 80 percent of them suffer from peripheral neuropathy. Some studies have shown that about 25 percent of the 80 million population of prediabetic patients (60 percent of them will become diabetic) also suffer from neuropathy. Therefore, over thirty million neuropathic people are diabetic or pre-diabetic.

There are many treatment options. Simple treatments such as vitamins, physical therapy, or topical analgesics have very limited efficacy. The most common drugs (Gabapentin, Pregabalin, etc.) used to treat the neuropathic symptoms have limited effectiveness with frequent substantial side effects. Overall, most patients are dissatisfied with their treatment and many of them are actively looking for better solutions.

Although the pathophysiology of this condition is complex and not fully understood, it seems to be very frequently associated with microvasculature deficiency and local hypoxia. Nanobubbles of gas are bubbles 50 to 200 nm in diameter; they are very stable and can be highly concentrated in solution. These nanobubbles will very readily diffuse into soft tissues. Nanobubbles of oxygen have been shown to reduce cellular hypoxia and to possess anti-inflammatory and neuro-protective properties. In addition, normal saline solution containing oxygen nanobubbles is effective for improving blood oxygenation. Thus, the use of oxygen fine, micro or nanobubbles containing fluids is a potentially effective novel method for improving blood oxygenation in cases involving hypoxia, ischemic diseases, infection control, and anticancer chemoradiation therapies.

2. Observational Study Design

The objective of the Ultrafine Bubbles observational, IRB-approved, study was to evaluate, using patient perceptions and clinician reported clinical assessments the efficacy and safety of UltraFine Bubbles of O2 and CO2 for the symptomatic treatment of Diabetic Peripheral Neuropathy. We used G*power (Faul, et al. 2007)[1] to estimate the required sample size to

[1] Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, *39*, 175-191.

detect an effect. We assumed a paired samples t-test design, estimating average pain reduction from baseline to one week and one month after completion of the treatments. A preliminary study with a similar design estimated an effect size of dz=1.03. Following van Zwet (2018)[2] --and to avoid undue optimism from preliminary results--we chose a sample size based on half that effect size, or dz=0.5. To detect an effect of size dz=0.5 at level 0.05 with 80 percent power, n=34 subjects are necessary.

The effect size is the minimum effect that we wanted to design the study to detect. The higher the effect size the stronger the relationship between variables.

G*Power is used to compute effect sizes for t tests, F tests, z tests and some exact tests.

3. Observational Study Implementation

Patients were recruited from four investigator clinics. After the clinician decided to prescribe UltraFine Bubbles (UFB) as part of the treatment plan, all patients that met the inclusion criteria were offered the option to participate in the study and receive this treatment free of charge. **Exhibit 1**, lists the eligibility criteria for inclusion in the study.

[2] van Zwet, E. (2018). A default prior for regression coefficients. *Statistical methods in medical research*, 0962280218817792.

Exhibit 1: Eligibility Criteria for inclusion in the study

Inclusion Criteria	
	Age: 20 – 85
	Patients with Type 1 or 2 diabetes mellitus and must have a documented clinical diagnosis of painful diabetic peripheral neuropathy with symptoms and signs for at least 6 months, and pain present at the time of screening with at least one usual pain score over 6.
	Diagnosis must include pain plus reduction or absence of pin sensibility and/or vibration sensibility on Total Neuropathy Score - Nurse (TNSn) examination in lower and/or upper extremities at screening. Semmes-Weinstein test score needs to be below 14.
	The investigator considers the patient's blood glucose to be controlled by diet, or hypoglycemics, or insulin for at least three months prior to enrolling in the study (as documented by figures of glycated hemoglobin (HbA1c) no greater than 11 percent at screening or documented within the past six months.
	Patients have signed an Informed Consent agreeing to participate in the observational study.
	Patients who are currently prescribed opioid medications must be taking daily doses of an opioid-based analgesic equivalent to 160mg or less of oral morphine.
Exclusion Criteria	
	Open wound, foot ulcer or scab on any part of the body surface than may be immersed into the gel.
	Patient unable to come to the medical office for at least 10 treatments over a period of 6 weeks.
	Patient unable to complete initial questionnaire, even with the help of a health care professional.
	Patients with any other causes of neuropathy, dialysis for renal insufficiency, active malignant diseases, uncontrolled hypothyroidism, or excessive alcoholic intake.
	Significant history of pulmonary, metabolic (except diabetes mellitus), neurological, psychiatric disorders (resulting in disorientation, memory impairment or inability to report accurately as in schizophrenia).
	Current Smokers (need to have quit smoking more than 2 years before enrolling).
	History of seizure disorder or epilepsy.
	History of any other clinically significant disease or condition that in the investigator's opinion may affect efficacy or safety assessments or may compromise patient safety during study participation.

Patients who met the inclusion criteria were given an informed consent form to review and any questions that they had were answered. Investigators explained in detail the requirements for

the 10 treatments, the questionnaires, and the required follow-up visits one week and one month after treatment was completed.

The Study included one treatment group using an UltraFine Bubbles (UFB) solution prepared by PeriphEX. Primary Treatment consisted of 10 treatments administered over a six-week period. Following the ten treatments, patients came back for a one-week and one-month follow-up. The 10 primary treatments were administered through physician visual and in-person testing and observation and through patient surveys.
In addition to the Enrollment Form, data were collected through five protocols:

- Toronto Neuropathy – completed by physician
- Semmes-Weinstein 5.07 (10g) Monofilament Examination – completed by physician
- Treatment Protocol – completed by physician
- Pain Questionnaire – completed by patient
- Quality of Life Questionnaire – completed by patient

The Toronto Neuropathy, Semmes-Weinstein 5.07 (10g) Monofilament Examination, and Quality of Life Questionnaire were completed before Treatment #1, Treatment #7, one-week after final treatment, and one month after final treatment. The Treatment Protocol and the Pain Questionnaire were completed for each treatment, one week after final treatment, and one month after final treatment.

The observational study collected data between the end of April 2019 and February 27, 2020. The study stopped data collection at the end of February 2020 due to the Corona Pandemic. Of the 31 patients enrolled in the study, 21 completed the 10 treatments and the one-week and one-month follow-up. The ten treatments were actually administered over a period of 19 and 42 days or between 2.7 and 6 weeks. This group of patients started to receive treatments between April 29, 2019 and October 22, 2019. They completed 10 treatments between May 21, 2019 and September 26, 2019. Their one-month follow-up was completed between June 19, 2019 to January 15, 2020. Data collection for seven of the ten patients who did not complete the entire regime of treatments and follow-up was terminated on February 27, 2020.

II. PATIENT DEMOGRAPHICS

The report focuses on 21 patients that received the 10 treatments and had both a one-week follow-up and a one-month follow-up after the 10th treatment.

The 21 patients are between 63 and 83 years old. Ten patients (47.6 percent) are between 63.4 and 70.5 years old, eight patients (38.1 percent) are between 71.4 and 79.7, two are between 82.2 and 82.5 (9.5 percent), and one patient (4.8 percent) did not have age data.

Thirteen of the patients (61.9 percent) are male and eight (38.1 percent) are female.

The patients are ethnically diverse: 14 of the patients (66.7 percent) are Caucasian, three (14.3 percent) are African American; another three (14.3 percent) are Hispanic, and one (4.8 percent) is Asian American.

III. MAJOR FINDINGS

The percentage of patients experiencing pain during the day or night and the percentage of patients experiencing different types of pain decreased with the treatments.

Patients experienced improvement (decrease) in all types and levels (Usual and Maximum) of pain after the final treatment, one week after the final treatment, and one month after the final treatment. The reduction in pain was statistically significant for all usual and maximum types of pain at one week and one month of follow-up after completion of treatments, according to the Paired Samples T-Test.

A Mixed Model Linear Regression tested the relationship between treatment and changes in usual and maximum tingling, numbness, and burning over time. It showed all negative and statistically significant average slopes, implying that the usual and maximum level of tingling, numbness and burning diminished as the number of treatments increased, and that the decrease is statistically significant.

A considerable percent of patients experienced 50 percent or more improvement in their pain levels across the different types of pain. Between 29 and 57 percent of patients experienced 50 percent or more improvement in usual pain and between 33 and 52 percent experienced 50 percent or more improvement in maximum pain.

Measurements of level of neuropathy (Toronto Neuropathy Score) showed that patients had severe neuropathy at the start of treatment. Their neuropathy level decreased from severe to moderate, as measured one week after they completed the treatments and remained so one month after treatment completion. The Paired Samples T-Test and Mixed model Linear Regression showed a statistically significant decrease in neuropathy levels during the measurement benchmarks.

According to the Semmes Weinstein 5.07 (10g) Monofilament Examination, patients were able to feel between 39.1 and 40.8 percent more of the checked spots on their feet. All had positive change and all changes were statistically significant, according to the Paired Samples T-Test and the Mixed Model Linear Regression implying an increase in the number of locations on the feet where patients felt the monofilament.

Patients' quality of life improved throughout the treatments and one week following treatment completion. The Paired Samples T-Test showed a decrease in neuropathy symptoms' interference in the different aspects of life, thus contributing to quality of life. The data shows statistically significant impact on general activity, mood, walking ability, normal work, feeling your feet while walking, normal work, relationships with other people, and sleep.

IV. OBSERVATIONAL STUDY RESULTS

1. Report Organization

The report focuses on the 21 patients who completed the ten treatments, one-week follow-up and one- month follow-up. The report is organized into four major sections.

- Pain Questionnaire
- Toronto Neuropathy
- Semmes-Weinstein 5.07 (10g) Monofilament Examination
- Quality of Life Questionnaire

Each section presents the data and results associated with the respective data collection protocol.

Not included in the report are results of analyses exploring the relationship between patients' age and pain improvement and analyses examining the relationship between frequency of treatments and pain improvement. These analyses did not show any pattern and results were not statistically significant.

2. Pain Questionnaire

2.1. Timing, Change, and Characteristics of Pain

The Pain Questionnaire was completed by the patients during each of the 10 treatments, one week following Treatment #10, and one month following Treatment #10. Patients were asked:

- Whether they have pain during the day;
- whether they have pain at night;
- whether the pain they are experiencing has one or more of these characteristics: shooting, tingling, electric shock, and burning.

At the onset of the treatments, 90.5 percent of the patients experienced pain during the day and 76.2 percent experienced pain at night. Between 42.9 and 90.5 percent also experienced different kinds of pain, as shown in **Exhibit 2**. The percentage of patients experiencing pain during the day or night and the percentage of patients experiencing different types of pain decreased with the treatments.

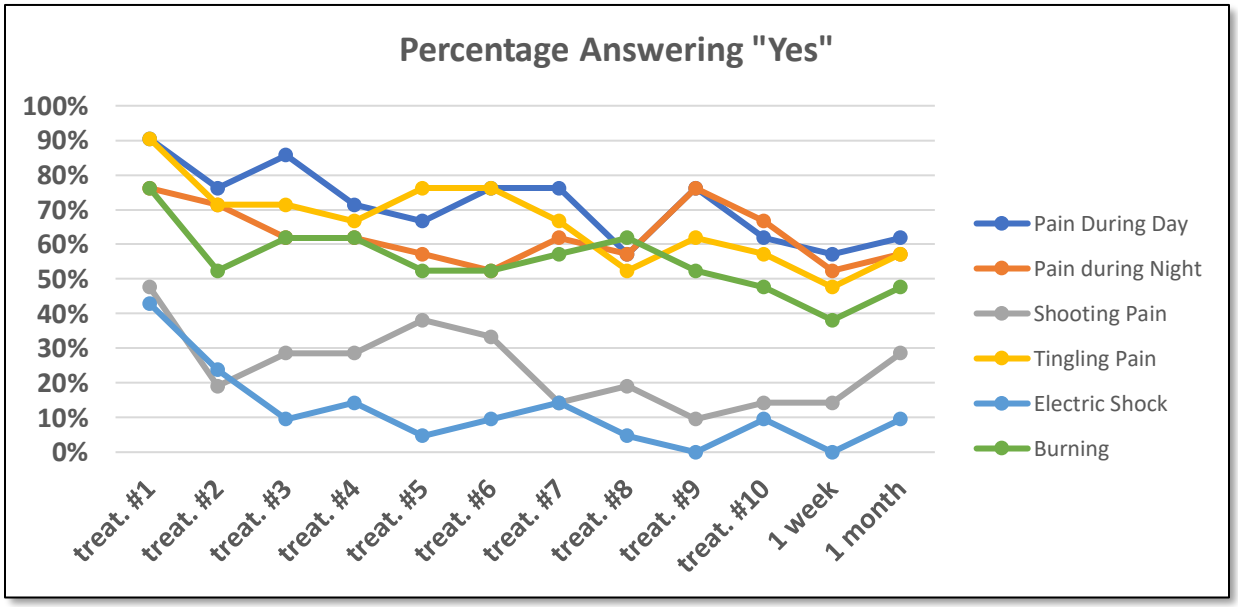
- After 10 treatments, 28.6 percent fewer patients experienced pain during the day and 9.5 percent fewer patients experienced pain at night. Between 28.6 percent and 33.4 percent fewer patients experienced the different types of pain.

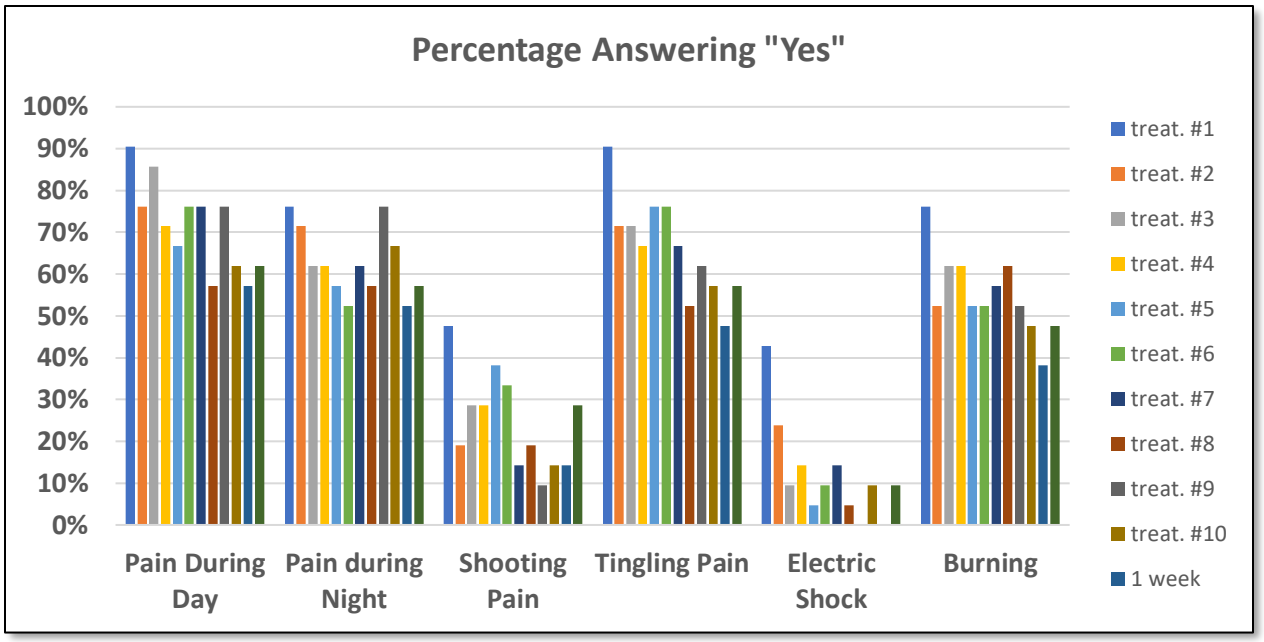
- One week following the last treatment, there was a further decrease in the percent of patients experiencing pain. For example, 33.4 percent fewer patients experienced pain during the day and 23.8 percent fewer patients experienced pain at night. Between 32.4 percent and 38.1 percent fewer patients experienced the different types of pain.

- One month following the last treatment, four of the six measures of pain were reported by the same percentage of patients as at the end of the 10 treatments. The percent of patients feeling pain at night decreased 19.1 percent and the percent of patients having shooting pains decreased 18.1 percent from the percent of respective patients experiencing pain at the start of treatments.

Exhibit 2: Percent of Patients Experiencing Pain

Experiencing Pain	T#1	T#7	T#10	1 Week After T#10	1 Month After T#10	Change in Percent of Patients Experiencing Pain		
						T#1 vs T#10	T#1 vs 1 Week After T#10	T#1 vs 1 Month After T#10
Pain during the day	90.5%	76.5%	61.9%	57.1%	61.9%	28.6%	33.4%	28.6%
Pain during the night	76.2%	61.9%	66.7%	52.4%	57.1%	9.5%	23.8%	19.1%
Shooting pain	46.7%	14.3%	14.3%	14.3%	28.6%	32.4%	32.4%	18.1%
Tingling pain	90.5%	66.7%	57.1%	47.6%	57.1%	33.4%	42.9%	33.4%
Electric shock	42.9%	14.3%	9.5%	0.0%	9.5%	33.4%	42.9%	33.4%
Burning	76.2%	57.1%	47.6%	38.1%	47.6%	28.6%	38.1%	28.6%





2.2 Changes in Usual and Maximum Pain

Patients were also asked to rate their usual and worst tingling pain, numbness, and burning pain on a 10-point scale ranging from "0-No Pain" to "10-Worst Pain."

Exhibit 3, shows mean scores calculated based on the ratings on the 10-point scale for usual and maximum tingling pain, numbness, and burning pain after the initial treatment, Treatment #7, Treatment #10 (final treatment), one week after Treatment #10, and one month after Treatment #10. The data shows improvement (decrease) in all types and levels of pain after the final treatment, one week after the final treatment, and one month after the final treatment.

- The level of improvement for usual pain after 10 treatments ranged from 27.9 percent (Numbness) to 53.2 percent (Tingling Pain). For maximum pain, level of improvement ranged from 31.4 percent (Numbness) to 51.5 percent (Tingling Pain).

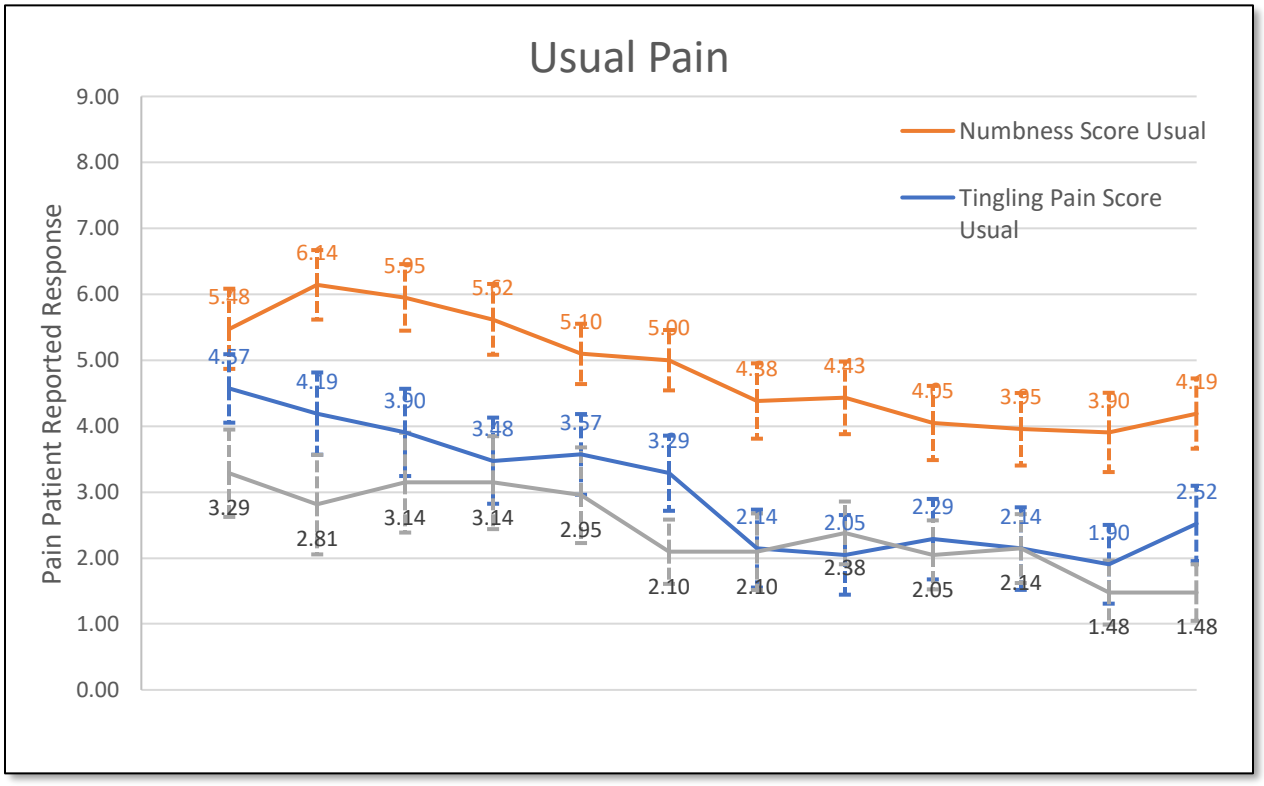
- One week after the final treatment, level of improvement for usual pain ranged from 28.8 percent (Numbness) to 58.5 percent (Tingling Pain). For maximum pain, level of improvement ranged from 31.4 percent (Numbness) to 56.6 percent (Tingling Pain).

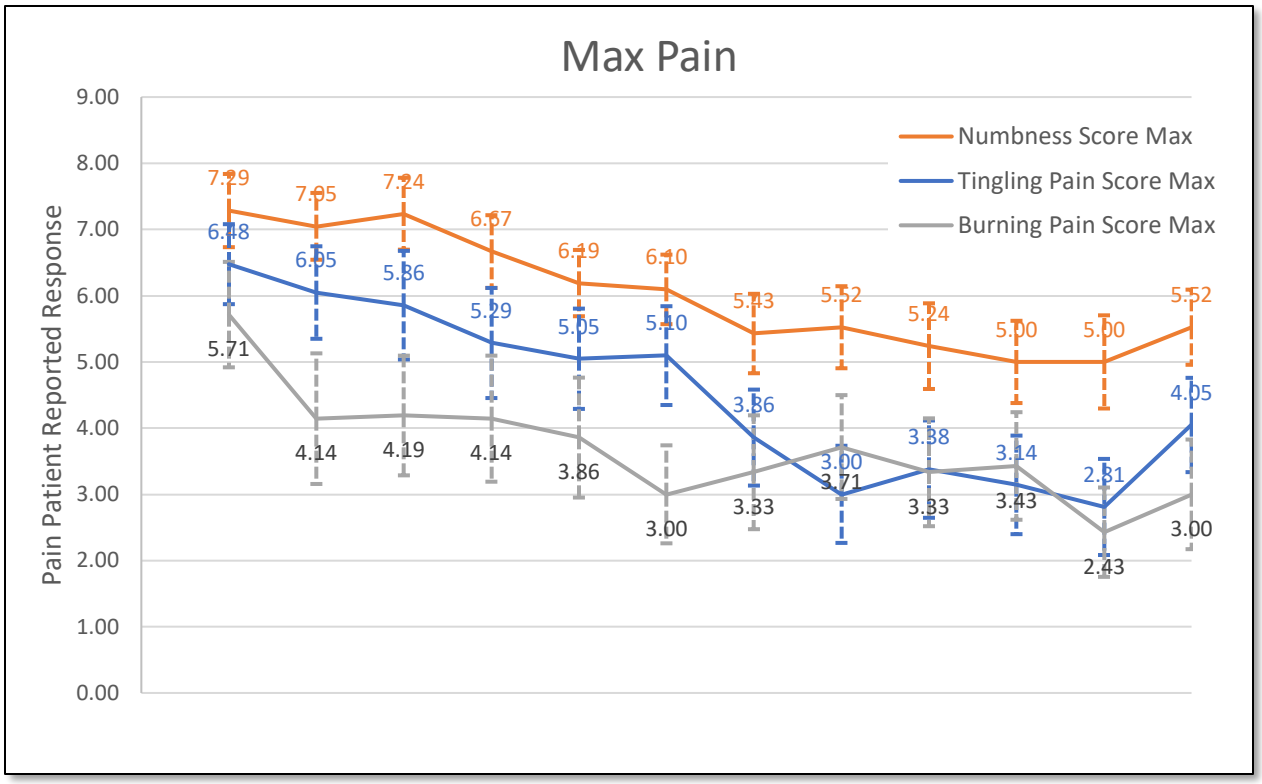
- One month after the final treatment, level of improvement for usual pain ranged from 23.5 percent (Numbness) to 55.0 percent (Burning Pain). For maximum pain, level for improvement ranged from 24.3 percent (Numbness) to 47.5 percent (Burning Pain).

Exhibit 3: Usual and Maximum Pain by Type of Pain, Benchmarks and % of Improvement

Type and Extent of Pain	T#1	T#7	T#10	One Week After T#10	One Month After T#10	Relative Improvement After 10 Ts	Relative Improvement 1 Week After 10 Ts	Relative Improvement After 1 Month
Tingling Pain – Usual	4.57 +/-0.52	2.14 +/- 0.59	2.14 +/-0.63	1.90 +/-0.60	2.52 +/-0.57	53.2%	58.4%	44.9%
Tingling Pain – Max	6.48 +/-0.60	3.86 +/- 0.72	3.14 +/-0.74	2.81 +/-0.73	4.05 +/-0.71	51.5%	56.6%	37.5%
Numbness – Usual	5.48 +/-0.61	4.38 +/- 0.57	3.95 +/-0.55	3.90 +/-0.60	4.19 +/-0.53	27.9%	28.8%	23.5%
Numbness – Max	7.29 +/-0.55	5.43 +/- 0.60	5.00 +/-0.62	5.00 +/-0.70	5.52 +/-0.57	31.4%	31.4%	24.3%
Burning pain – Usual	3.29 +/-0.66	2.10 +/- 0.58	2.14 +/-0.52	1.48 +/-0.49	1.48 +/-0.43	35.0%	55.0%	55.0%
Burning Pain – Max	5.71 +/-0.80	3.33 +/- 0.96	3.43 +/-0.81	2.43 +/-0.67	3.00 +/-0.83	39.9%	57.4%	47.5%

** +/- refers to the standard error.*





2.3 Paired Samples T-Test

In the Paired Samples T-Test each subject is measured twice (at baseline and after the administration of treatment) thus creating pairs of observations. The test is used in repeated measures designs to determine whether the mean difference between two sets of observations is zero. The null hypothesis assumes that the true mean difference between the paired observations is zero; that is, all observable differences can be explained by random variation. The expected outcome for this study is that the difference between baseline and treatment will be greater than zero (an upper tailed hypothesis) thus increasing the power of the test.

Paired Samples T-Tests were conducted on the three types of pain at the usual and maximum levels comparing mean scores from baseline to Treatment #7, Treatment #10, one week after the final treatment and one month after the final treatment. As shown in **Exhibit 4**, all showed negative average changes (reduction in pain) across the four time benchmarks. All showed a statistically significant reduction in pain after the 7th and 10th treatments except in two cases: usual burning pain 7th and 10th treatments and usual numbness 7th treatment. The reduction in pain was statistically significant for all usual and maximum types of pain at one week and one month of follow-up after completion of treatments.

Exhibit 4: Paired Samples T-Tests by Type of Pain and Interval

Type of Pain	Time	Average Change	T-Test P Value
Tingling Pain - Usual	7th Treatment	-2.42857	0.000847
Tingling Pain - Usual	10th Treatment	-2.42857	0.001493
Tingling Pain - Usual	1 Week Follow-up	-2.66667	0.000458
Tingling Pain - Usual	1 Month Follow-up	-2.04762	0.0054
Tingling Pain – Max	7th Treatment	-2.61905	0.000614
Tingling Pain – Max	10th Treatment	-3.33333	0.00024
Tingling Pain – Max	1 Week Follow-up	-3.66667	0.000113
Tingling Pain – Max	1 Month Follow-up	-2.42857	0.001789
Numbness – Usual	7th Treatment	-1.09524	0.107867*
Numbness – Usual	10th Treatment	-1.52381	0.013015
Numbness – Usual	1 Week Follow-up	-1.57143	0.027392
Numbness – Usual	1 Month Follow-up	-1.28571	0.047874
Numbness – Max	7th Treatment	-1.85714	0.002128
Numbness – Max	10th Treatment	-2.28571	0.000593
Numbness – Max	1 Week Follow-up	-2.28571	0.001495
Numbness – Max	1 Month Follow-up	-1.7619	0.007232
Burning Pain - Usual	7th Treatment	-1.19048	0.133425*
Burning Pain - Usual	10th Treatment	-1.14286	0.162298*
Burning Pain - Usual	1 Week Follow-up	-1.80952	0.013906
Burning Pain - Usual	1 Month Follow-up	-1.80952	0.004612
Burning Pain – Max	7th Treatment	-2.38095	0.018059
Burning Pain – Max	10th Treatment	-2.28571	0.011853
Burning Pain – Max	1 Week Follow-up	-3.28571	0.001192
Burning Pain – Max	1 Month Follow-up	-2.71429	0.002467

**Not statistically significant.*

Eight of the 21 patients were followed six months after they completed the 10 treatments (**Exhibit 5**). The UFB product significantly reduced their usual and maximum tingling pain and numbness and their maximum burning pain even after six months. Although there was also a reduction in usual burning pain it was not statistically significant.

Exhibit 5: Paired Samples T-Test: Six Months Follow-up

Type of Pain	Time	Average Change	T-Test P Value
Tingling Pain – Usual	6 Months Follow-up	-3.5	0.003799
Tingling Pain – Max	6 Months Follow-up	-3.5	0.0136
Numbness – Usual	6 Months Follow-up	-2.625	0.034015
Numbness – Max	6 Months Follow-up	-3	0.018452
Burning Pain – Usual	6 Months Follow-up	-1.875	0.069341
Burning Pain – Max	6 Months Follow-up	-3.375	0.026862

2.4 Mixed Model Linear Regression – Pain Questionnaire

A mixed model linear regression is particularly useful in settings where repeated measurements are made over time on the same statistical units (longitudinal study), or where measurements are made on clusters of related statistical units. When using such measurements we have to account for both within-person and across-person variability.

A standard linear regression model has only fixed effects while a mixed model has both random and fixed effects. The use of both fixed and random effects in the same model can be thought of hierarchically. The hierarchy arises because we can think of one level for subjects and another level for measurements within subjects. The basic idea is that the fixed effects parameters tell how population means differ between any set of treatments, while the random effect parameters represent the general variability among subjects or other units.

A mixed model linear regression was used to test for usual and maximum tingling, numbness, and burning per the Pain Questionnaire data. The mixed model linear regression was run with the score regressed on the measurement occasion. Measurement occasions consist of baseline, 10^{th} treatment, one-week follow-up, and one-month follow-up with random intercepts and slopes for subjects. Note that the average slope refers to estimated change of that measurement over time. The null hypothesis is that the average slope is equal to 0.

All the average slopes are negative, and all are statistically significant (**Exhibit 6**).

Exhibit 6: Mixed Model Linear Regression – Type and Level of Pain

Type of Pain	Average Slope	Standard Error	t Value	P Value
Tingling Pain – Usual	-0.24332	0.043544	-5.5878	2.05E-05
Tingling Pain – Max	-0.31756	0.072917	-4.35503	0.000314
Numbness – Usual	-0.22002	0.051684	-4.2571	0.000394
Numbness – Max	-0.23533	0.052002	-4.52545	0.000212
Burning Pain – Usual	-0.1611	0.050521	-3.18874	0.00476
Burning Pain – Max	-0.19567	0.064526	-3.03248	0.00676

In mathematics, the slope of a line describes how rapidly or slowly change is occurring and in which direction, whether positive or negative. In statistics, a graph with a negative slope (the

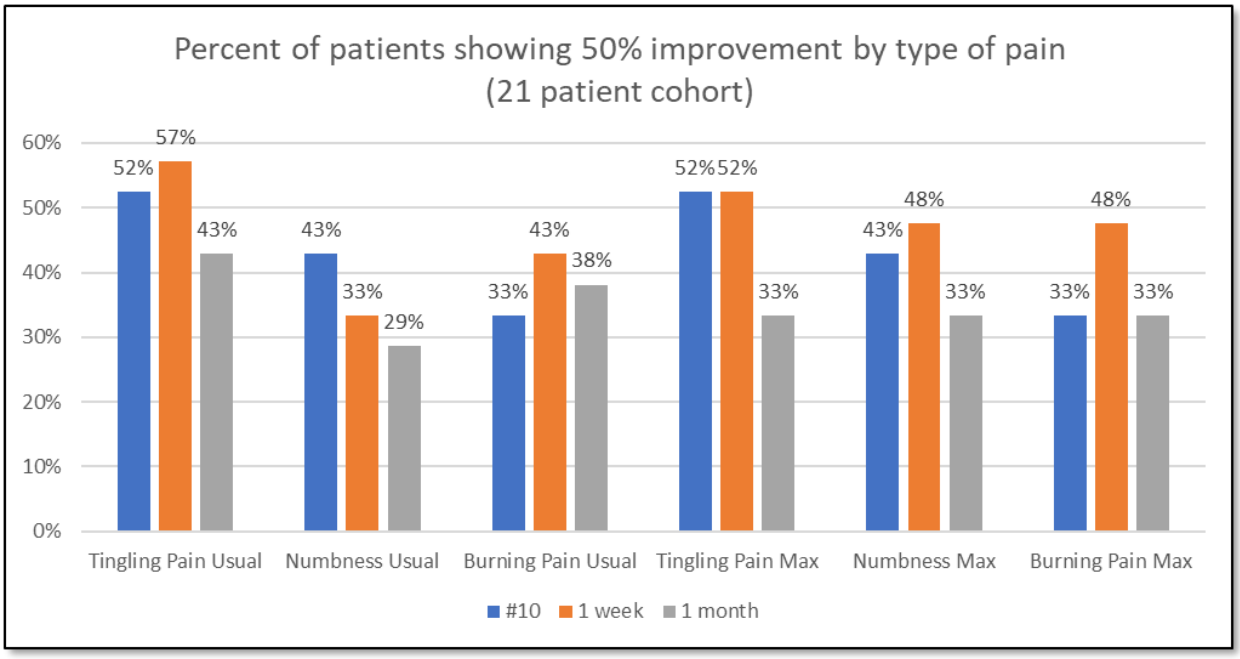
line moves down when going from left to right) represents a negative correlation between two variables. This means that as one variable increases, the other decreases—and vice versa. Negative correlation indicates a clear relationship between the variables, meaning one affects the other in a meaningful way.

In this study, the negative slope implies that the usual and maximum level of tingling, numbness and burning diminished as the number of treatments increased, and that the decrease is statistically significant.

2.5 Fifty Percent or More Improvement in Pain

A considerable percentage of patients experienced a 50 percent or greater improvement in their usual and maximum pain. This was measured at three benchmarks: after completing the ten treatments, one week after completing the treatments, and one month after completing the treatments. As shown in the graph below:

- Between 43 percent and 57 percent of patients experienced a 50 percent or greater improvement in their usual tingling pain and between 33 percent and 52 percent experienced such improvement in their maximum tingling pain.
- Between 29 percent and 43 percent of patients experienced 50 percent or more improvement in usual numbness and between 33 percent and 48 percent experienced such improvement in their maximum numbness.
- Between 33 percent and 43 percent experienced 50 percent or more improvement in usual burning pain and between 33 percent and 48 percent experienced such improvement in their maximum burning pain.



3. Toronto Neuropathy Score

The Toronto Neuropathy Score protocol is used by physicians to measure the level of the patient's neuropathy in the right foot and left foot based of patient's three scores:

- Symptoms score – absence (0) or presence (1) – of pain, numbness, weakness, ataxia, and upper-limb symptoms;
- Reflex score – normal (0), reduced (1), absent (2) – of knee reflexes and ankle reflexes;
- Sensory test score – normal (0) or abnormal (1) – of pinprick, temperature, light touch, vibration sense, and position sense.

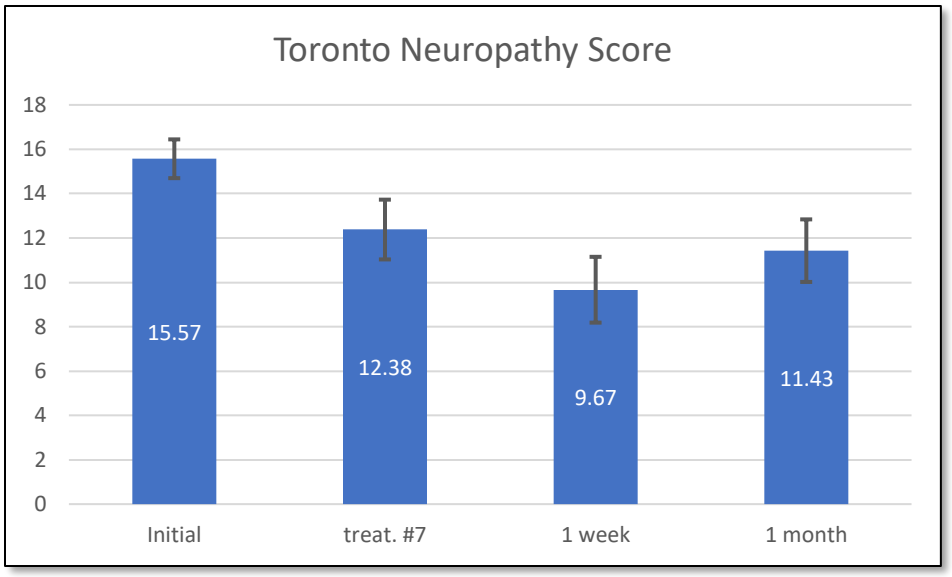
The scores are totaled and classified into four levels:

- No neuropathy: 0-5 points
- Mild neuropathy: 6-8 points
- Moderate neuropathy: 9-11 points
- Severe neuropathy: 12+ points

The Toronto Neuropathy Score protocol was administered at baseline before Treatment #1, after Treatment #7, one week after Treatment #10, and one month after Treatment #10. As shown in **Exhibit 7**, patients had severe neuropathy at the start of treatment. Their neuropathy level decreased from severe to moderate, as measured one week after they completed the treatments and remained so one month after treatment completion.

Exhibit 7: Toronto Neuropathy Score

Administration	Mean Score	Level of Improvement from Baseline	Standard Error	Standard Deviation
Baseline before Treatment #1	15.57		0.874	4.007
Treatment #7	12.38	20.5%	1.348	6.176
One week after Treatment #10	9.67	37.9%	1.484	6.800
One month after Treatment #10	11.43	26.65	1.410	6.462



The Paired Samples T-Test shows negative changes (reduction in level of neuropathy) at all time benchmarks, including a six months follow-up that was performed on eight of the 21 patients. All changes are statistically significant (**Exhibit 8**).

Exhibit 8: Toronto Neuropathy Score – Paired Samples T-Test

Time	Average Change	T-Test P Value
7th Treatment	-3.19048	0.025982
1 Week Follow-up	-5.90476	0.000173
1 Month Follow-up	-4.14286	0.001488
6 Months Follow-up*	-6.125	0.032037

**Data available for 8 patients only.*

The Mixed Model Linear Regression analysis was run with the neuropathy score regressed on the measurement occasion – Treatment #7, one week after Treatment #10, and 1 month after Treatment #10. Note that the average slope refers to change of that measurement over time with the null hypothesis that the average slope is equal to 0. A shown in **Exhibit 9**, the average slope is negative, implying a reduction in level of neuropathy, and statistically significant.

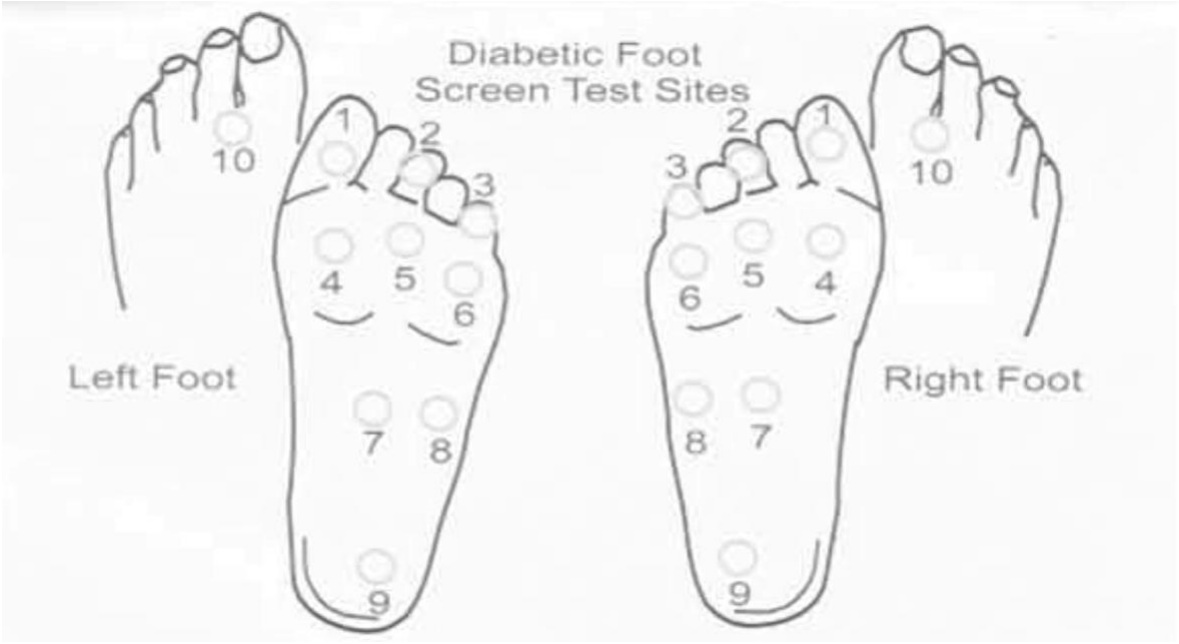
Exhibit 9: Toronto Neuropathy Score – Mixed Model Linear Regression

Average Slope	Standard Error	t Value	P Value
-0.44643	0.103304	-4.32154	0.000229

4. Semmes-Weinstein 5.07 (10g) Monofilament Examination

The steps involved in the Semmes-Weinstein 5.07 (10g) Monofilament Examination are described as follows:

1. Explain Procedure to the patient
2. Position the patient in a comfortable position, for ease of performing the exam, and occlude patient vision such that he cannot see when the wire is applied to the skin of his feet.
3. Demonstrate the use of the monofilament on the patient's hand so that she or he will know what to expect.
4. Hold the probe by the plastic or cardboard handle.
5. Apply the filament perpendicular to the skin. Apply sufficient force to cause the monofilament to buckle of bend, using a smooth not jabbing, motion.
6. Touch the monofilament
7. Ask the patient to respond with a "yes" each time he or she feels the monofilament touching the skin.
8. Apply the monofilament along the margin of a callus, ulcer, scar or necrotic tissue; do NOT apply the monofilament over these lesions.
9. Record the results on the form below by filling in the circle (●) if the patient felt the monofilament, and an "X" if there is no sensation.

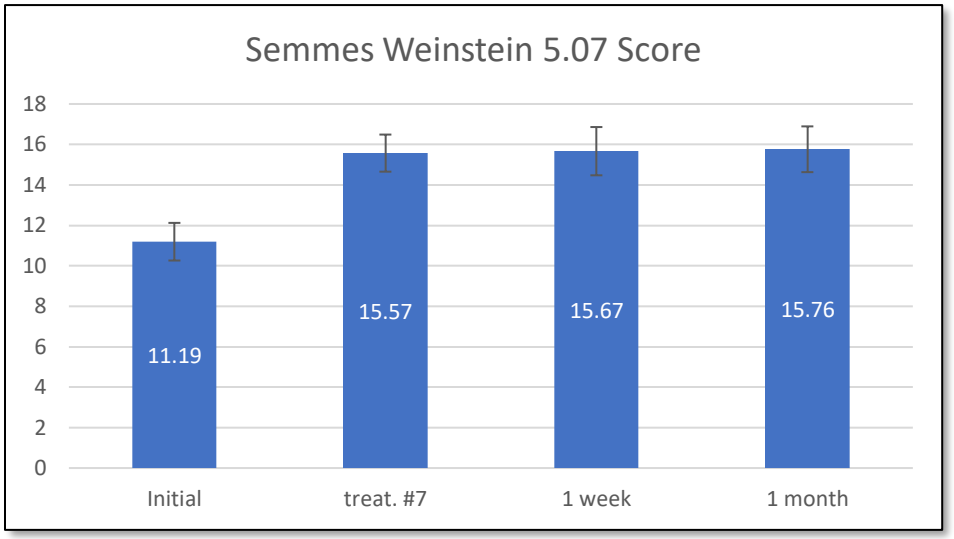


Score Left Foot: _____ (0-10) Score Right Foot: _____ (0-10)

Exhibit 10 shows the mean score representing the number of times patients felt the monofilament touching their skin. Between the baseline and Treatment #7, one week after Treatment #10 and one month after Treatment #10, patients were able to feel between 39.1 and 40.8 percent more of the checked spots on their feet.

Exhibit 10: Semmes-Weinstein

Administration	Mean Score	Level of Improvement from Baseline	Standard Error	Standard Deviation
Baseline before Treatment #1	11.19		0.930	4.262
Treatment #7	15.57	39.1%	0.917	4.202
One week after Treatment #10	15.67	40.0%	1.194	5.471
One month after Treatment #10	15.76	40.8%	1.132	5.186



The Paired Samples T-Test compared baseline to seven treatments, baseline to one week after completion of the ten treatments and baseline to one month after treatment completion; all had positive change and all were statistically significant (**Exhibit 11**). The six months follow-up, performed on eight of the 21 patients, also showed a positive change and was statistically significant.

Exhibit 11: Semmes-Weinstein 5.07 (10g) Monofilament Examination – Paired Samples T-Test

Time	Average Change	T-Test P Value
7th Treatment	4.380952	5.37E-05
1 Week Follow-up	4.47619	0.002103
1 Month Follow-up	4.571429	9.42E-05
6 Months Follow-up*	5.375	0.029018

**Data available for 8 patients only.*

The Mixed Model Linear Regression analysis was run with the score regressed on the measurement occasion – Treatment #7, one week after Treatment #10, and 1 month after Treatment #10. Note that the average slope refers to change of that measurement over time with the null hypothesis that the average slope is equal to 0. A shown in **Exhibit 12**, the average slope is positive and statistically significant, implying an increase in the number of locations on the feet that patients' felt the monofilament.

Exhibit 12: Semmes Weinstein – Mixed Model Linear Regression

Average Slope	Standard Error	t Value	P Value
0.435645	0.081742	5.329538	2.88E-05

5. Quality of Life Questionnaire

The Quality of Life Questionnaire asks patients to assess the level of interference of neuropathy symptoms in the past 48 hours on their quality of life on eight daily activities. The eight daily activities include:

- General activity
- Mood
- Walking ability
- Feeling your feet while walking
- Normal work (includes both work outside the home and housework)
- Relations with other people
- Sleep
- Enjoyment of life

Patients were asked to rate each activity on a 10-point scale ranging from "0 – Does Not Interfere" to "10 – Completely Interferes."

Thirteen patients completed the Quality of Life Questionnaire at four benchmarks during the observational study: at baseline before the first treatment, after Treatment # 7, one week after Treatment #10, and one month after the Treatment #10.

Exhibit 13 shows the improvement patients experienced in each of the eight Quality of Life activities after they started the treatment regime. Their quality of life improved throughout the treatments and one week following treatment completion. Even though all experienced a decrease in quality of life one-month after treatment completion, their quality of life was still better than when they started the treatments.

Exhibit 13: Quality of Life

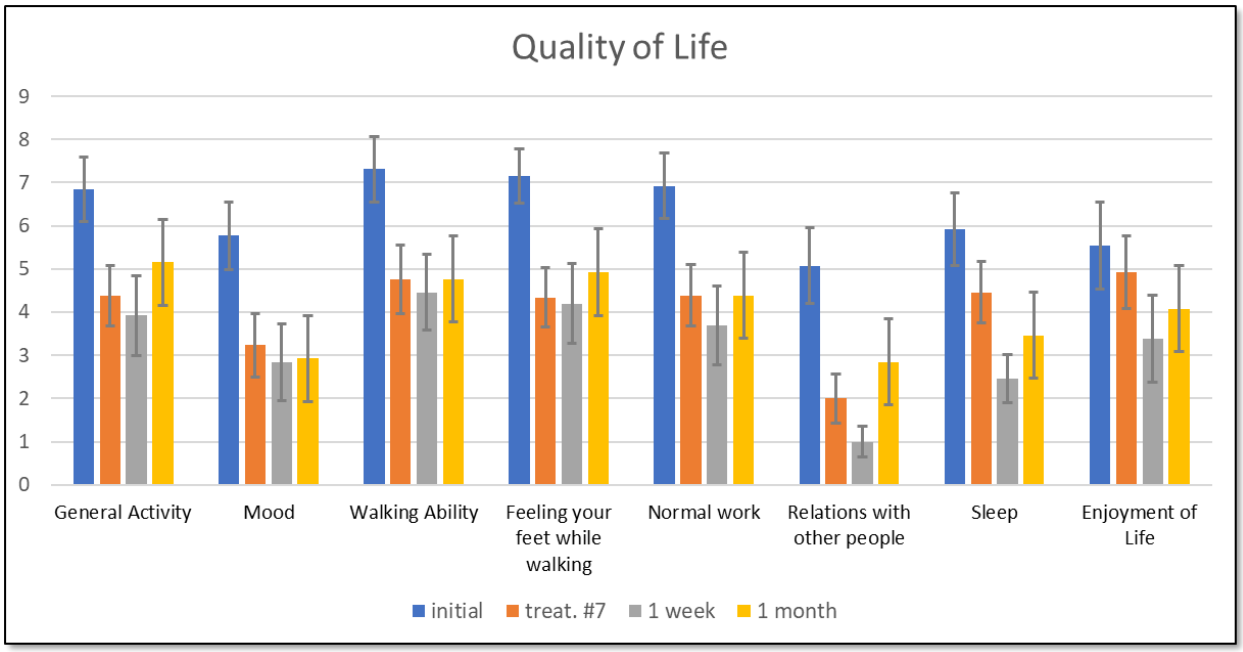
Quality of Life Areas	Mean Scores			
	Initial Treatment	Treatment #7	One week After Treatment 10	One Month After Treatment #10
General Activity	6.85	4.38	3.92	5.15
Mood	5.77	3.23	2.85	2.92
Walking Ability	7.31	4.77	4.46	4.77
Feeling your feet while walking*	7.15	4.33	4.20	4.92
Normal work	6.92	4.38	3.69	4.38
Relations with other people	5.08	2.00	1.00	2.85
Sleep	5.92	4.46	2.46	3.46
Enjoyment of Life	5.54	4.92	3.38	4.08

**Nine patients rated this quality of life component during Treatment #7 and 10 patients rated it one week following Treatment #10.*

Exhibit 14 shows percent improvement in each of the Quality of Life activities during three benchmarks. Improvement after the Treatment #7 ranged from 11.2 percent – Enjoyment of life – to 60.6 percent – Relations with other people. Improvement increased further one week following treatment completion, ranging from 39.0 percent – Walking ability and Enjoyment of life – to 80.3 percent – Relations with other people. One month after initial treatment, improvement ranged from 24.8 percent – General activity – to 49.4 percent – Mood.

Exhibit 14: Rate of Improvement in Quality of Life

Quality of Life Areas	Percent Improvement		
	Treatment #7 vs. Initial Treatment	One Week Follow-up vs. Initial Treatment	One Month vs. Initial Treatment
General Activity	36.1%	42.8%	24.8%
Mood	44.0%	50.6%	49.4%
Walking Ability	34.7%	39.0%	34.7%
Feeling your feet while walking*	39.4%	41.3%	31.2%
Normal work	36.7%	46.7%	36.7%
Relations with other people	60.6%	80.3%	43.9%
Sleep	24.7%	58.4%	41.6%
Enjoyment of life	11.2%	39.0%	26.4%



A Paired T-Test was conducted on 13 patients comparing baseline to 7th treatment, one-week follow-up after treatment completion, and one-month follow-up after treatment completion.

The null hypothesis implies no change over time. The data disproves the null hypothesis, showing a decrease in neuropathy symptoms' interference in the different aspects of life, thus contributing to quality of life. The data shows statistically significant impact on general activity, mood, walking ability, normal work, feeling your feet while walking, normal work, relationships with other people, and sleep. The only area that did not achieve statistical significance is enjoyment of life (**Exhibit 15**).

Exhibit 15: Quality of Life – Paired Samples T-Tests

Type of Pain	Time	Means Difference	T-Test P Value
General Activity	7th Treatment	2.461538	0.018414
	1 Week Follow-up	2.923077	0.014346
	1 Month Follow-up	1.692308	0.0238
Mood	7th Treatment	2.538462	0.001422
	1 Week Follow-up	2.923077	0.000869
	1 Month Follow-up	2.846154	0.003669
Walking Ability	7th Treatment	2.538462	0.01094
	1 Week Follow-up	2.846154	0.014157
	1 Month Follow-up	2.538462	0.012644
Feeling Your Feet While Walking	7th Treatment	3.444444	0.006522
	1 Week Follow-up	3.2	0.007841
	1 Month Follow-up	2.230769	0.00719
Normal Work	7th Treatment	2.538462	0.008846
	1 Week Follow-up	3.230769	0.004109
	1 Month Follow-up	2.538462	0.005712
Relations with Other People	7th Treatment	3.076923	0.006241
	1 Week Follow-up	4.076923	0.000746
	1 Month Follow-up	2.230769	0.002964
Sleep	7th Treatment	1.461538	0.027812
	1 Week Follow-up	3.461538	0.000113
	1 Month Follow-up	2.461538	0.000536
Enjoyment of Life	7th Treatment	0.615385	0.264296*
	1 Week Follow-up	2.153846	0.074508*
	1 Month Follow-up	1.461538	0.068544*

**Not statistically significant.*