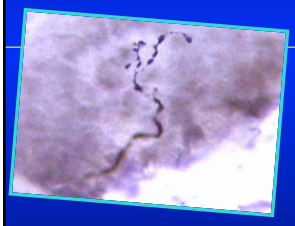
What is the role of punch skin biopsy in analgesic clinical trials and the development of improved analgesic treatments?



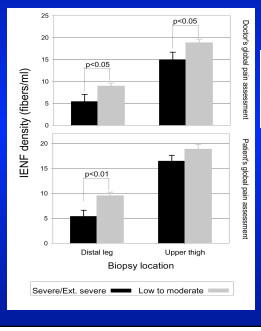
Michael Polydefkis, MD MHS
Associate Professor, Neurology
Director Cutaneous Nerve Laboratory,
Director, JH Bayview EMG Laboratory

IMPACT XV Meeting
Washington, DC June 21, 2012

Outline

- IENFD as a biomarker of neuropathic pain
- IENFD as a measure of neuropathy
  - N of 1 experiments
  - Early marker of neuropathy
  - Natural history studies
- Clinical trials
- Measure of regeneration

IENFD as a biomarker of neuropathic pain



Reduced intraepidermal nerve fiber density in HIV-associated sensory neuropathy

M. Polydefkis, MD; C.T. Yiannoutsos, PhD; E.A. Cohen, MD; E. Hollander, MD; G. Schiffito, MD; D.B. Clifford, MD; D.M. Simpson, MD; D. Katzenstein, MD; S. Shriver, MS; P. Haase, BA; A. Brown; A.E. Hedrick, MSc; L. Moe, MD; and J.C. McArthur, MBBS, MPH

Neurology 2002;58:115-9.

Skin biopsy results may predict response to treatment

- PHN: patients with relatively preserved sensation had less epidermal denervation and less allodynia. Such subjects responded to topical treatments.
- In contrast, IENFD did not predict response to topical lidocaine.

Postherpetic Neuralgia: Irritable Nociceptors and Deafferentation

Howard L. Fields,*,[1] Michael Rowbotham,*,[1] and Ralf Baron*,[2]

TABLE 1
Clinical Features of Different Types of PHN Pain

	Irritable nociceptor	Deafferentation
Thermal sensory deficit	+/−	+++
Allodynia	+++	or +++
Local skin block	Relief	

Neurobiol Dis. 1998;5(4):209-27.

Skin biopsy results may predict response to treatment

- PHN: patients with relatively preserved sensation had less epidermal denervation and less allodynia. Such subjects responded to topical treatments.
- In contrast, IENFD did not predict response to topical lidocaine.

SKIN BIOPSY AND QUANTITATIVE SENSORY TESTING DO NOT PREDICT RESPONSE TO LIDOCAINE PATCH IN PAINFUL NEUROPATHIES

DAVID N. HERRMANN, MB, BCh,[1,2] VALERIE PANNONI, BS,[1] RICHARD L. BARBANO, MD, PhD,[1] JANET PENNELLA-VAUGHAN, MS,[3] and ROBERT H. DWORKIN, PhD[1,3]

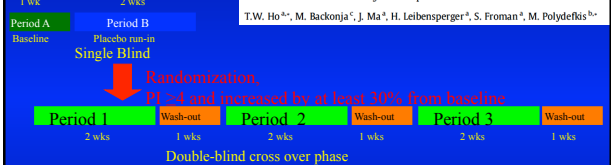
Variables*	Responders (n = 33)	Nonresponders (n = 1
Age (mean ± SD)	56.1 (10.5)	58.7 (13.7)
Gender (M/F)	5/28	6/13
Distal-leg epidermal innervation Skin biopsy grade (mean ± SD)	2.42 (1.43)	2.36 (1.5)

Pain Med. 2005;6(5):379-84

STUDY DESIGN AND DURATION

Efficient assessment of neuropathic pain drugs in patients with small fiber sensory neuropathies ☆

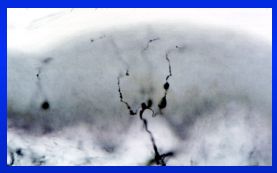
T.W. Ho[a,*], M. Backonja[c], J. Ma[a], H. Leibensperger[a], S. Froman[a], M. Polydefkis[b,*]



PAIN 141 (2009) 19–24 PAIN

Epidermal nerve fiber swellings: a harbinger of future axonal loss

- Swellings
  - Predictive for development of neuropathy
  - Lauria et al. Neurology. 2003;61(5):631-6.
  - Herrmann et al. Muscle & Nerve. 29(3): 420 - 427
  - Gibbons et al. Neurology. 2006 24;66(2):256-8

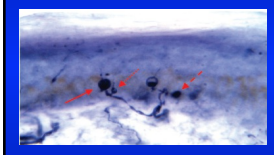


Epidermal nerve fiber swellings: a harbinger of future axonal loss

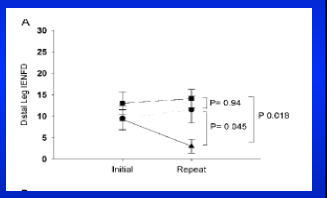
Lauria et al. Neurology. 2003;61(5):631-6.

			Baseline			Follow-up			Age-matched controls		
		Neuropathic signs at baseline	IENF density			IENF density			IENF density		
Patient	Diagnosis		Pth	DI	Swelling ratio, DI	Pth	DI	Swelling ratio, DI	Pth	DI	Swelling ratio, DI
1	Diabetes	+	12.7	6.0	0.60	11.5	3.0	0.26	27.0	12.5	0.29
2	Diabetes	+	26.6	5.2	0.83	22.0	3.9	0.72	21.5	9.0	0.31
3	Diabetes	+	20.7	4.7	0.54	18.0	3.6	0.51	32.6	14.2	0.26
4	Diabetes	+	14.0	4.9	0.55	13.5	4.1	0.32	28.5	10.7	0.23
5	Diabetes	+	37.9	11.4	0.62	23.4	6.8	0.50	25.1	16.4	0.22
6	Diabetes	+	15.7	5.0	0.43	10.5	0.0	0.00	13.5	15.9	0.13
7	Idiopathic	+	13.8	5.8	0.43	12.5	3.2	0.27	25.0	12.7	0.21
8	Idiopathic	+	18.9	6.6	0.57	17.7	3.4	0.48	18.2	10.4	0.16
9	AIDS	+	14.0	6.2	0.60	12.1	2.8	0.40	20.2	15.2	0.43
10	Taxol	–	13.1	8.3	0.54	12.3	5.4	0.36	15.6	11.8	0.30
11	Idiopathic	–	10.1	3.2	0.61	10.0	2.3	0.59	22.0	18.0	0.56
12	Idiopathic	–	22.0	18.0	0.55	19.6	13.0	0.45	25.2	16.1	0.46
13	Idiopathic	–	25.5	13.5	0.68	21.1	6.4	0.59	21.1	19.1	0.42
14	Idiopathic	–	30.4	17.3	0.64	28.0	9.0	0.38	21.9	18.2	0.22
15	Idiopathic	–	13.5	8.9	0.72	12.6	6.2	0.52	21.9	15.4	0.32
Mean(SD)			18.4 (6.5)	8.4 (4.6)	0.59 (0.1)	16.3 (5.5)	4.9 (3.1)	0.42 (0.2)	22.6 (4.8)	14.4 (3.0)	0.30 (0.12)

Morphological changes in epidermal nerve can predict neuropathy development

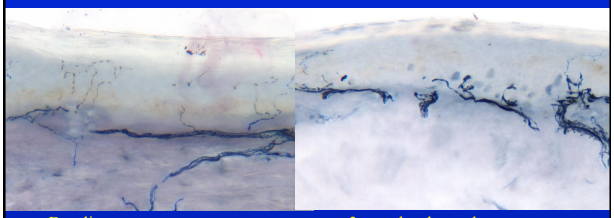


Gibbons et al. Neurology. 2006 24;66(2):256-8



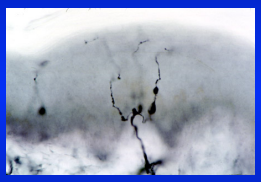
- ▲ large swellings
- • no swellings
- ■ small-medium sized swellings

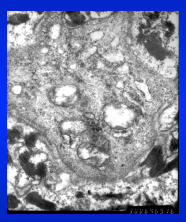
Are swellings irritable nociceptors, microneuromas?



Baseline 3 months chemotherapy

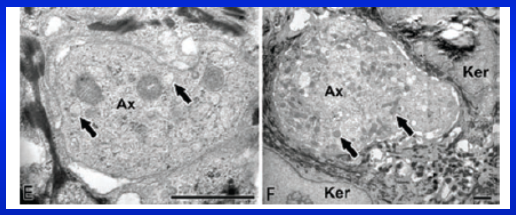
Are swellings irritable nociceptors, microneuromas?





Polydefkis et al., Neurology. 2000;55(8):1115-21.

Are swellings irritable nociceptors, microneuromas?



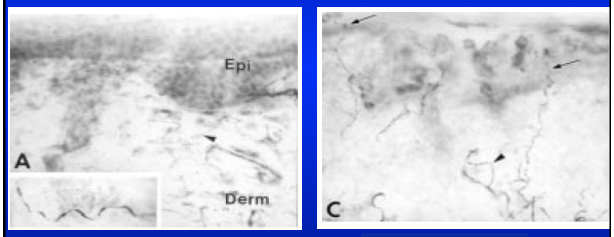
Ebenezer et al., Brain. 2007;130:2703-14

IENFD in the clinic and cross sectional studies

- 'N of 1' experiments
- Sensitive marker of early neuropathy
- Correlates with sensory thresholds
  - Inverse correlation with heat pain, cold detection threshold
  - Correlates with other measures of peripheral neuropathy: NIS-LL, NCV parameters

71 year old man with DM trunkal neuropathy

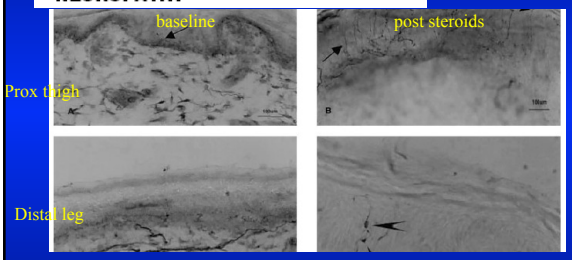
J Neurol Neurosurg Psychiatry. 1998 Nov;65(5):762-6



Symptomatic side

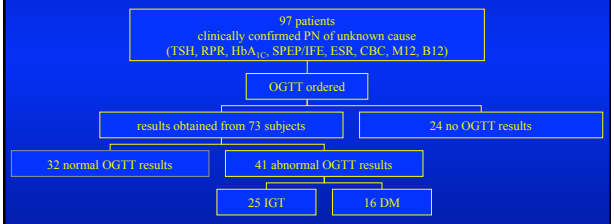
Symptomatic side f/u

EPIDERMAL REINNERVATION CONCOMITANT WITH SYMPTOMATIC IMPROVEMENT IN A SENSORY NEUROPATHY



Muscle Nerve. 2003(4):507-9.

Epidermal innervation in early diabetes and IGT



Polydefkis et al., Neurology 2003

Results: electrophysiology

Measure	IGT (n=25)	DM (n=16)	p
Sural nerve amplitude (μV)	10.3 + 9.5	7.3 + 13.6	0.056
Sural nerve velocity (m/s)	46.2 + 5.9	39.8 + 4.2	0.03
Peroneal nerve amplitude (mV)	3.0 + 3.2	2.2 + 1.8	0.28

Results: skin biopsy

Measure	IGT	DM	p	Norm.
ENF density (fibers/ mm)				
distal leg	3.5 ± 5.9	0.3 ± 0.2	0.03	13.8 ± 6.7
distal thigh	10.5 ± 8.8	11.4 ± 6.0	0.41	
proximal thigh	16.5 ± 5.0	15.4 ± 6.5	0.73	21.1 ± 10.4

ENFD was a more sensitive marker of neuropathy than large fiber measures

Oxaliplatin and neuropathy

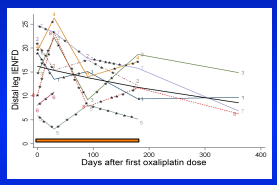
- 3rd gen. platinum derivative that has enhanced inhibition of DNA repair and replication
- a/w acute and chronic symptoms.
  - Acute: cold-induced paresthesias in distal extremities, oral and laryngeal
  - Chronic: distal paresthesias, numbness.
- First-line therapy for colorectal cancer
- Peripheral neuropathy is described as the dose limiting toxicity
- Ca/Mg infusions routinely given as a 'neuroprotective' treatment
- Very few rigorous studies characterized Ox- pn.

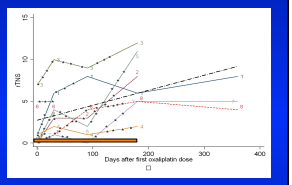
NCI-CTCv3 enhanced with patient-recorded outcomes (PRO)

Nikcevich, ASCO 2008

Grade	I	II	III	IV
NCI-CTC AE v3.0	loss of deep tendon reflexes or paresthesia, including tingling, but not interfering with function	objective sensory alteration or paresthesia, including tingling, interfering with function, but not with activities of daily living	sensory alteration or paresthesia interfering with activities of daily living	permanent sensory losses that are disabling
Questions	**Sample answers for each toxicity grade**			
Do you have problems tying your shoe laces, buttoning your shirts, fastening buckles or pulling up zippers?	"No, I might feel some tingling in my hands, but I have no problems tying laces, buttoning shirts, fastening buckles or pulling up zippers"	"It is a bit harder than before, but I can still tie laces, button shirts, fasten buckles or pull up zippers"	"I have severe difficulties tying shoe laces, buttoning shirts, fastening buckles or pulling up zippers" or "I cannot tie laces, button shirts, fasten buckles or pull up zippers anymore"	"I haven't been able to tie laces, button shirts, fasten buckles or pull up zippers for weeks"
Do you have problems writing?	"No, I might feel some tingling in my hands, but I have no problems writing"	"It is a bit harder than before, but I can still write"	"I have severe difficulties writing" or "I cannot write anymore"	"I haven't been able to write for weeks"
Do you have problems putting on your jewelry or your watch?	"No, I might feel some tingling in my hands, but I have no problems putting on my jewelry or my watch"	"It is a bit harder than before, but I can still put on my jewelry or my watch"	"I have severe difficulties putting on my jewelry or my watch" or "I cannot put on my jewelry or my watch anymore"	"I haven't been able to put on my jewelry or my watch for weeks"
Do your have problems walking?	"No, I might feel some tingling in my feet, but I have no problems walking"	"It is a bit harder than before, but I can still walk"	"I have severe difficulties walking" or "I cannot walk anymore"	"I haven't been able to walk for weeks

Distal leg IENFD rTNS





Burakgazi et al., Neurology. 2011;77(10):980-6.

Results

Measure	coefficient	p	95% CI
Distal leg IENFD (fibers/mm)	-0.00176	<0.001	(-0.00266, -0.000853)
Distal thigh IENFD (fibers/mm)	-0.000333	0.455	(-0.00121, -0.000541)
Peroneal motor CV (m/s)	0.0018	0.7	(-0.000725, 0.0109)
Peroneal motor amplitude (mV)	-0.0032	0.02	(-0.00587, -0.000536)
Sural amplitude (μV)	-0.0277	0.005	(-0.4717, -0.00816)
TNS	0.02015	<0.001	(0.0115, 0.0288)

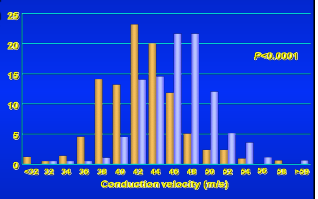
Longitudinal assessment of oxaliplatin-induced neuropathy
A.Z. Burakgazi, W. Messersmith, D. Vaidya, et al.
Neurology 2011;77:980; Published online before print August 24, 2011;

Conclusion

- Oxaliplatin is associated with sensory & motor axon loss though it is relatively mild.
- IENFD yielded objective evidence of axon loss and has potential to simply future studies and reduce subject 'test fatigue'.
- Ox CIPN lends itself to neuroprotection studies
- There is a distinction between axon loss and neuropathic symptoms.
- CTC Oncologic neuropathy scales may preferentially focus on neuropathic symptoms which may or may not be neuropathy

Trial in DPN

- Phase III multicenter clinical trial in DPN
- Based upon promising phase II NCV data
- Powered to detect a difference between placebo and treatment groups of 1.2 m/s (DCCT)
- 1° outcome measure: composite NCV: sensory: sural & ulnar and motor: peroneal & median
- Several secondary measures
  - IENFD – first large multicenter DPN study



NCV DCCT—Cohorts After 5 Years

Average A1C in conventional Tx group was 9.2%

Demographics

	Mean	SD	Range
Age	58.4	8.33	24-71
Gender (%female)	37		
A1C (%)	7.20	0.93	4.7 - 9
Diabetes duration (yr)	12.3	10.5	0.5 - 56
BMI	31.4	4.8	16.9 – 41.4
cNCV (m/s)	44.1	3.6	28.3 – 54.0

Different outcome measures progressing in different directions: why?

- Noise: variation about the mean?
- There was no treatment effect- so unlikely to represent differential behavior of one nerve fiber subtype
- Assessed factors associated with neuropathy at baseline
- Associations of progression of PN

Recent Neuropathy Studies: NCV Observations in Placebo Groups

Study	# Pts	Duration	Placebo Δ from bsl
Rochester DPN Study Dyck 1997	*183 pt (58 had DP)*	*2 year*	
rhNGF Apfel 2000	515 (74% type 2) HbA1c 8.7	1 year	Sural peak latncy 0 ± 0.4 ms Ulnar peak ltncy 0 ± 0.4
Ruboxistaurin Tesfaye 2007	248 (74% Type 2) HbA1c 7.6	1 year	0.38 ± 2.2 peroneal m/s ↓ 0.33 ± 2.4 tibial F ↑ 1.12 ± 3.7 sural amp↓
Alpha Lipoic Acid (NATHAN 1) Ziegler 2007	224 (79% Type 2) HbA1c 8.8	@ 2 year @ 4 year	0.01mV improvemnt peroneal amp 0.15 mV worsening tibial amp
Ruboxistaurin (MBBR) Bastyr 2007	348 (48% Type 1) HbA1c 7.5	2-3 years (stopped for futility)	

Recent Neuropathy Studies: NIS-LL Observations in Placebo Groups

Study	# Pts	Duration	Placebo Δ from bsl
Rochester DPN Study Dyck 1997	*183 pt (58 had DP)*	*2 year*	*81.4% unchanged 9.8% worse*
rhNGF Apfel 2000	515 (74% type 2) HbA1c 8.7	1 year	-0.3 (± 2.7) [Bsl: 8.2 (± 4.0)]
Ruboxistaurin Tesfaye 2007	248 (74% Type 2) HbA1c 7.6	1 year	-0.63 (± 3.4) [Bsl: 6.95 (± 5.0)]
Alpha Lipoic Acid (NATHAN 1) Ziegler 2007	224 (79% Type 2) HbA1c 8.8	@ 2 year @ 4 year	+ 0.0 (± 4.2) + 0.4 (± 4.5)* [Bsl: 8.5 (± 3.2]
Ruboxistaurin (MBBR) Bastyr 2007	348 (48% Type 1) HbA1c 7.5	2-3 years (stopped for futility)	-0.39 [Bsl: 4.73(± 4.1)]

* Estimated from slide

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

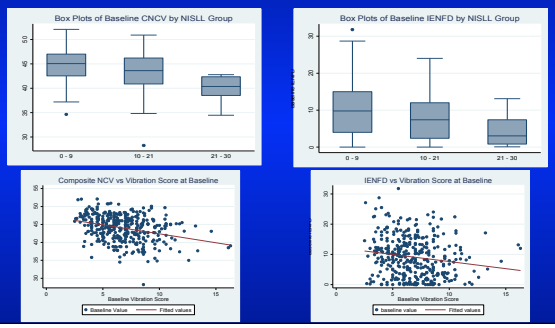
Vascular Risk Factors and Diabetic Neuropathy

Solomon Tesfaye, M.D., Nish Chaturvedi, M.D., Simon E.M. Eaton, D.M., John D. Ward, M.D., Christos Manes, M.D., Constantin Ionescu-Tirgoviste, M.D., Daniel R. Witte, Ph.D., and John H. Fuller, M.A., for the EURODIAB Prospective Complications Study Group*

Drug approval for peripheral neuropathy is very difficult

- Changes in NCV or IENFD are surrogate measures of nerve function, but regulatory agencies are most concerned with functional measures.

Functional measures vs IENFD, CNCV at Baseline

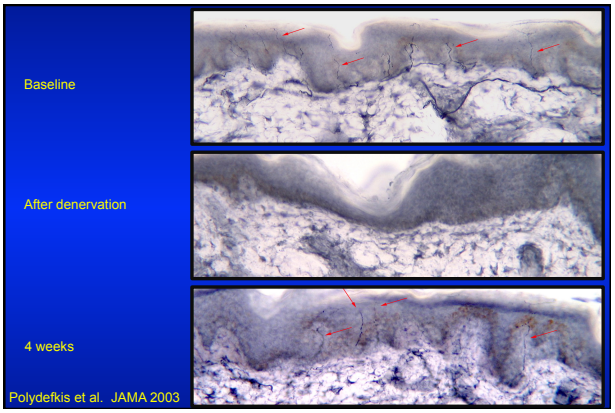


Conclusions

- IENFD outperformed NCV
  - Direct measure of axonal density
- Is peripheral neuropathy a moving target?
  - Powered on data from the 1980s
  - Diabetes was a different disease: AIC higher, BP goals were higher, tobacco, no statins.
- Clinical trials in 2012 should use contemporary natural history data to power studies.

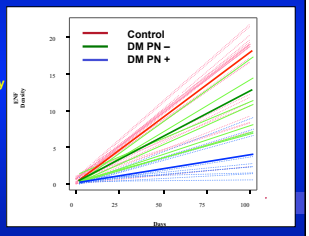
Regeneration Models in Human Skin

- Animal models limited in predicting human clinical trial results
- Regenerative sprouting, collateral sprouting, dermal structures: vessels, Schwann cells, regenerative growth from a transected stump



Capsaicin Model: Nerve Regeneration in DM

- After 100 days of regeneration neither group regenerated to baseline levels.
- Baseline ENFD and the presence of DM were predictive of the regeneration rate.
- In DM subjects the presence of PN was associated with a reduced regeneration rate while the baseline HbA_{1c} was not.



Polydefkis et al. Brain 2004

Capsaicin Model in Clinical Trials

- 6 multicenter clinical trials
- Robust, consistent data across different studies
- Fast, economical
- Unbiased measure of regeneration
- Basis 'go, no-go decisions'
- Allowing us to define factors that influence regeneration in humans

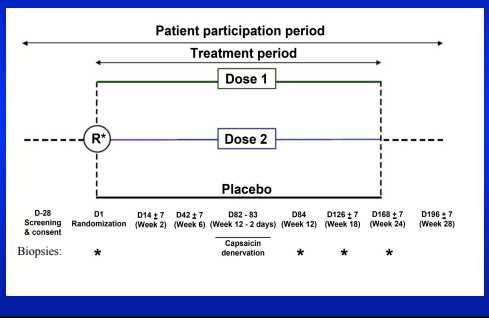
Neurology. 2006;66(2):259-61.

Trial using return of IENFD as primary outcome measure

- Design
  - Multi-center, 24 week double-blinded, randomized placebo controlled, parallel group trial
- Study population
  - Type 1 or Type 2 DM
  - Age: 18-65
  - HbA1c ≤ 9, on stable DM regimen
  - No hospitalizations for DM complications in last 3 months
  - Measurable sural nerve response
  - No LE amputation
  - No severe comorbid condition including CHF, unstable angina, ACS, CABG or PCI in the last 3 months
  - No other causes of neuropathy
- Dose
  - Placebo, 25, 100mg dose daily

Table 1. Patient demographics

Characteristic	Screened (n=726)	Enrolled (n=308)
Median age (range)	56.3 (19.8-69.7)	57.1 (31.8-65.9)
Female (%)	41.3	39.6
African American (%)	16.5	10.4
Median duration of diabetes in yrs (range)	4.2 (0-42.4)	4.5 (0.1-39.0)
Mean glycated haemoglobin (SD)	8.0 (1.9)	7.3 (1.0)
Number of points on hypertension severity scale (%)		
0	23.4	19.7
1	31.0	30.8
2	29.1	31.5
3	13.8	14.8
4	2.8	3.3
Median duration of dyslipidemia in yrs (range)	0.84 (0-30.6)	1.2 (0-19.9)
Mean height in cm (SD)	171.5 (10.4)	172.2 (10.3)



Linear regression analysis of factors associated baseline epidermal nerve fiber density

Characteristic	Unadjusted regression coefficient (95% Confidence Interval (CI); p-value)	Adjusted coefficient (95% CI; p-value)*
Duration of diabetes (in yrs)	-0.169,(-0.341, 0.004); 0.056	-0.178, (-0.347, -0.008); 0.040
Number of hypertension points (scale 0-4)	-1.06, (-1.98, -0.143); 0.024	-1.56, (-2.50, -0.621); 0.001
Female gender	3.59, (1.70, 5.47); <0.001	3.56, (1.56, 5.55); 0.001

*Multivariate model adjusted for the above characteristics as well as age as a continuous variable.

Linear regression analysis of nerve fiber regeneration using factors associated baseline epidermal nerve fiber density *

Characteristic	Unadjusted regression coefficient (95% CI; p-value)	Adjusted regression coefficient (95% CI; p-value)**
Treatment group		
Placebo	(Ref)	(Ref)
25mg dose	-0.956, (-1.87, -0.036), 0.042	-0.909, (-1.78, -0.038); 0.041
100mg dose	-0.931, (-1.87, 0.011); 0.053	-0.743, (-1.67, 0.188); 0.117
Duration of diabetes (in yrs)	-0.091, (-0.147 ,-0.035); 0.002	-0.097, (-0.151, -0.044); <0.001
Baseline number of hypertension points (scale 0-4)	-0.142, (-0.509, 0.224); 0.445	-0.307, (-0.656, 0.042); 0.084
Female gender	1.20, (0.429, 1.98); 0.002	1.20, (0.428, 1.97); 0.002

*All models derived using generalized estimating equations which accounted for clustering by subject. **Multivariate model adjusted for the above characteristics as well as age, and time since denervation as continuous variables.

Linear regression analysis of nerve fiber regeneration.*

Characteristic	Unadjusted regression coefficient (95% CI; p-value)	Adjusted regression coefficient (95% CI; p-value) **
Baseline ENF density	0.253, (0.217, 0.288); <0.001	0.214, (0.173, 0.254); <0.001
Duration of diabetes (in yrs)	-0.091, (-0.147 ,-0.035); 0.002	-0.072, (-0.123, -0.021); 0.006
Duration of dyslipidemia (in yrs)	-0.0747, (-0.191, 0.042); 0.207	-0.132, (-0.218, -0.0451); 0.003
Height (in cm)	-0.0649, (-0.100,-0.029), <0.001	-0.034, (-0.068, -0.001); 0.047

*All models derived using generalized estimating equations which accounted for clustering by subject.

**Multivariate model adjusted for the above characteristics as well as time since denervation as a continuous variable and treatment group as a categorical variable.

- Ability to measure human axonal regeneration in a standardized, uniform fashion
- Amenable to multicenter clinical trials
- Fast
- The rate of IENFD return is associated with factors that make biologic sense

- Are we using the best outcome measures in peripheral nerve clinical trials?

Development of neuropathy

diabetes

↓

Reduction in regeneration

↓

Swellings (abnormal morphology)

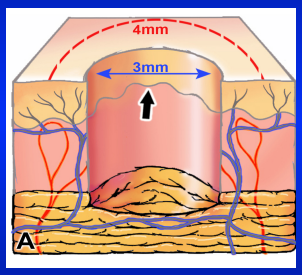
↓

Sensory axon loss (small fiber)

↓

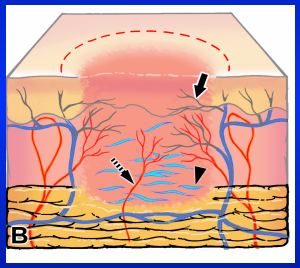
Sensory axon loss (large fiber)

Skin biopsy: not just for nerves



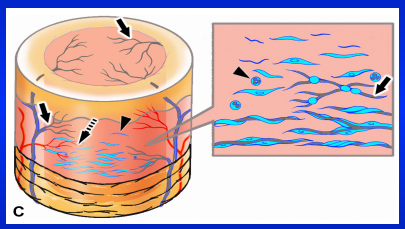
BRAIN
A JOURNAL OF NEUROLOGY

Skin biopsy: not just for nerves



BRAIN
A JOURNAL OF NEUROLOGY

Skin biopsy: not just for nerves



DM related reduction in
collateral sprouting
regenerative sprouting
Schwann cell growth
Vessel growth

Hierarchy of regrowth that recapitulates what is seen during development with vessels first followed by Schwann cells and then axons.

BRAIN
A JOURNAL OF NEUROLOGY


Thank you