Sensitivity and specificity of skin biopsy

Giuseppe Lauria

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Milan, Italy

1997

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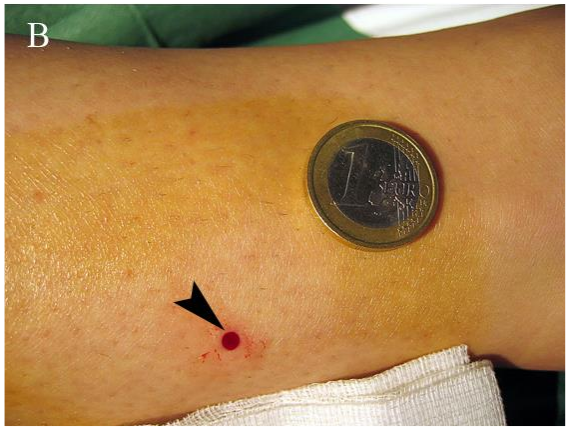
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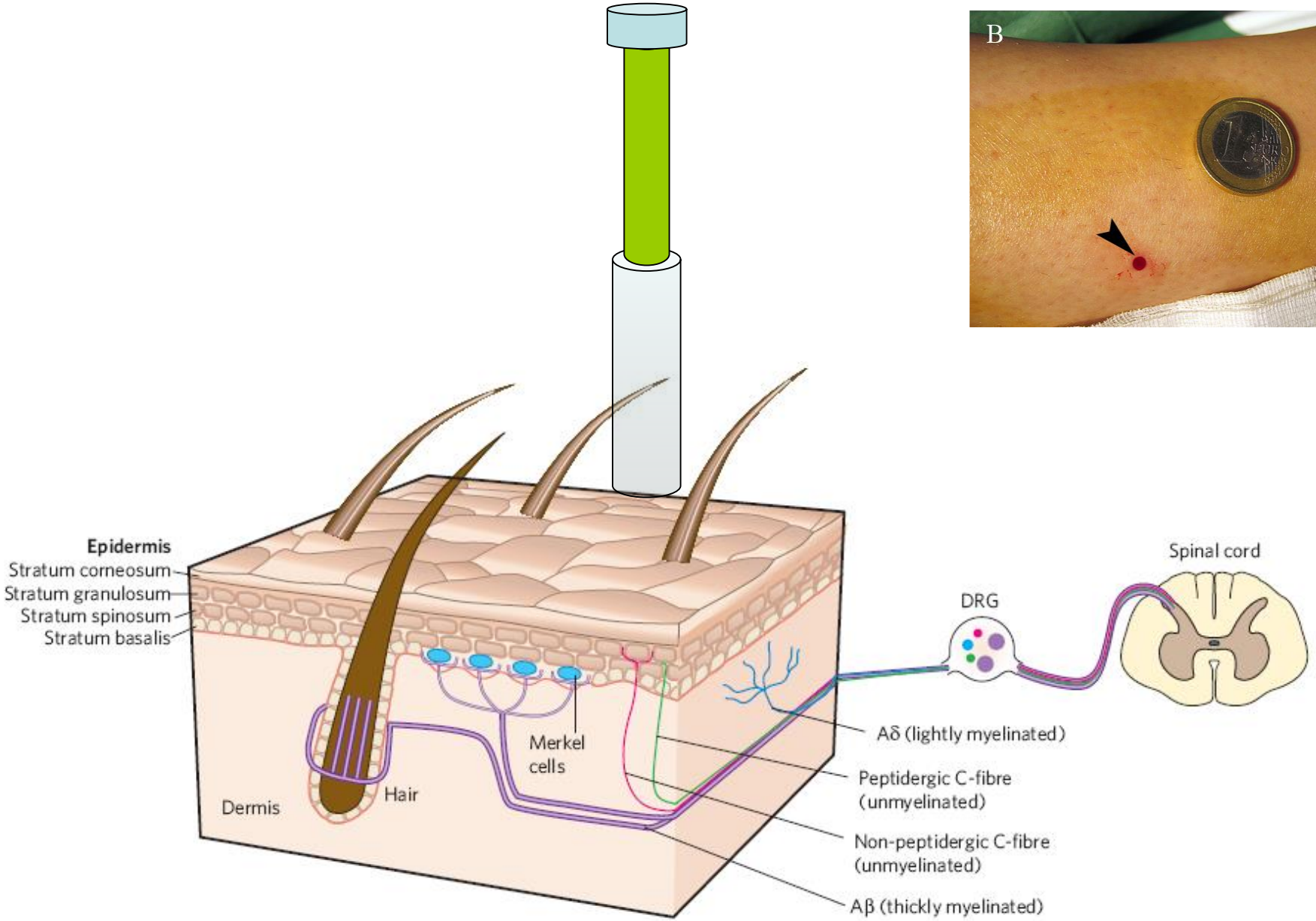
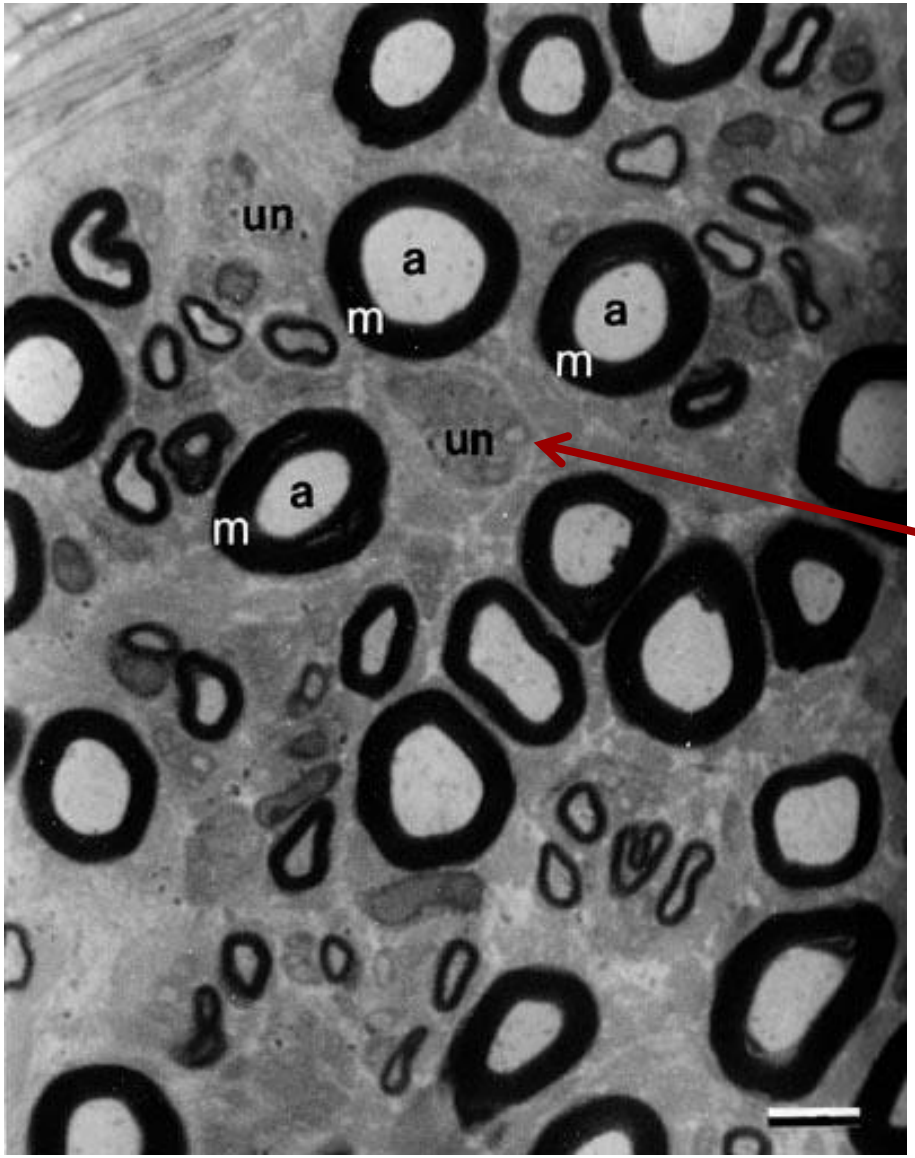
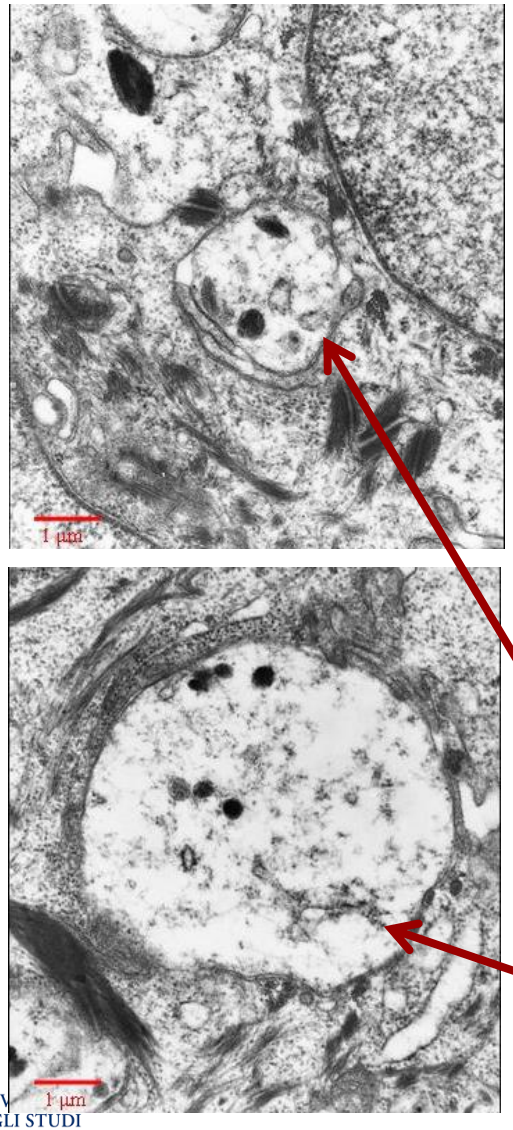

Epidermis
Stratum corneosum
Stratum granulosum
Stratum spinosum
Stratum basalis
Dermis
Hair
Merkel cells
DRG
Spinal cord
Aδ (lightly myelinated)
Peptidergic C-fibre (unmyelinated)
Non-peptidergic C-fibre (unmyelinated)
Aβ (thickly myelinated)

Sural nerve biopsy



Remak bundle

Skin biopsy

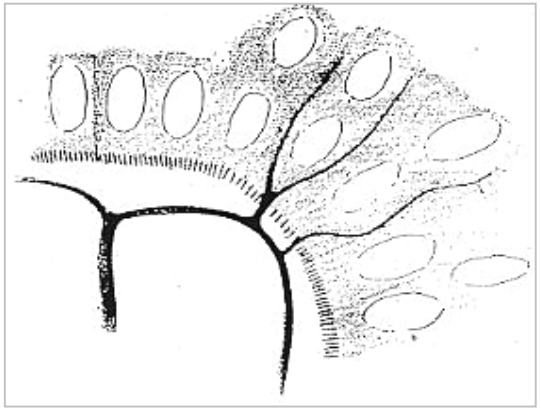


Unmyelinated axon

Remak bundle

Historical notes

1868: Langerhans' first description of human IENF



1959: Arthur & Shelley's - first quantification of human IENF showing that proximal sites of the body (neck and back) have higher innervation density than lower sites (fingers and ankle)

Historical notes

1989: availability of anti-PGP 9.5 antibodies

1990: Karolinska's lab first description of IENF staining

1993: Kennedy's lab – IENFD by IF confocal microscope

1998: JHU's lab – IENFD normative values by BF

2005: EFNS guidelines

2009: AAN recommendations

2010: PNS/EFNS guidelines

2010: inter-lab BF normative reference values for clinical use

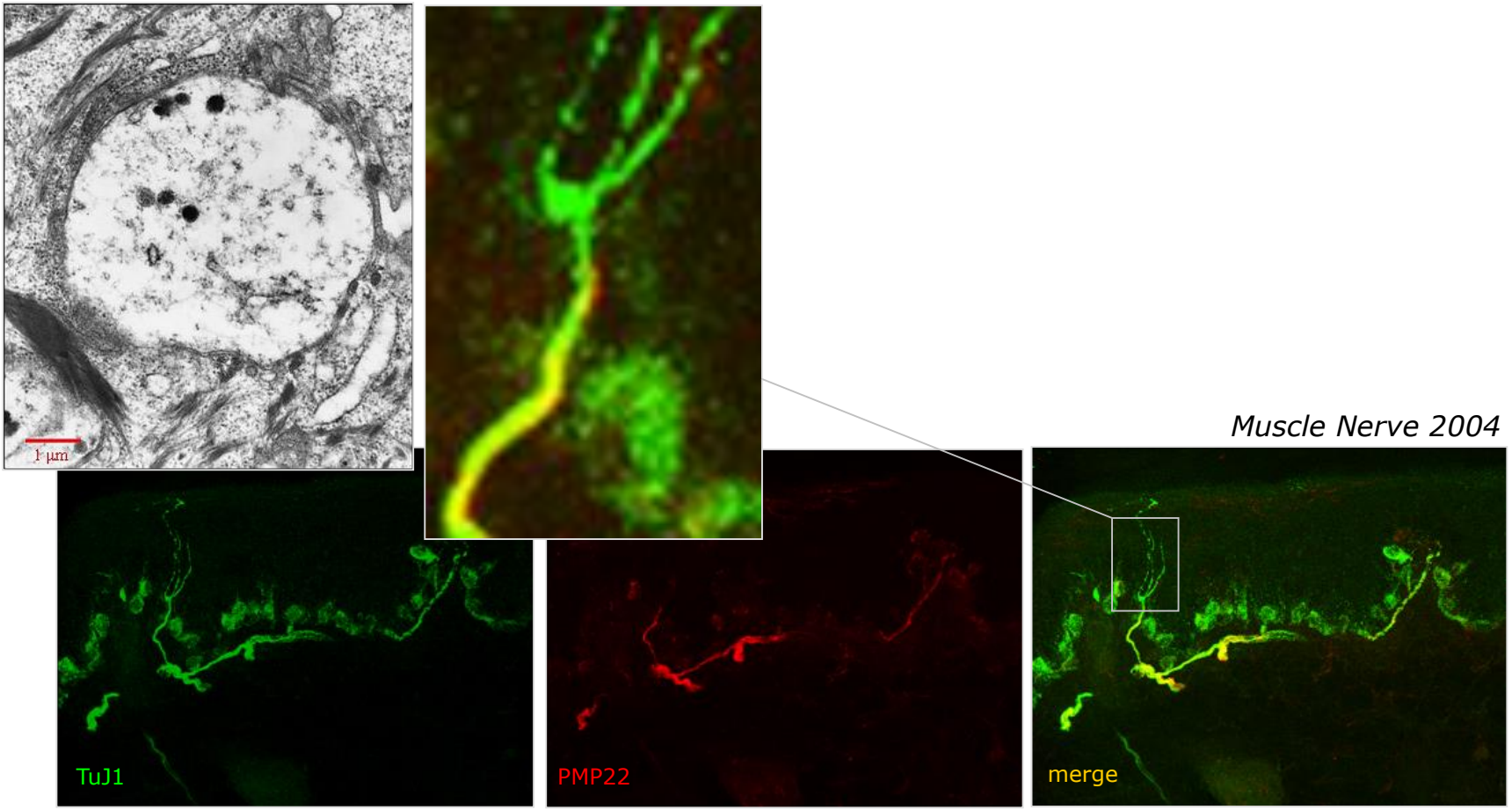
2015: BF-IF comparative study on normative reference values

2015: side and time variability of IENFD

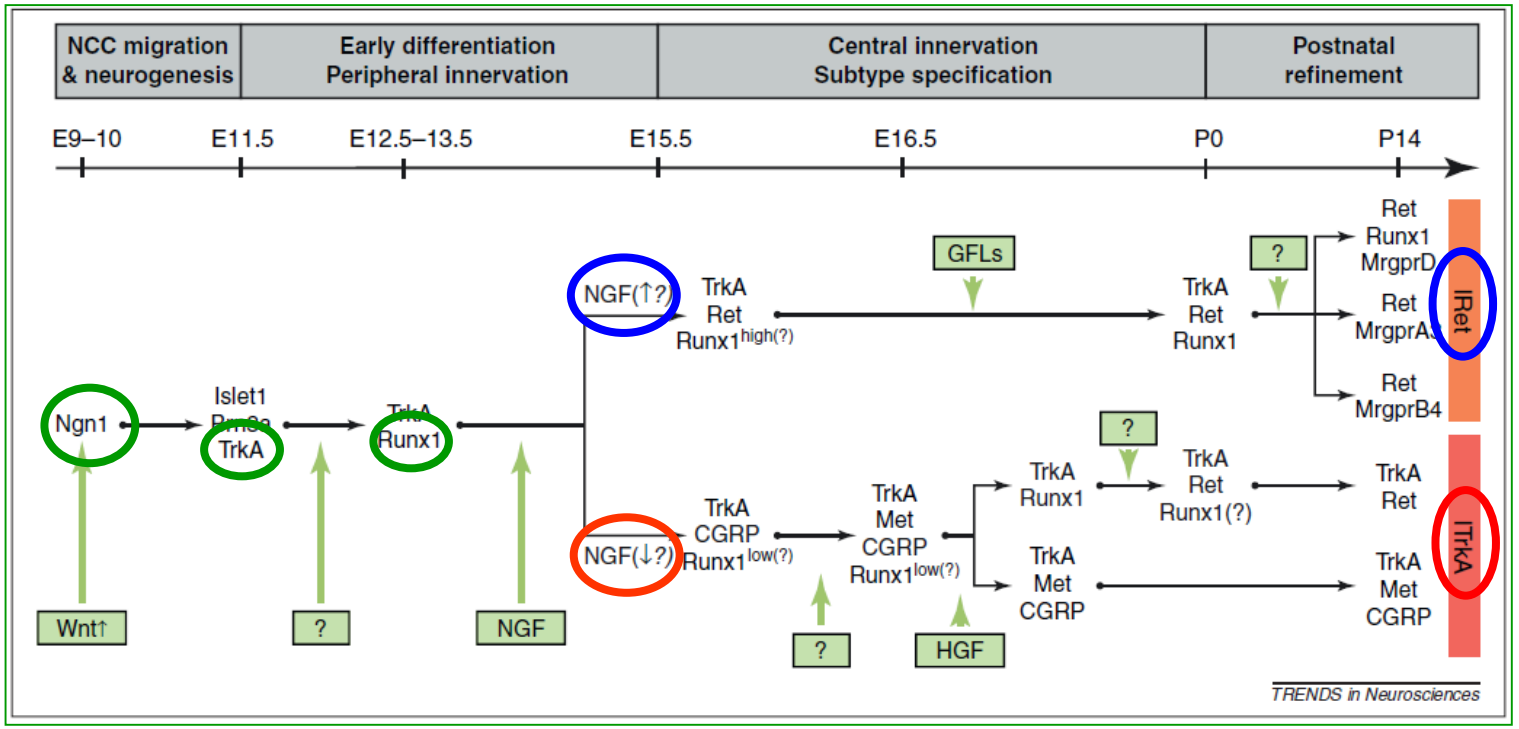
2016: inter-lab IF normative reference values for clinical use

Epidermal nerve fibers

Naked axons *(no Schwann cell ensheathment)*



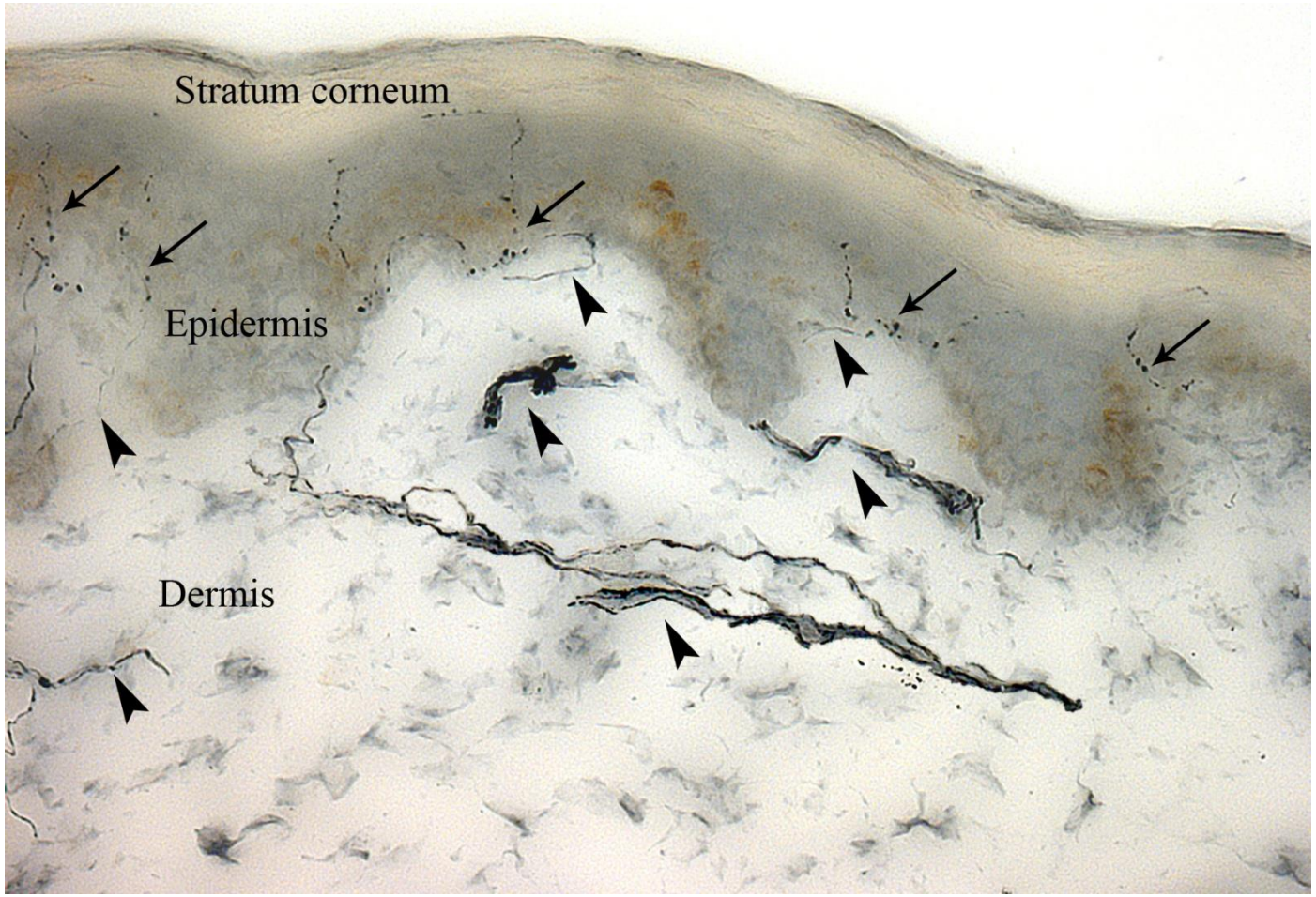
Small-size DRG neuron differentiation promoting cutaneous nerve fibre segregation



- Neurogenesis → Neurogenin 1
- TrkA and Runx1 expression promote the differentiation

- Upper epidermal layer → high NGF → high Runx1 and Ret (**non-peptidergic - IRet**)
- Lower epidermal layer → low NGF → low Runx1 and high CGRP (**peptidergic -ITrkA**)

Innervation of human hairy skin



bright-field immunohistochemistry with PGP9.5

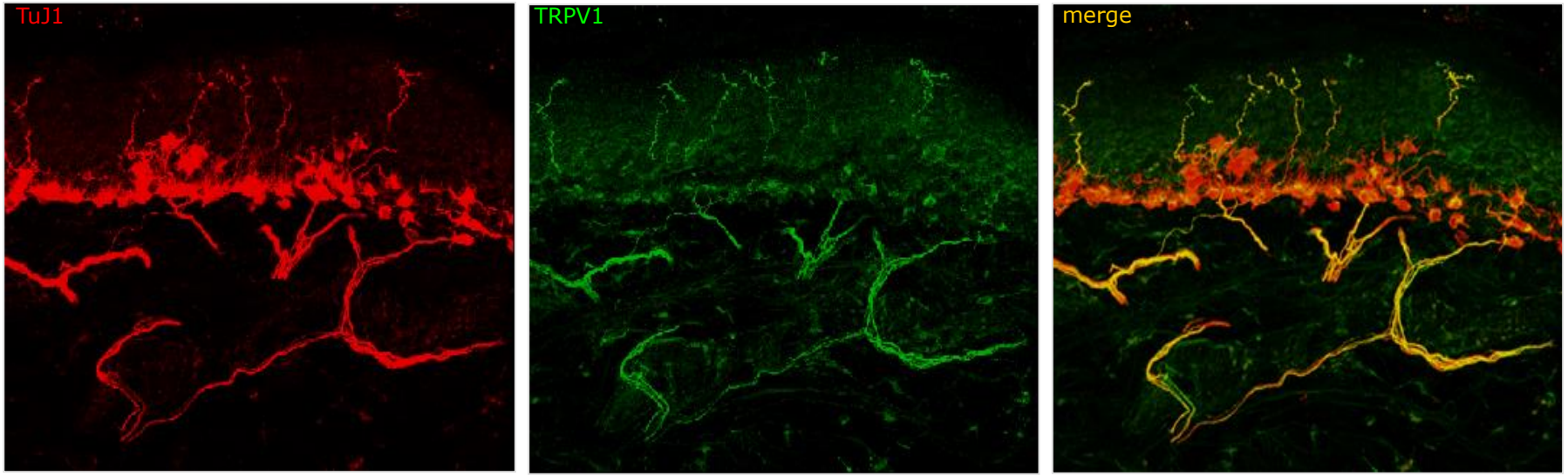
Protein gene product 9.5

- PGP9.5 is a soluble cytoplasmic protein of 25,000 kD MW
  - Neuronal form of ubiquitin carboxyl-terminal hydrolase
  - Cytosolic enzyme that removes ubiquitin and is transported within the slow component of the axonal transport

- Being abundant in nerves, it is used as a marker for peripheral nerve fibers.

Epidermal nerve fibers

Widely expression of TRPV1 receptor



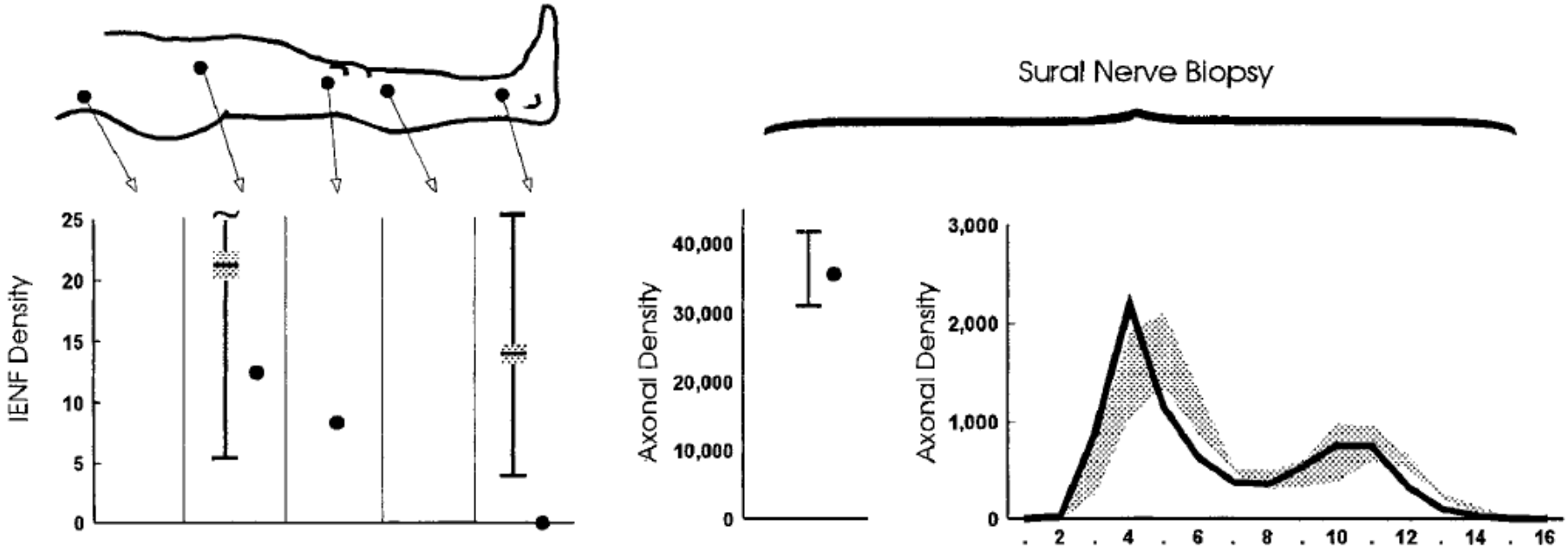
J Periph Nerv Syst 2006

Point #1

- Skin nerves are stained using a cytoplasmatic marker not specific for fiber functions

- IENF are the terminal nociceptors

SMALL FIBER NEUROPATHY



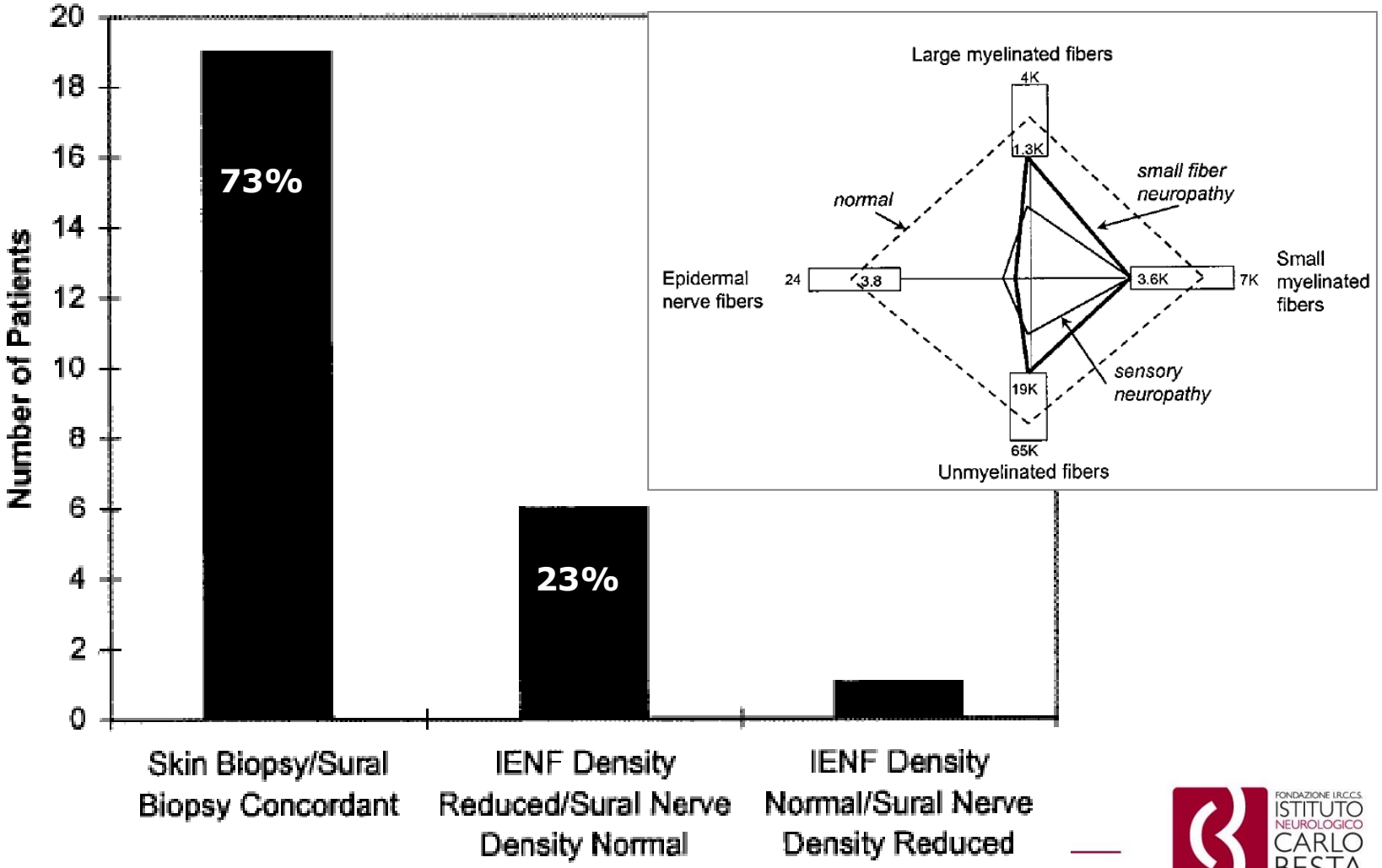
Neil R. Holland, MB BS,* Thomas O. Crawford, MD,* Peter Hauer, BS,* David R. Cornblath, MD,*
John W. Griffin, MD,*† and Justin C. McArthur, MB BS, MPH*‡

Ann Neurol 1998;44:47–59

Skin biopsy *vs* Sural Nerve biopsy

D.N. Herrmann, MBBCh; J.W. Griffin, MD; P. Hauer, BS; D.R. Cornblath, MD;
and J.C. McArthur, MBBS, MPH

NEUROLOGY 1999;53:1634–1640



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FONDAZIONE I.R.C.C.S. ISTITUTO NEUROLOGICO CARLO BESTA

Epidermal nerve fiber density:

Normative reference range and diagnostic efficiency

Arch Neuro 1998;55:1513

Table 2. Intraepidermal Nerve Fiber Density by Age Decile

Skin Biopsy Site	No. of Fibers per Millimeter by Age, y						
	10-19 (n = 8)	20-29 (n = 18)	30-39 (n = 20)	40-49 (n = 19)	50-59 (n = 12)	60-69 (n = 9)	70-79 (n = 12)
Distal part of leg*							
Mean ± SD	20.3 ± 7.4	11.3 ± 5.3	11.9 ± 7.1	14.6 ± 5.6	14.4 ± 5.7	13.3 ± 6.5	14.7 ± 8.2
Range	12.7-36.5	3.8-23.0	4.2-31.2	2.7-25.2	6.7-24.6	0.6-20.2	1.9-32.2
Median	19.1	9.7	10.6	14.8	13.4	15.9	12.4
80th percentile interval	13.5-32.5	6.1-20.6	4.3-21.8	8.8-22.6	8.4-23.6	3.4-20.0	5.7-26.8
Thigh†							
Mean ± SD	31.6 ± 13.2	15.8 ± 5.9	17.8 ± 9.4	22.3 ± 7.4	21.4 ± 14.1	24.0 ± 8.4	23.4 ± 8.3
Range	14.6-50.5	2.9-27.1	4.2-40.6	3.0-32.7	9.7-57.5	16.3-38.1	11.2-39.5
Median	27.3	15.7	17.4	23.6	16.5	20.3	22.9
80th percentile interval	16.6-49.2	8.3-24.0	5.6-29.6	14.5-31.5	10.2-46.4	16.4-37.9	11.9-36.4
Thigh/distal part of leg							
Mean ± SD	1.6 ± 0.6	1.6 ± 0.7	1.8 ± 1.1	1.6 ± 0.7	1.6 ± 0.8	5.1 ± 10.0	2.3 ± 2.2
Range	0.9-2.5	0.3-2.7	0.2-4.5	0.9-3.1	0.7-2.9	0.9-31.8	0.9-9.1
Median	1.4	1.5	1.7	1.4	1.4	1.9	1.6
80th percentile interval	1.0-2.4	0.8-2.4	0.9-3.7	1.0-2.6	0.7-2.9	1.0-20.3	0.9-4.9
P (Mann-Whitney test), distal part of leg vs thigh	.06	.02	.03	<.001	.13	.01	.02

Epidermal nerve fiber density:
Normative reference range and diagnostic efficiency

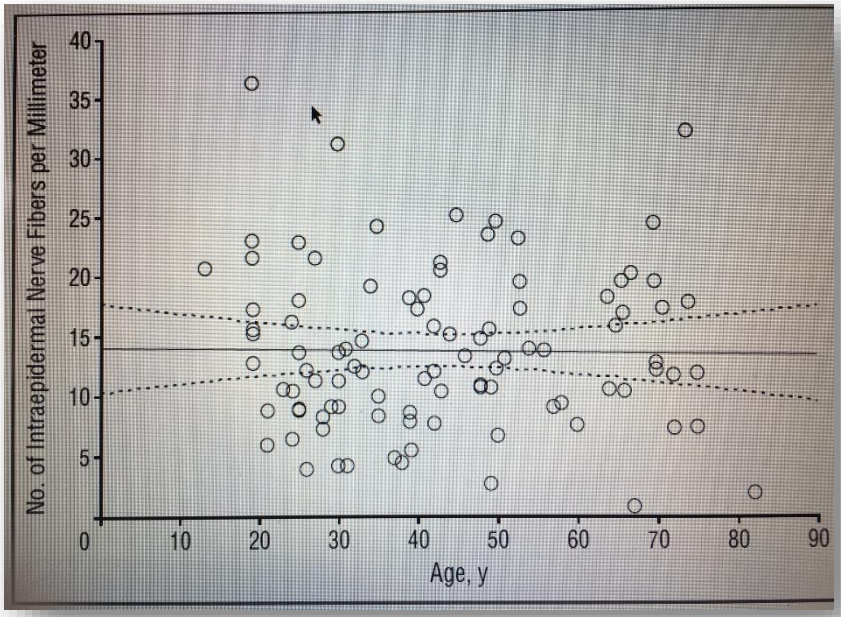
Arch Neuro 1998;55:1513

Table 3. Performance Characteristics of Intraepidermal Nerve Fiber Linear Density Measure*

	Sensitivity, %		Specificity, %		Efficiency, %		Positive Predictive Value, %		Negative Predictive Value, %	
Percentile	Thigh	Distal Part of Leg	Thigh	Distal Part of Leg	Thigh	Distal Part of Leg	Thigh	Distal Part of Leg	Thigh	Distal Part of Leg
10th	45	60	88	90	82	86	45	55	89	75
5th	25	45	96	97	84	88	56	92	86	90

Epidermal nerve fiber density:
Normative reference range and diagnostic efficiency

Arch Neuro 1998;55:1513



13.8 ± 6.7/mm (mean ± SD; lower 5th percentile 3.8)

Point #2

- No age-related changes → for >10 years all labs made reports based on a mean value applied to both sexes at any age

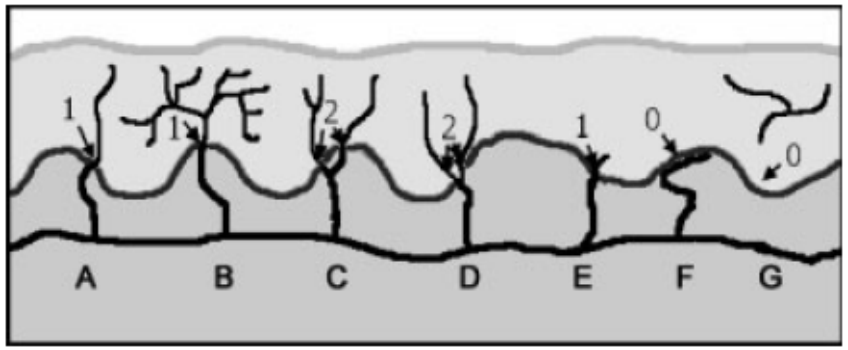
- More false positive or false negative ?

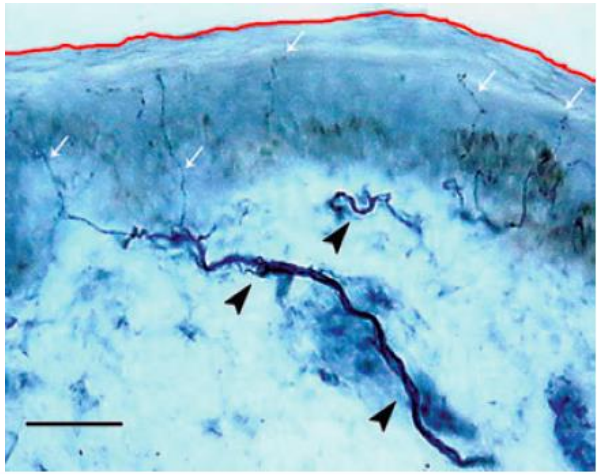
European Journal of Neurology 2005, **12:** 747–758

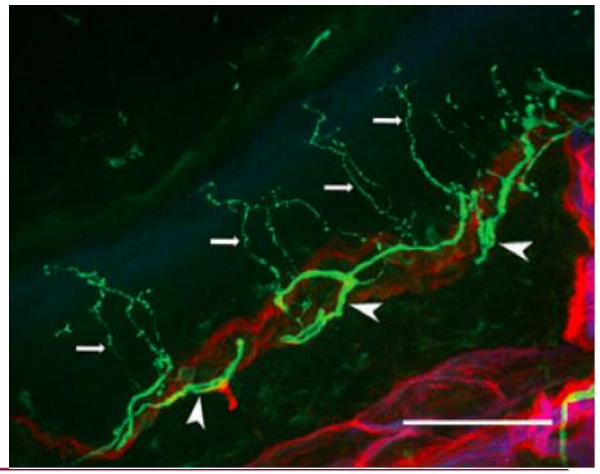
EFNS TASK FORCE/CME ARTICLE

EFNS guidelines on the use of skin biopsy in the diagnosis of peripheral neuropathy

G. Lauria[a], D. R. Cornblath[b], O. Johansson[c], J. C. McArthur[b], S. I. Mellgren[d], M. Nolano[e], N. Rosenberg[f] and C. Sommer[g]

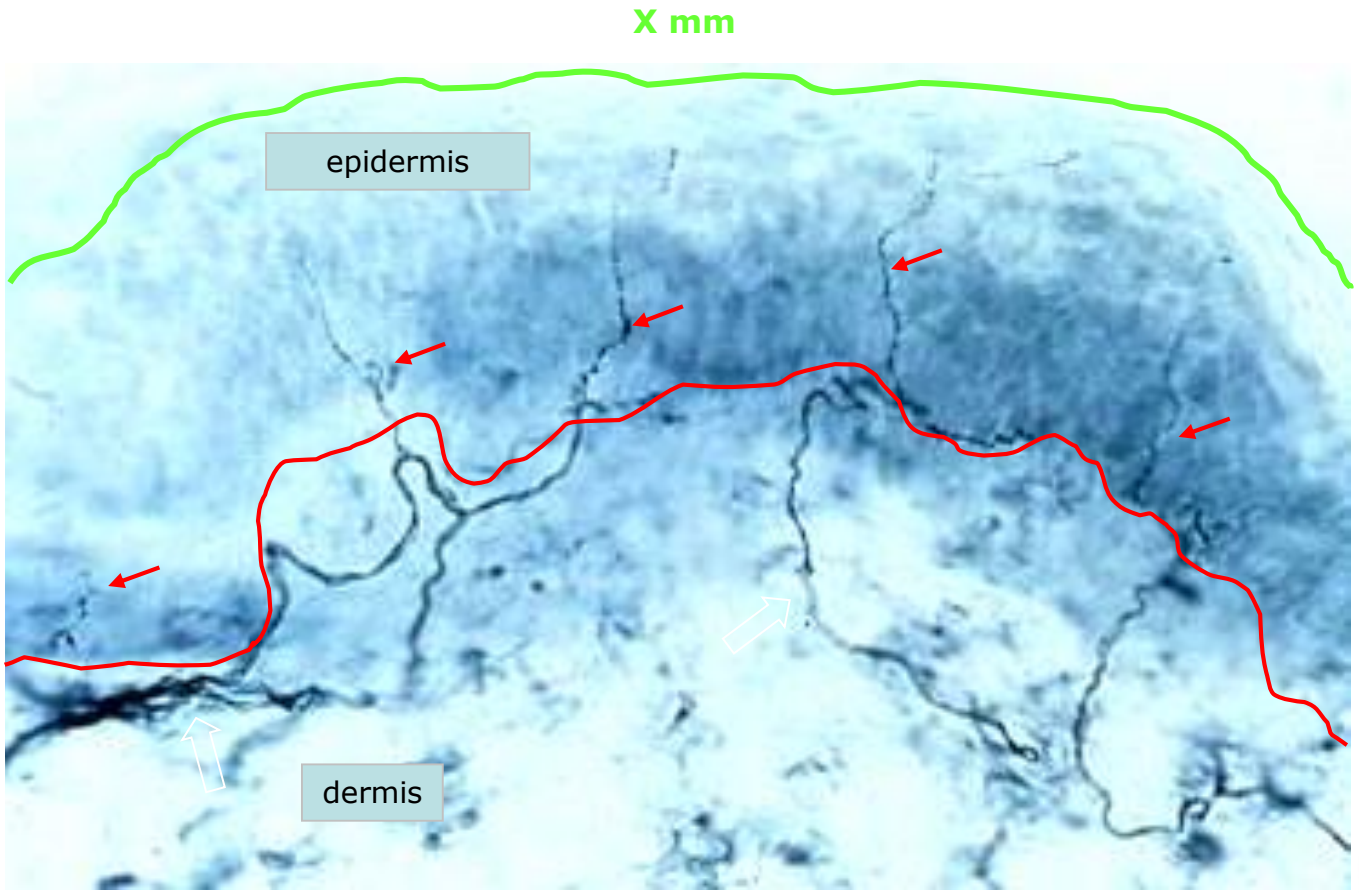






Quantification of epidermal nerve fibres

1. Count IENF (not branching) in at least 3 sections
2. Measure surface length
3. Calculate linear density (IENF/mm)



Practice Parameter: Evaluation of distal symmetric polyneuropathy: Role of autonomic testing, nerve biopsy, and skin biopsy (an evidence-based review)

Report of the American Academy of Neurology, American Association of Neuromuscular and Electrodiagnostic Medicine, and American Academy of Physical Medicine and Rehabilitation

Neurology® 2009;

J.D. England, MD
G.S. Gronseth, MD, FAAN
G. Franklin, MD
G.T. Carter, MD
L.J. Kinsella, MD
J.A. Cohen, MD
A.K. Asbury, MD
K. Szigeti, MD, PhD
J.R. Lupski, MD, PhD
N. Latov, MD
R.A. Lewis, MD
P.A. Low, MD
M.A. Fisher, MD
D.N. Herrmann, MD
J.F. Howard, Jr, MD
G. Lauria, MD
R.G. Miller, MD
M. Polydefkis, MD, MHS
A.J. Sumner, MD

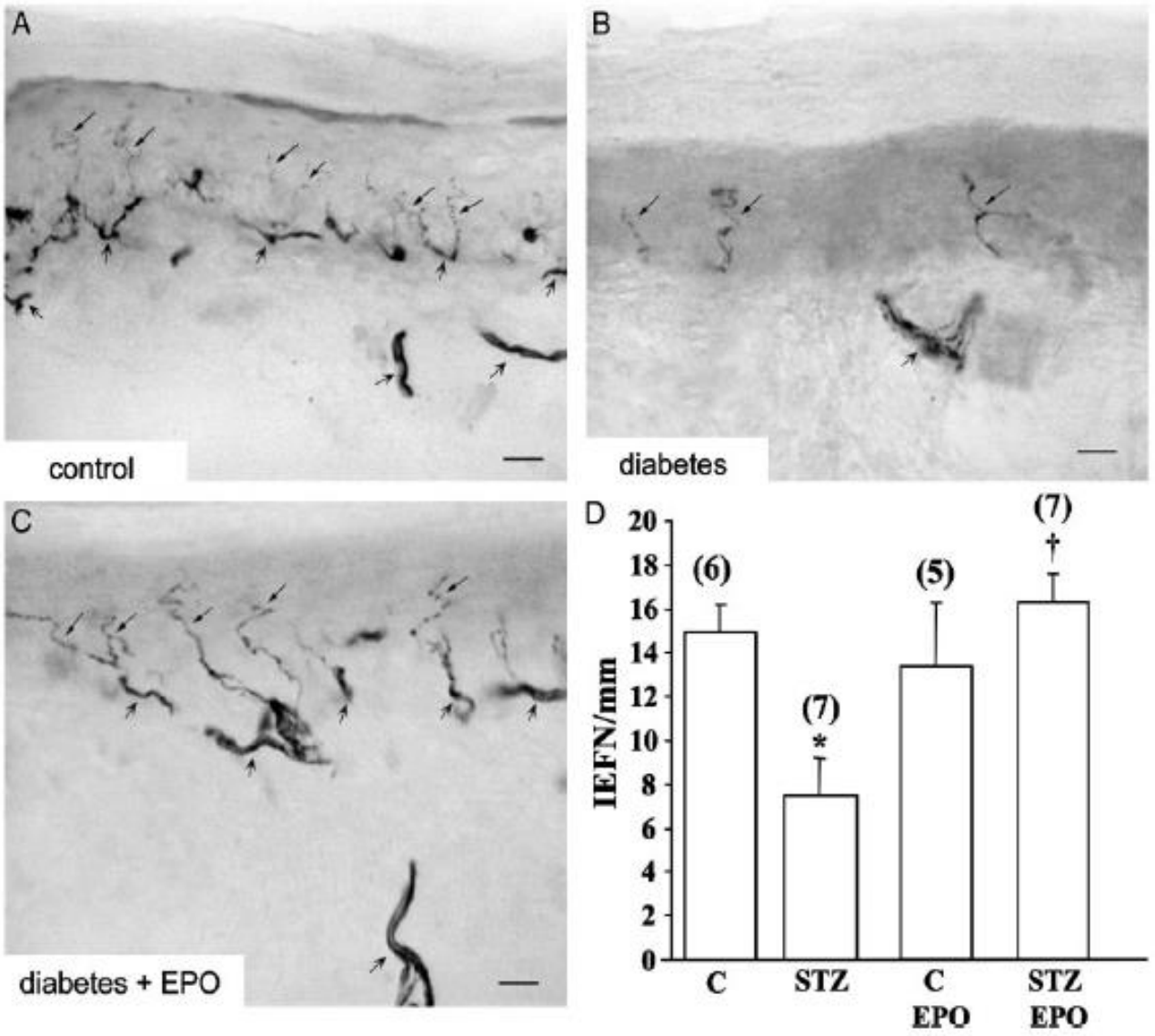
EFNS/PNS SKIN BIOPSY GUIDELINE

European Federation of Neurological Societies/Peripheral Nerve Society Guideline on the use of skin biopsy in the diagnosis of small fiber neuropathy. Report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society

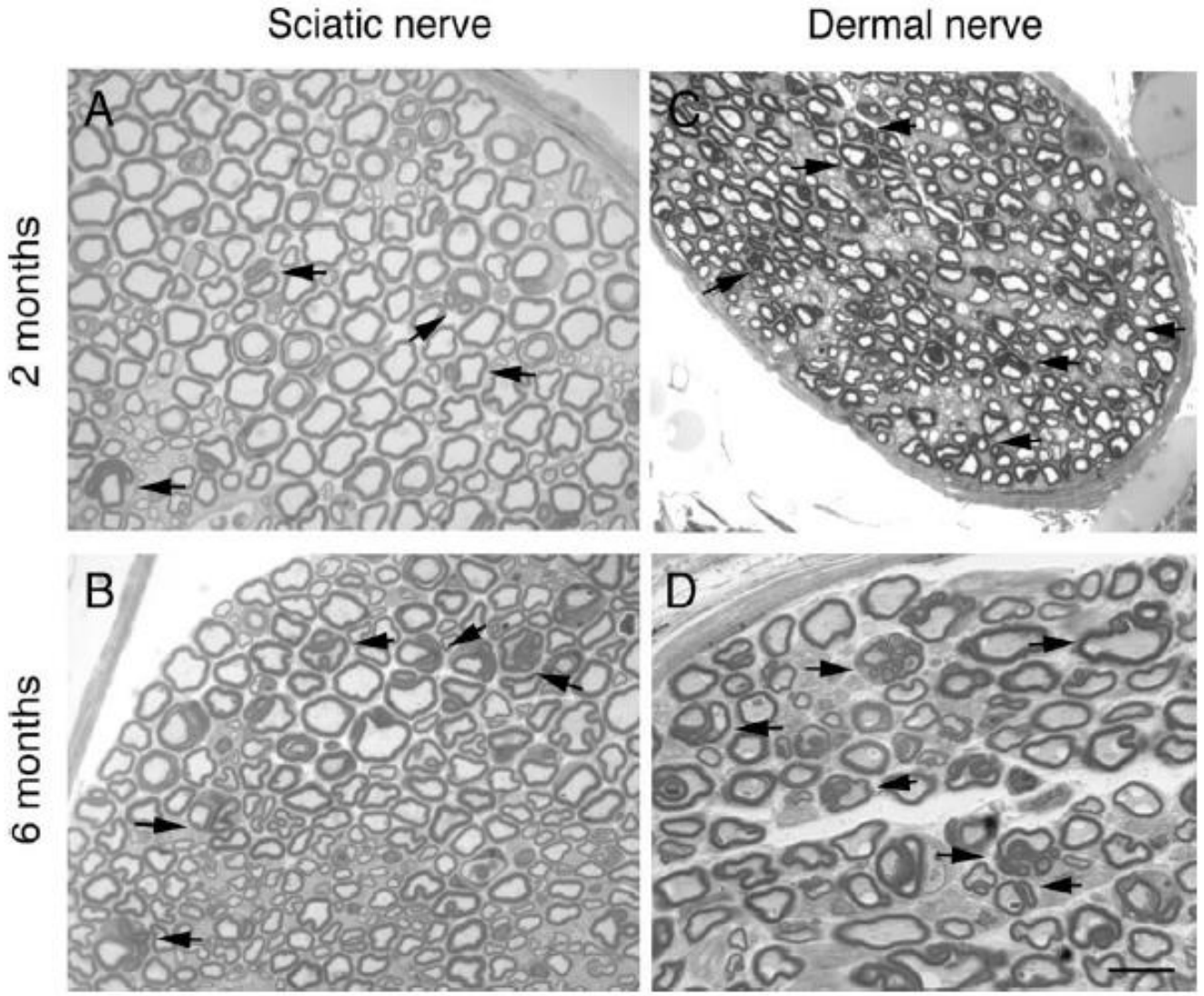
Joint Task Force of the EFNS and the PNS†

Journal of the Peripheral Nervous System 15:79–92 (2010)

Skin biopsy in animal models



Skin biopsy in animal models



Point #3

- 2005 and 2010 guidelines: standardization of procedure and counting rules for both BF and IF technique
- Distal leg biopsy for clinical purposes

- Preclinical models of peripheral neuropathies

- Inter-lab stardardization on procedure and counting is a relevant issue

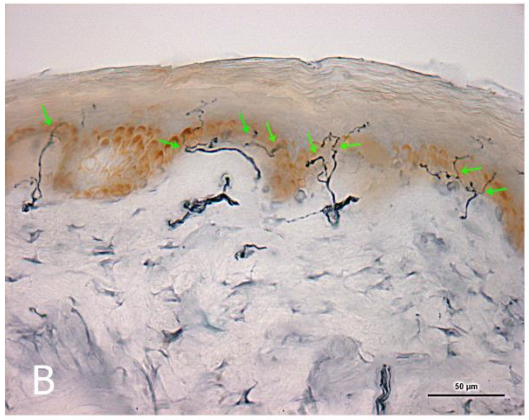
Intraepidermal nerve fiber density at the distal leg: a worldwide normative reference study

Journal of the Peripheral Nervous System 15:202–207 (2010)

	Females (n=285)			Males (n=265)		
Age (years)	Number of Subjects	0.05 quantile IENFD values per age span	median IENFD values per age span	Number of Subjects	0.05 quantile IENFD values per age span	Median IENFD values per age span
20 – 29	57	8.4	13.5	36	6.1	10.9
30 – 39	47	7.1	12.4	40	5.2	10.3
40 – 49	70	5.7	11.2	62	4.4	9.6
50 – 59	59	4.3	9.8	53	3.5	8.9
60 – 69	32	3.2	8.7	43	2.8	8.3
70 – 79	16	2.2	7.6	22	2.1	7.7
≥ 80	4	1.6	6.7	9	1.7	7.2

- IRCCS “Carlo Besta” Neurological Institute, Milan (Italy)
- University of Ferrara (Italy)
- University of Utah (USA)
- University of Tromsø (Norway)
- German Diabetes Center, University of Düsseldorf (Germany)
- University Medical Centre of Maastricht (The Netherlands)
- National Taiwan University Hospital, Taipei (Taiwan)
- National Neuroscience Institute of Singapore (Singapore)
- Department of Neurology, Spaarne Hospital, Hoofddorp (The Netherlands)

BFI



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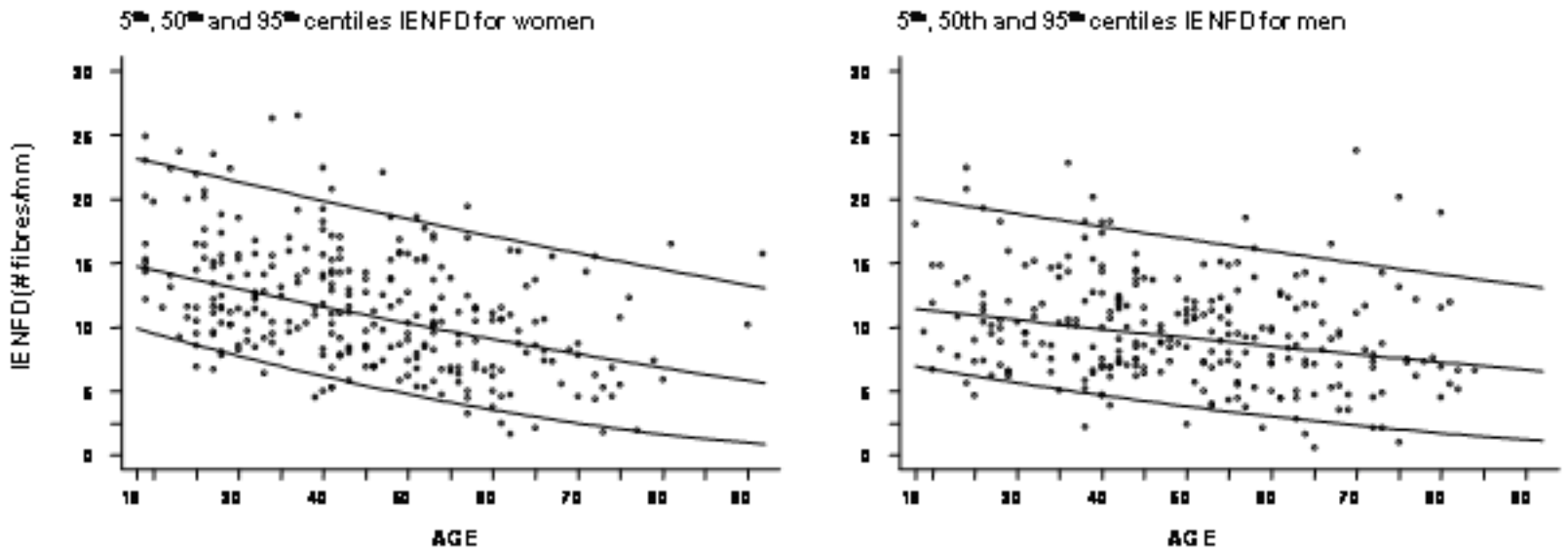
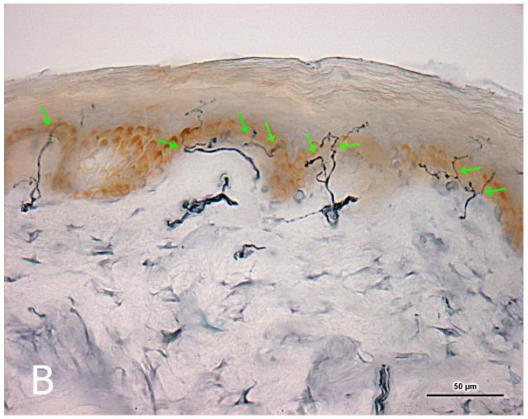
Journal of the Peripheral Nervous System 15:202–207 (2010)



- **IENFD decrease** 0.9 IENF/mm per decade
- **Height** does not influence IENFD
- **Weight and BMI** have mild influence on IENF density in men
  - 12% variation (R^2=0.12; p<0.001).

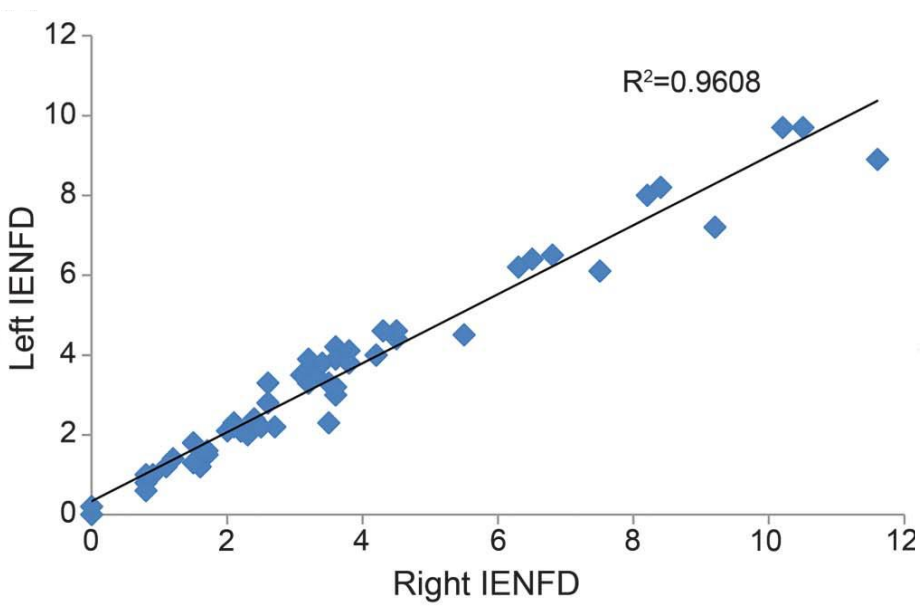
BFI

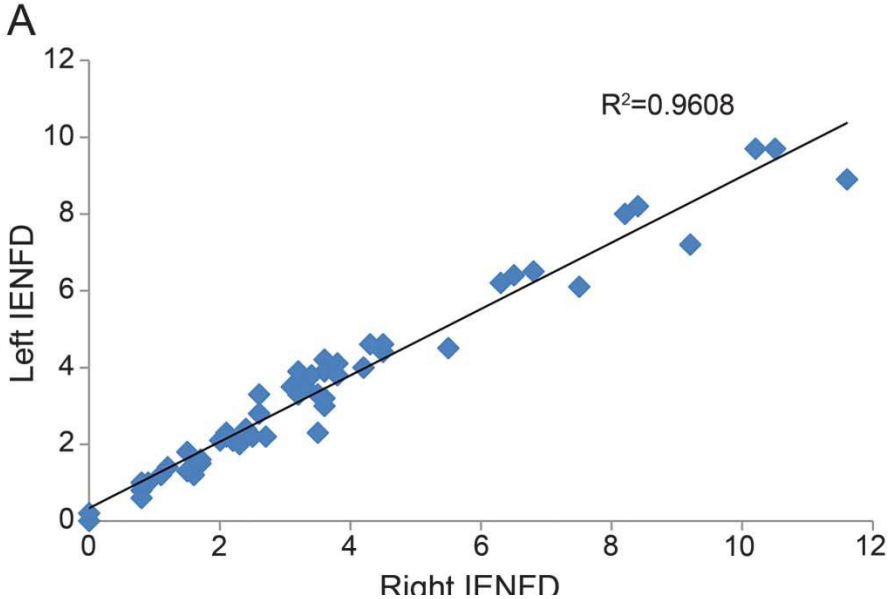


Side and time variability of intraepidermal nerve fiber density

Neurology® 2015;84:1–4

40 SFN patients and 17 healthy subjects → bilateral biopsies
15 SFN patients and 8 healthy subjects → 20-day follow-up biopsies





Unilateral skin biopsy is reliable

and

IENFD is not expected to vary within 3 weeks

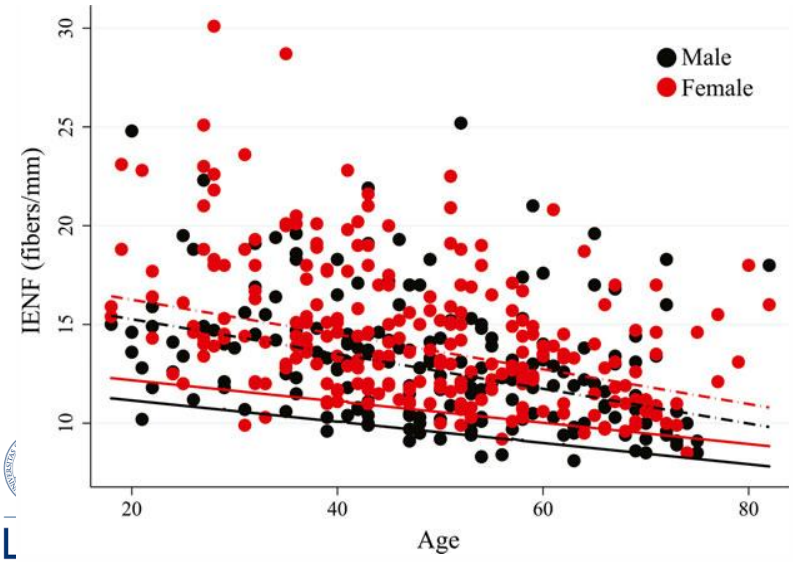
A multi-center, multinational age- and gender-adjusted normative dataset for immunofluorescent intraepidermal nerve fiber density at the distal leg

V. Provitera[a], C. H. Gibbons[b], G. Wendelschafer-Crabb[c], V. Donadio[d], D. F. Vitale[a], A. Stancanelli[a], G. Caporaso[a], R. Liguori[d], N. Wang[b], L. Santoro[e], W. R. Kennedy[c] and M. Nolano[a]

European Journal of Neurology 2016, **23:** 333–338

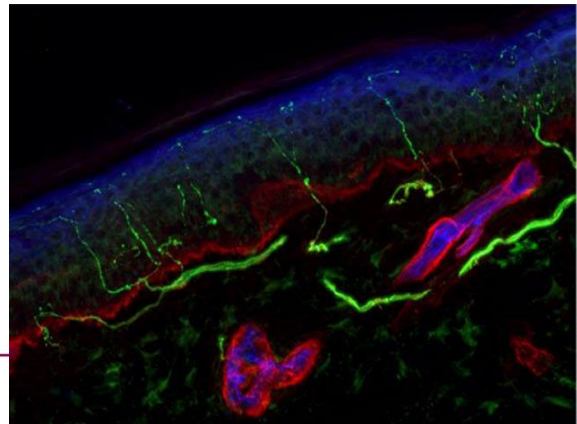
	Age (years) 18–29	30–39	40–49	50–59	60–69	>70
Female	11.9 (11.1–12.7)	11.4 (10.8–12.0)	10.8 (10.3–11.4)	10.3 (9.8–10.8)	9.8 (9.2–10.3)	9.2 (8.5–9.9)
Male	10.9 (10.1–11.6)	10.3 (9.7–11.0)	9.8 (9.3–10.3)	9.3 (8.8–9.8)	8.7 (8.2–9.3)	8.2 (7.5–8.9)

Cutoffs are referred to the midpoint of each decade and their 95% CIs (in parentheses) are reported for male and female.



- **IENFD decrease** 0.54 IENF/mm per decade
- **BMI and ethnicity** do not influence IENFD

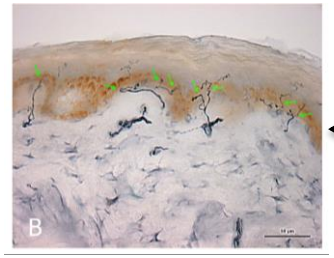
IF



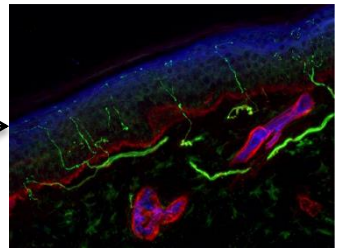
Epidermal innervation morphometry by immunofluorescence and bright-field microscopy

Journal of the Peripheral Nervous System 20:387–391 (2015)

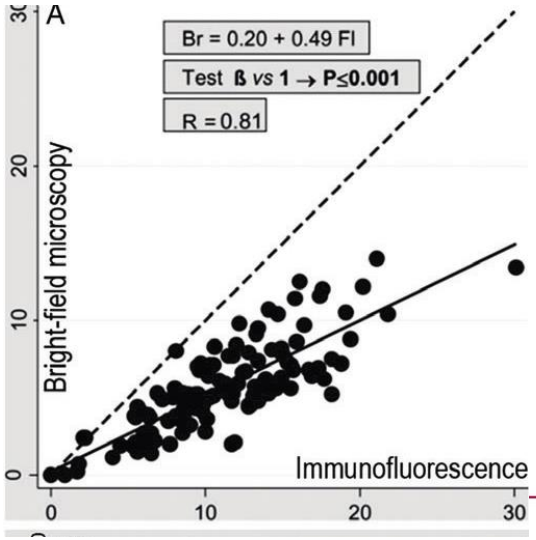
BFI



IF



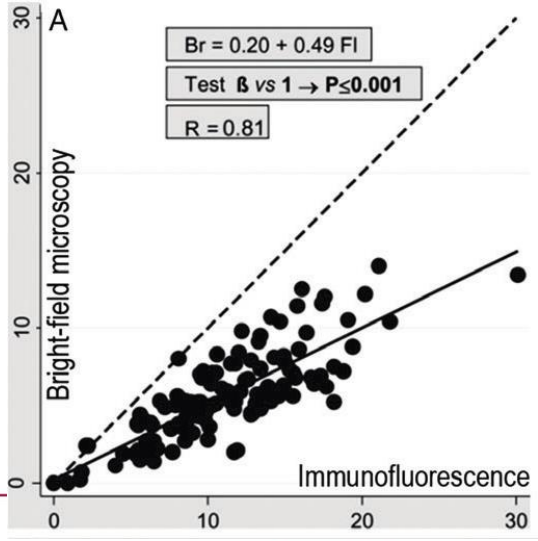
	Age (years)	Sex (M/F)	IENF/mm IF	IENF/mm (BFI)
SFN	58.7 ± 13	31/32	9.4 ± 4.8	4.6 ± 2.8
Healthy subject	42.6 ± 14	28/27	13.2 ± 4.2	6.9 ± 2.6



BFI/IF ratio = 1:2

Diagnostic agreement = 93.3%

(<1 IENF from 5th cut-off tolerated)



Diagnostic judgement

- The analysis of **concordance** between BF and IF, based on each method's cut-off showed **an agreement in 93.3% of cases if a variation of less than 1 IENF from the 5% cut-off was tolerated.**

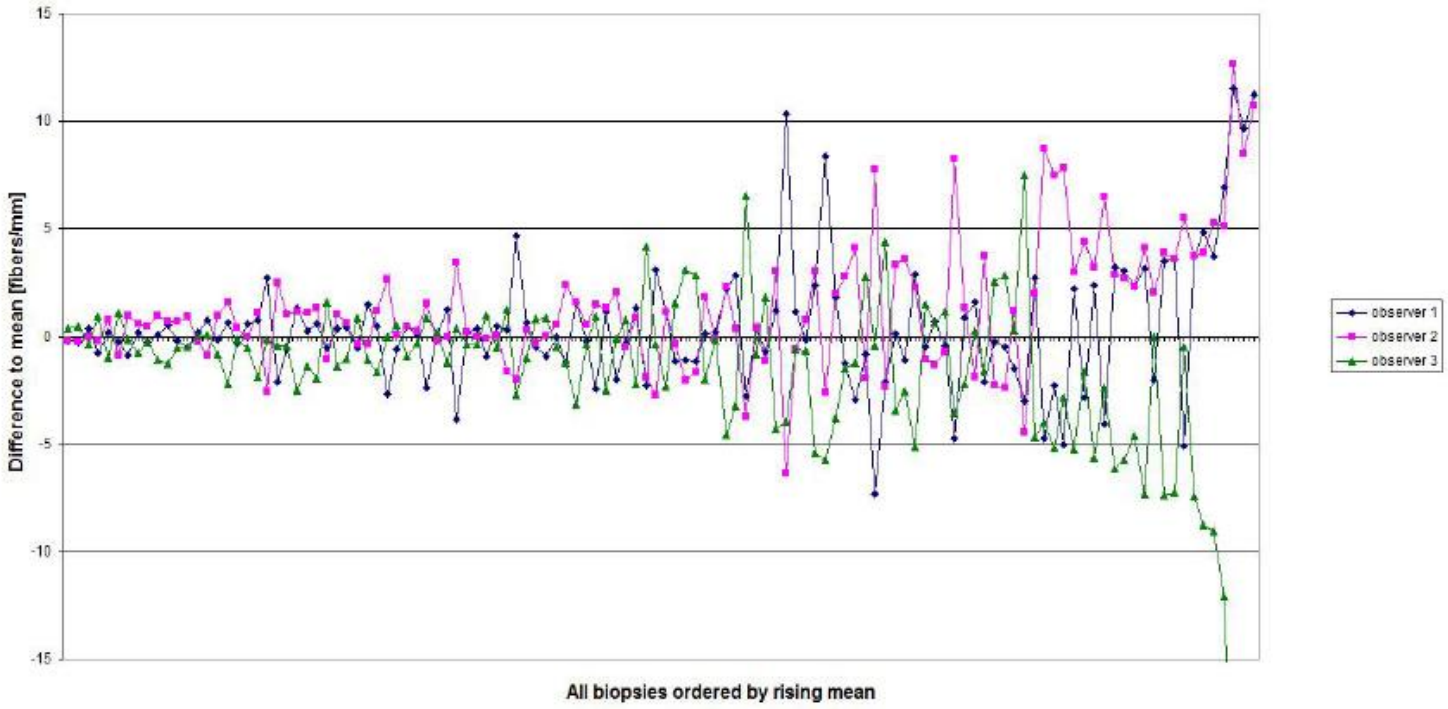
- One IENF is comparable for magnitude to the inter-rater variation 0.4±1.5 IENF/mm as calculated on the same sections (Goransson et al., 2004)

- **Values** of IENFD **very close to the cut-off** (just normal or just abnormal) must be **considered with caution** before providing a diagnostic judgment (Engelstad et al., 2012)

Significant difference between three observers in the assessment of intraepidermal nerve fiber density in skin biopsy

Sigrid Wöpking[†1], Andrea Scherens[*†1], Ida S Haußleiter[2], Helmut Richter[1], Julia Schüning[1], Sabrina Klauenberg[1] and Christoph Maier[1]

BMC Neurology 2009, 9:13 doi:10.1186/1471-2377-9-13



Point #4

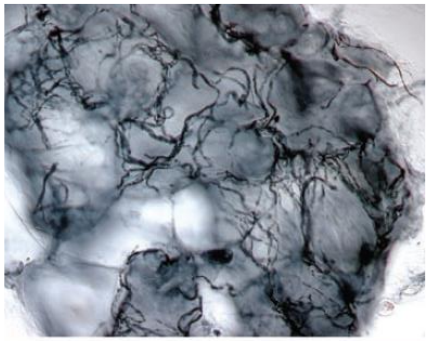
- Age and sex-adjusted normative values for both BF and IF
- Excellent agreement
  - right and left side of DL
  - 3-week follow-up
  - between BF and IF

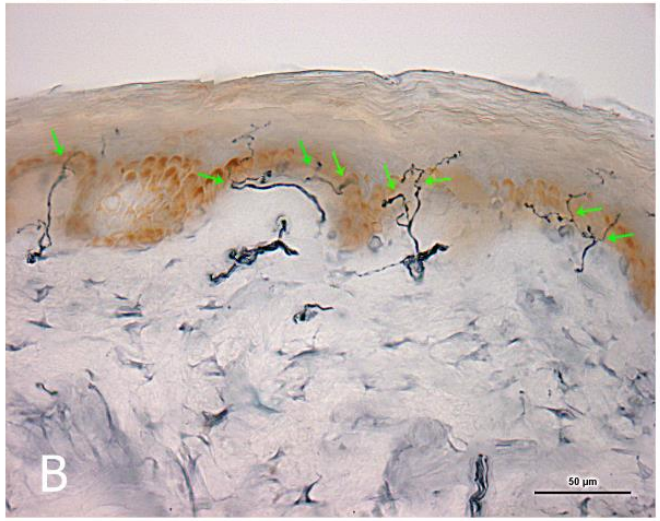
- IENFD is a virtual calculation based on:
  - Technique used
  - Agreement on its intrinsic variability

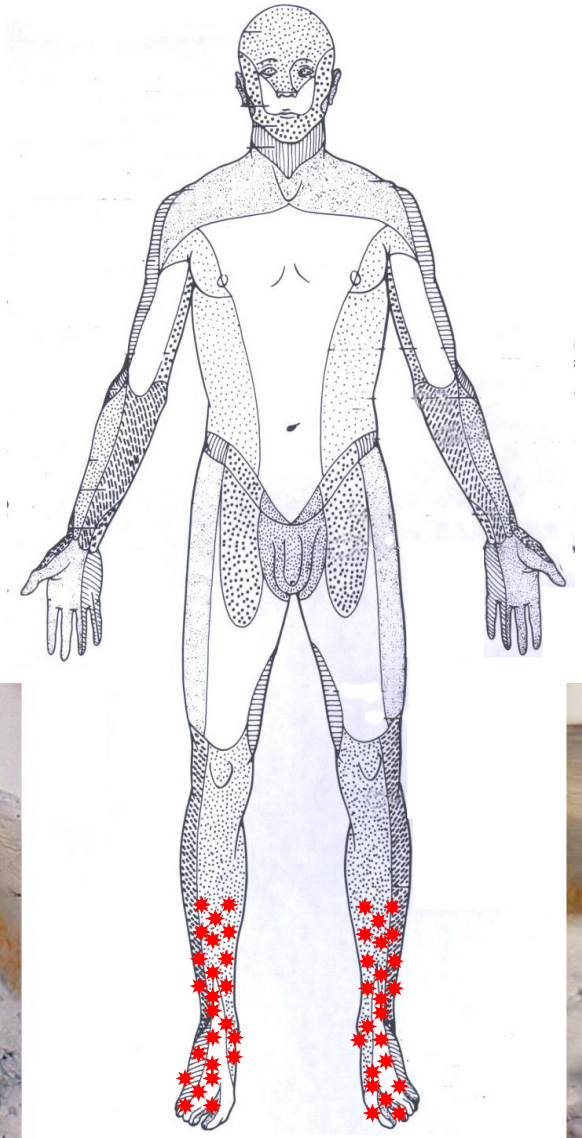
- Mandatory training and external quality control of skin biopsy laboratories

Specificity and sensitivity

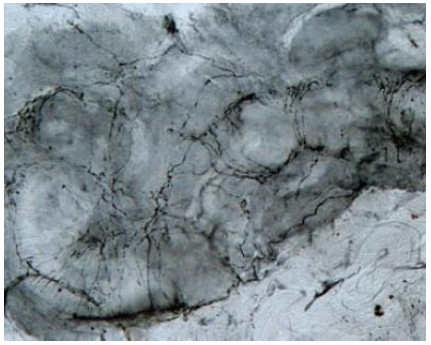
HEALTHY SUBJECT

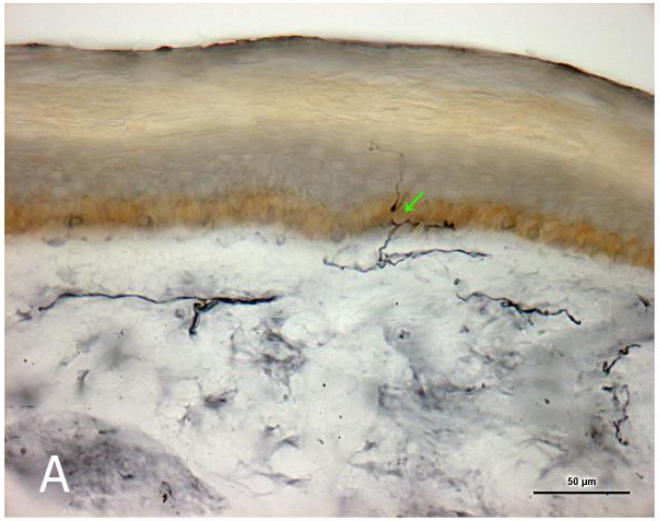






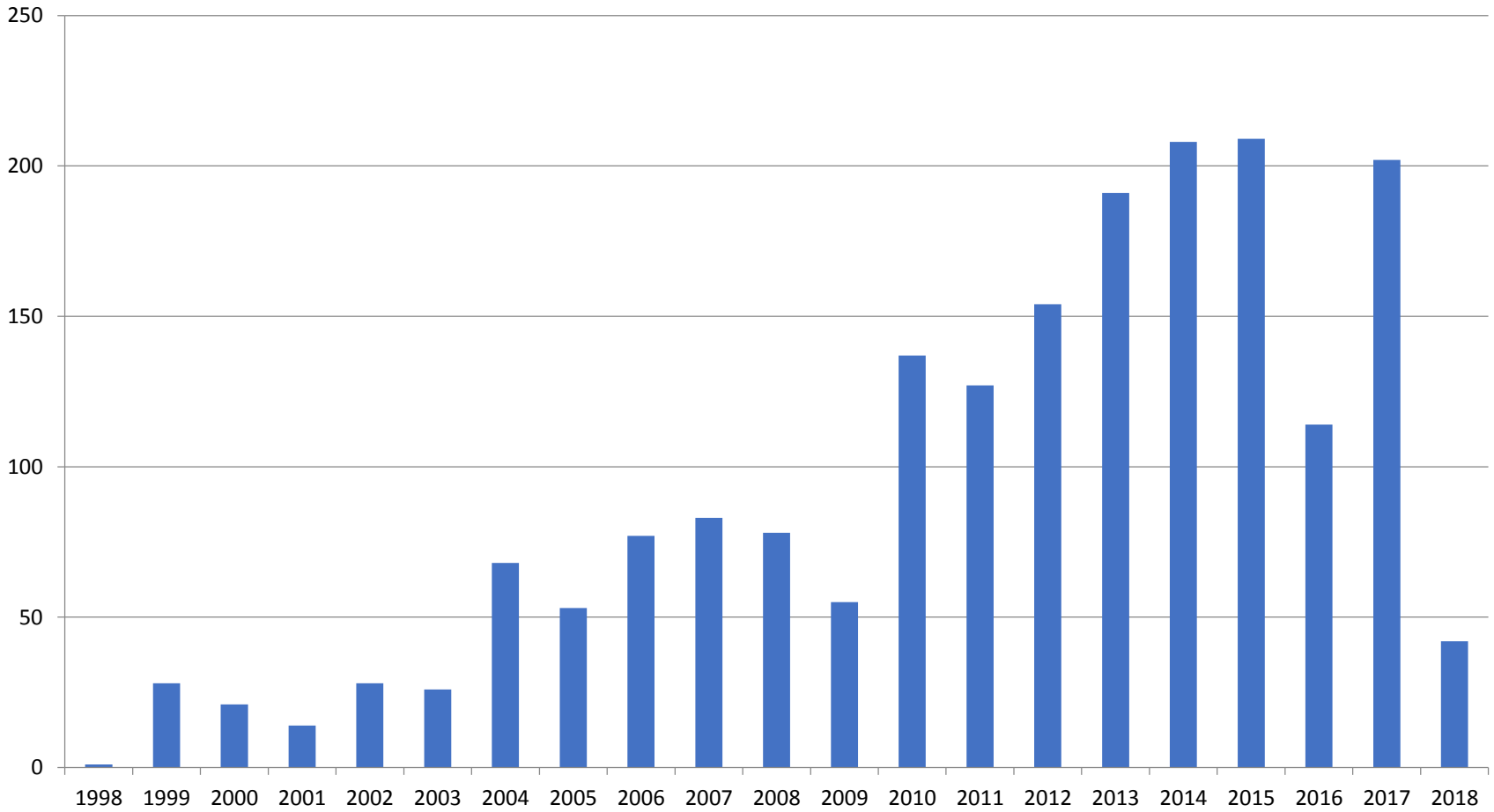
SFN





DL biopsies at the Besta lab over 20 years

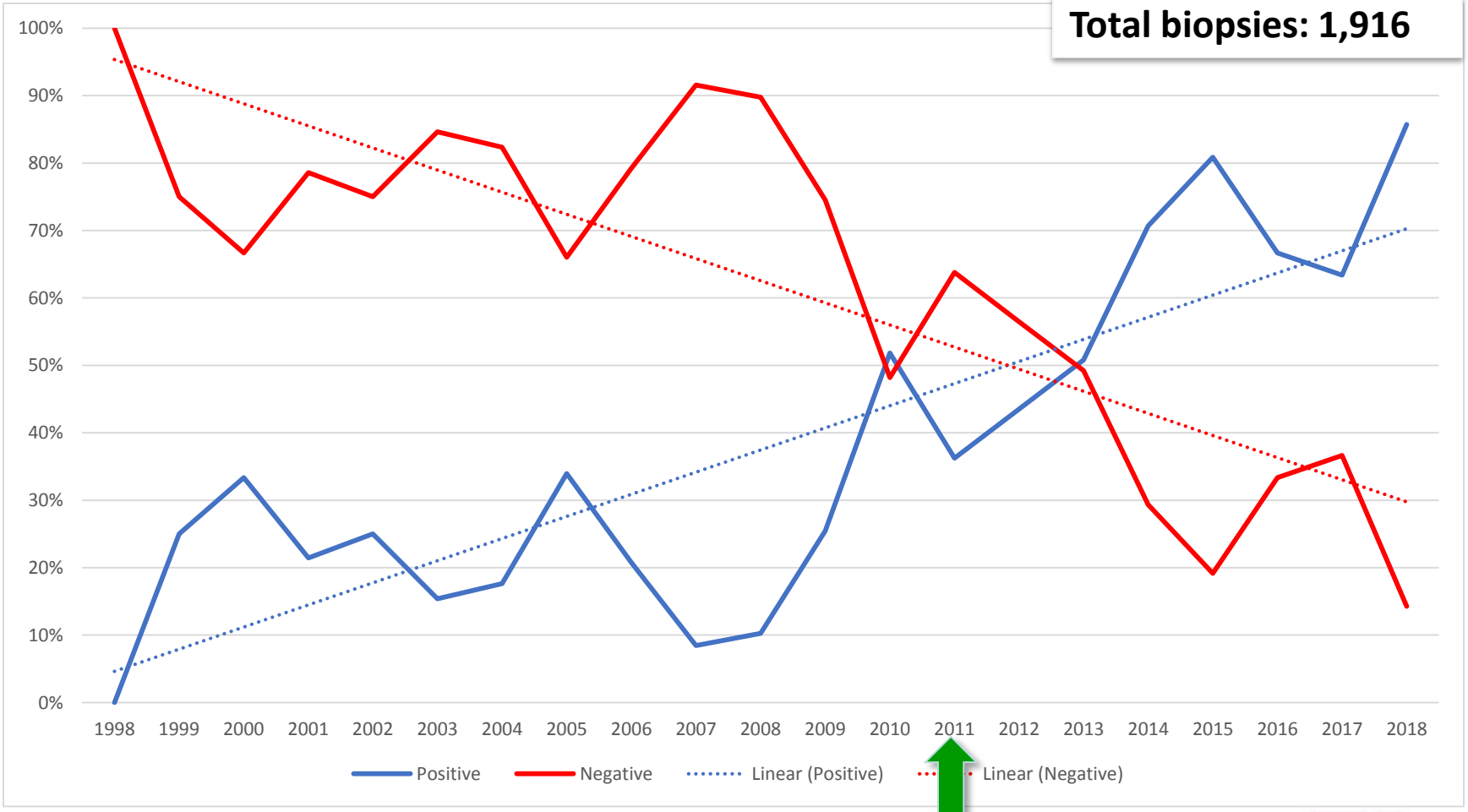
Total: 3,341



Rate of positive *vs* negative DL biopsy

based on 2010 normative values

N° positive: 940
N° negative: 976
Total biopsies: 1,916



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Point #5

- Based on skin biopsy alone with non-adjusted cut-off, for >10 years our lab (and many others) reported high rate of false positive (and likely lower of false negative)

- The figures started changing after 2010, likely for two reasons:
  - Availability of sex- and age-adjusted cutoff based on 5th percentile
  - Focused clinical approach to patients

doi:10.1093/brain/awn093 *Brain* (2008), **131**, 1912–1925

The diagnostic criteria for small fibre neuropathy: from symptoms to neuropathology

SFN when *at least two* were *abnormal*:

1. **clinical signs** of small fibre impairment (pinprick and thermal sensory loss and/or allodynia and/or hyperalgesia), which distribution was consistent with peripheral neuropathy (length or non-length dependent neuropathy);
2. **QST** abnormal warm and/or cooling threshold at the foot;
3. **reduced IENFD** at the distal leg

Diagnostic efficiency of composite approach against "modular gold standard"

Brain (2008), **131**, 1912–1925

	Clinical examination (%)	QST (%)	* Skin biopsy (%)
Sensibility	62.6	56.7	88
Specificity	46	36.5	88.8
Positive predictive value	55.3	48.7	89.4
Negative predictive value	53.7	44.2	87.5

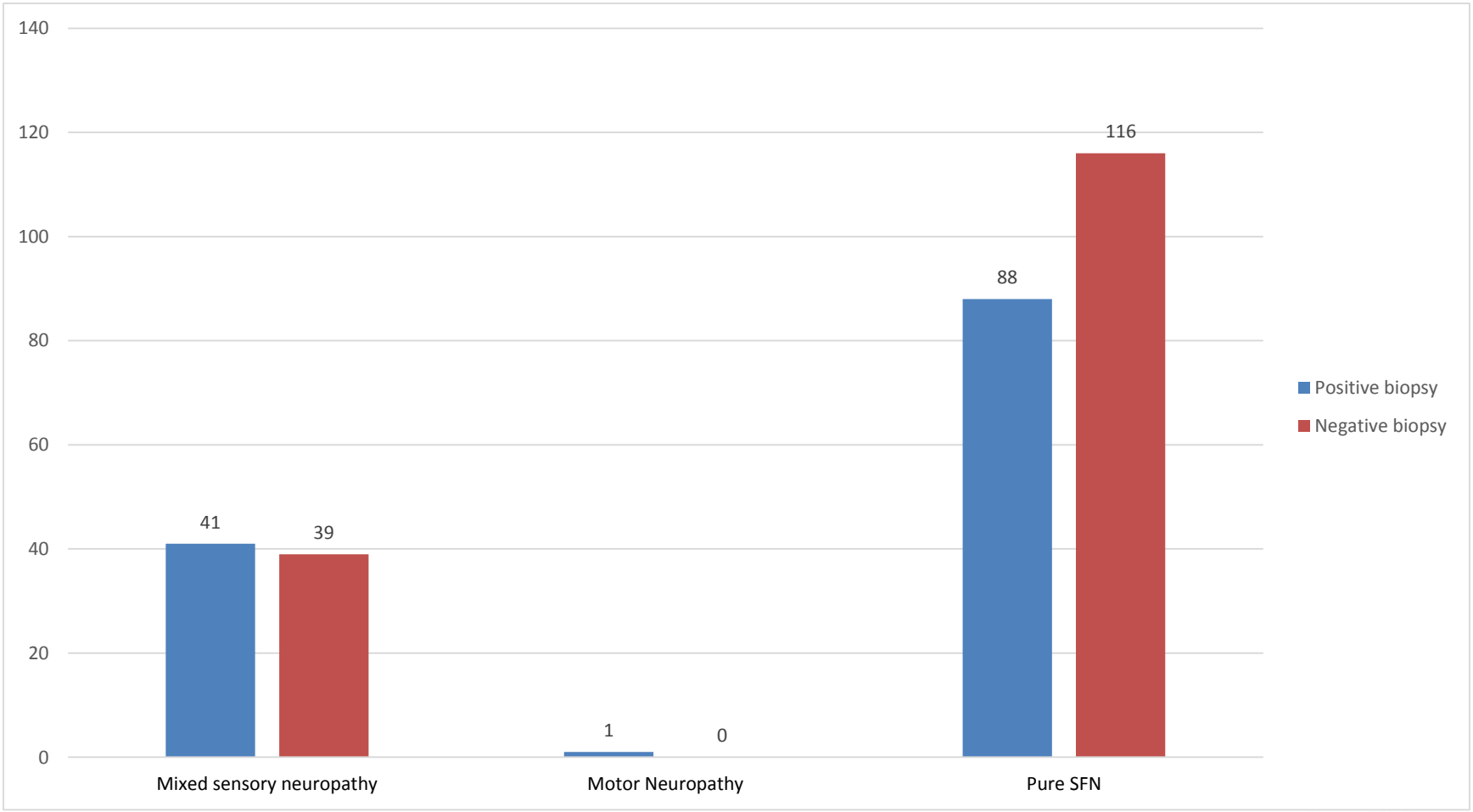
** Cut-off values calculated by ROC curve analysis vs 47 healthy subjects. Value of 7.63 IENF/mm at DL distal had specificity of 90% and sensibility of 82.8%*

Comparison of diagnostic efficiency across labs

Site	Sn	Sp	PPV	PPN	No. patients	Reference	Year	Author	Journal
DL	0,60	0,90	0,55	0,75	118	CE, NCS	1998	McArthur	Arch Neurol
PTH	0,45	0,88	0,45	0,89	118	CE, NCS	1998	McArthur	Arch Neurol
DL	0,90	0,95	0,95	0,91	30	CE, NCS	2001	Koskinen	J Neurol
DL	0,80	0,95	0,84	0,93	90	CE, NCS	2001	Chien	Acta Neuropathol
Arm	0,70	0,94	0,84	0,90	90	CE, NCS	2001	Chien	Acta Neuropathol
DL	0,69	0,95	-	-	99	CE, QST, NCS	2008	Vlckova-Moravcova	Muscle nerve
DL	0,88	0,88	0,89	0,87	124	CE, QST, NCS	2008	Devigili	Brain
DL	0,35	0,95	-	-	210	CE, QST, NCS	2009	Nebuchennykh	J Neurol

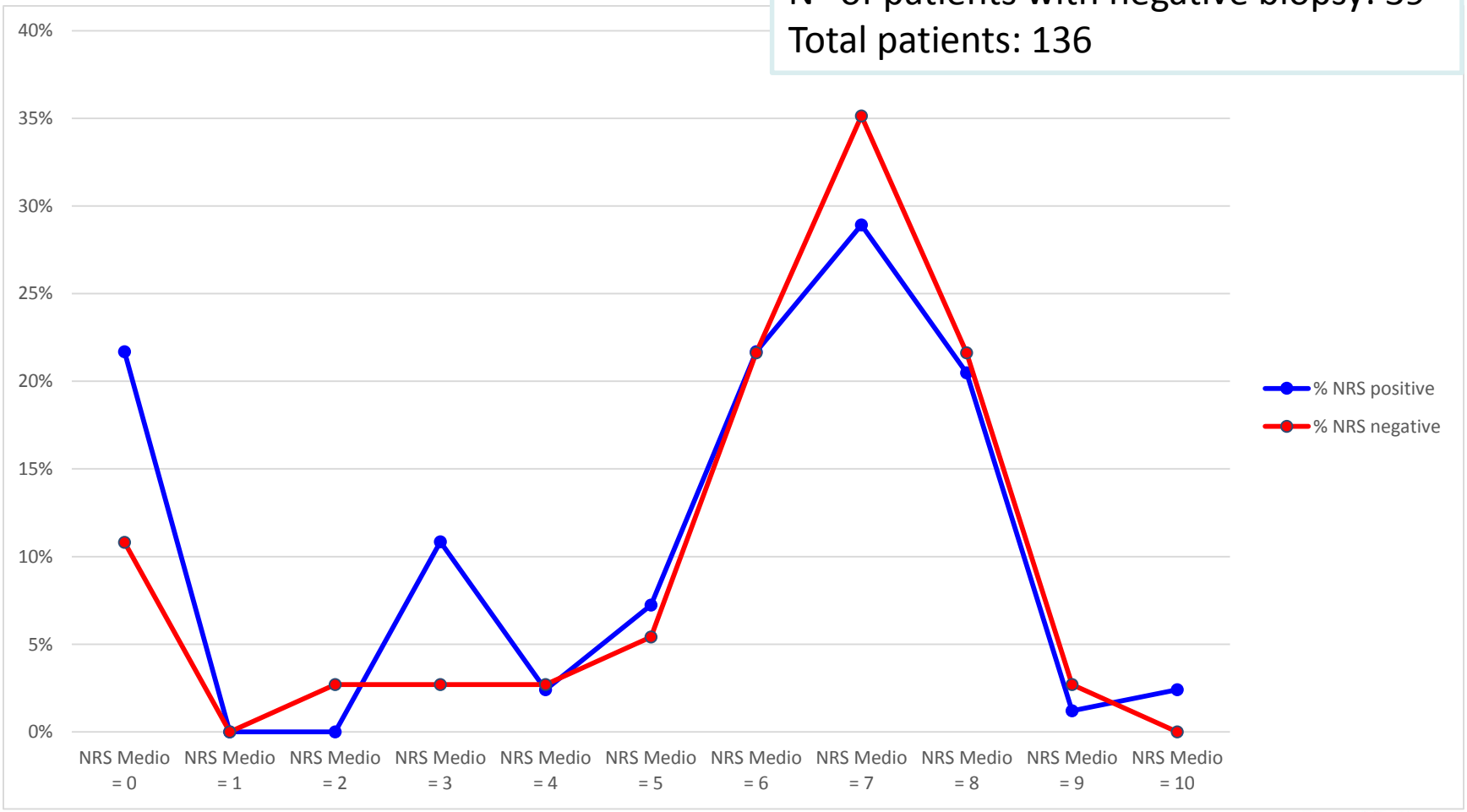
?

IENFD and type of neuropathy:
positive and negative in pure vs mixed neuropathy

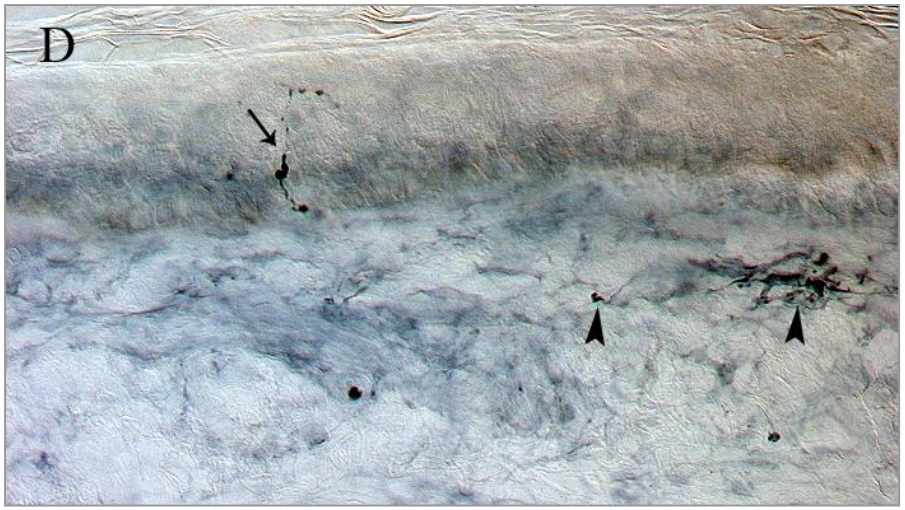


IENFD and PAIN:
mean NRS score in positive and negative biopsy

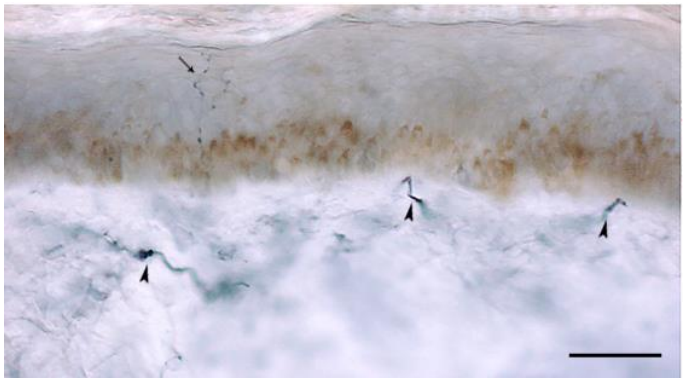
N° of patients with positive biopsy: 97
N° of patients with negative biopsy: 39
Total patients: 136

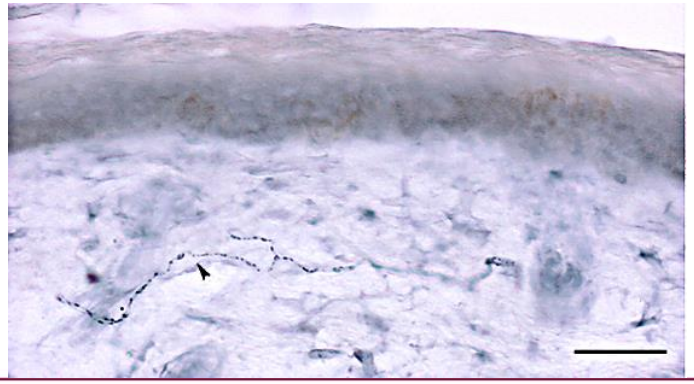


To pain, or not to pain, that is the *punching* question



IENFD and risk of neuropathic pain





Lancet Neurol 2007; 6: 632–42

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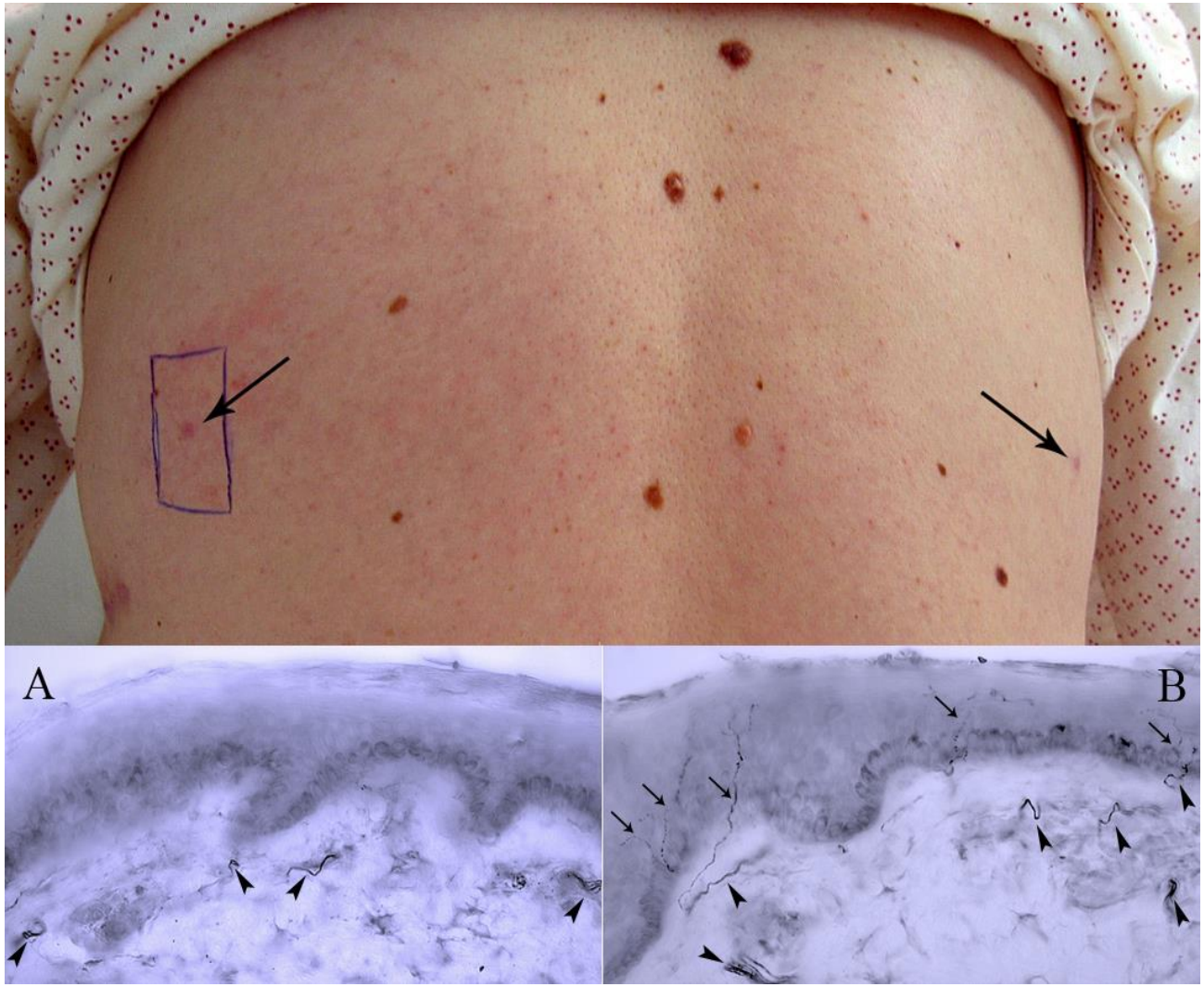
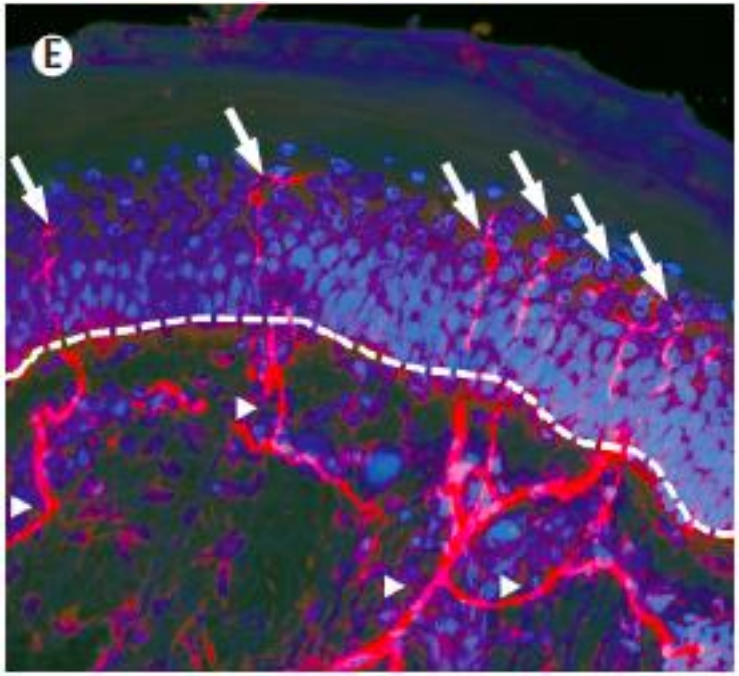
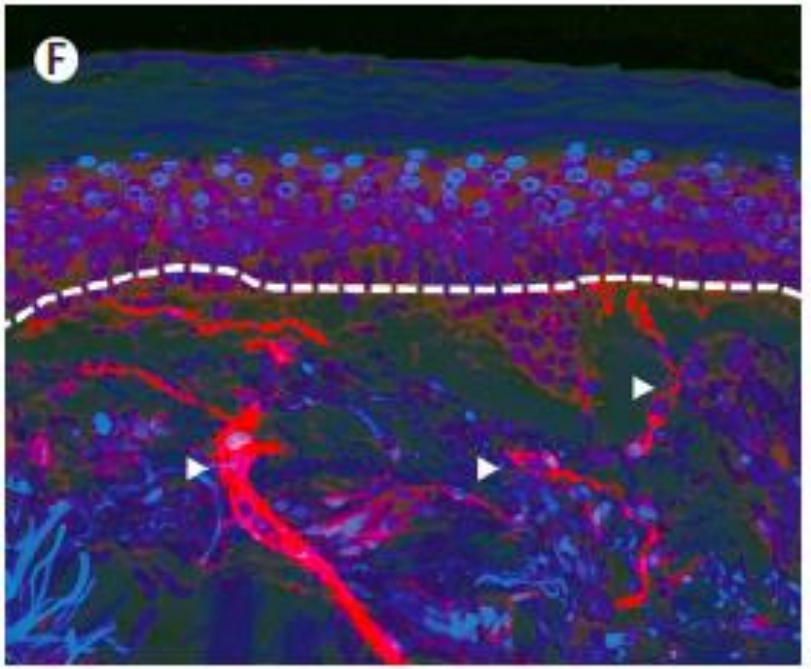

A
B

IENFD and risk of neuropathic pain

Inherited erythromelalgia



SFN

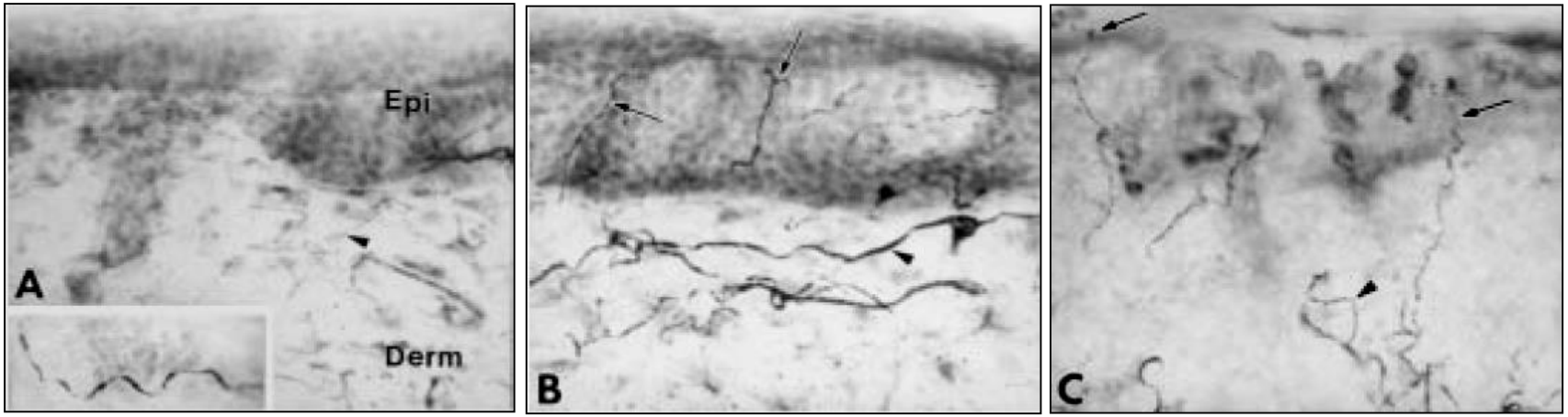


David L H Bennett, C Geoffrey Woods
Lancet Neurol 2014; 13: 587–99

Neuropathological alterations in diabetic truncal neuropathy: evaluation by skin biopsy

Neurol Neurosurg Psychiatry 1998;65:762–766

Giuseppe Lauria, Justin C McArthur, Peter E Hauer, John W Griffin, David R Cornblath



Acute phase

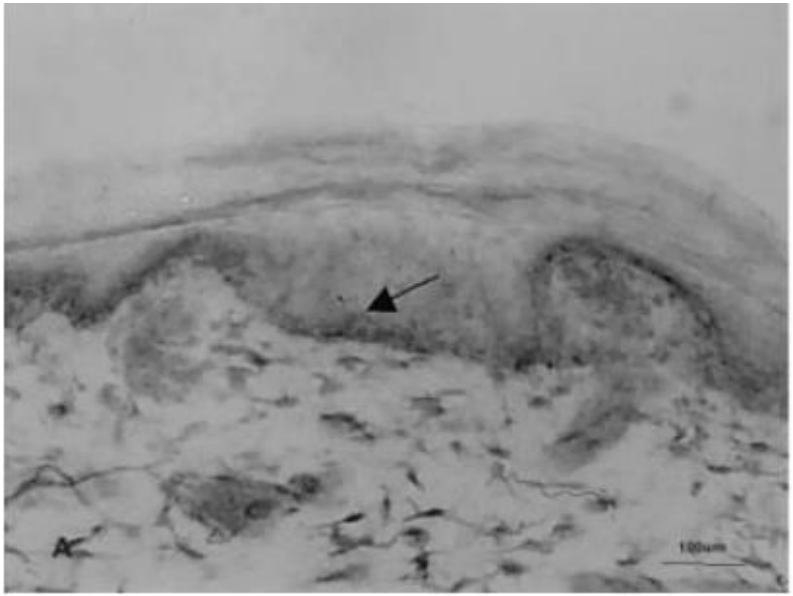
Healthy subject

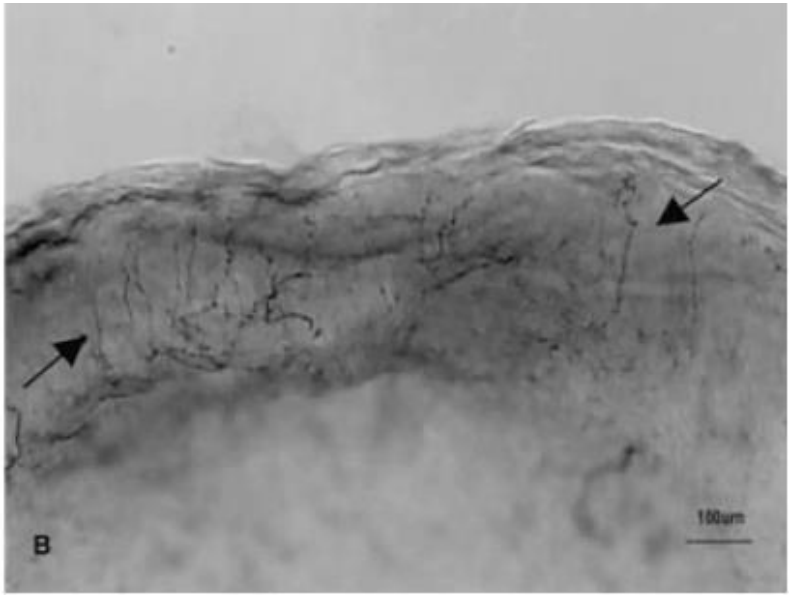
18 months follow-up

EPIDERMAL REINNERVATION CONCOMITANT WITH SYMPTOMATIC IMPROVEMENT IN A SENSORY NEUROPATHY

Hiroyuki Nodera, MD
Richard L. Barbano, MD, PhD
Don Henderson
David N. Herrmann, MB, BCh

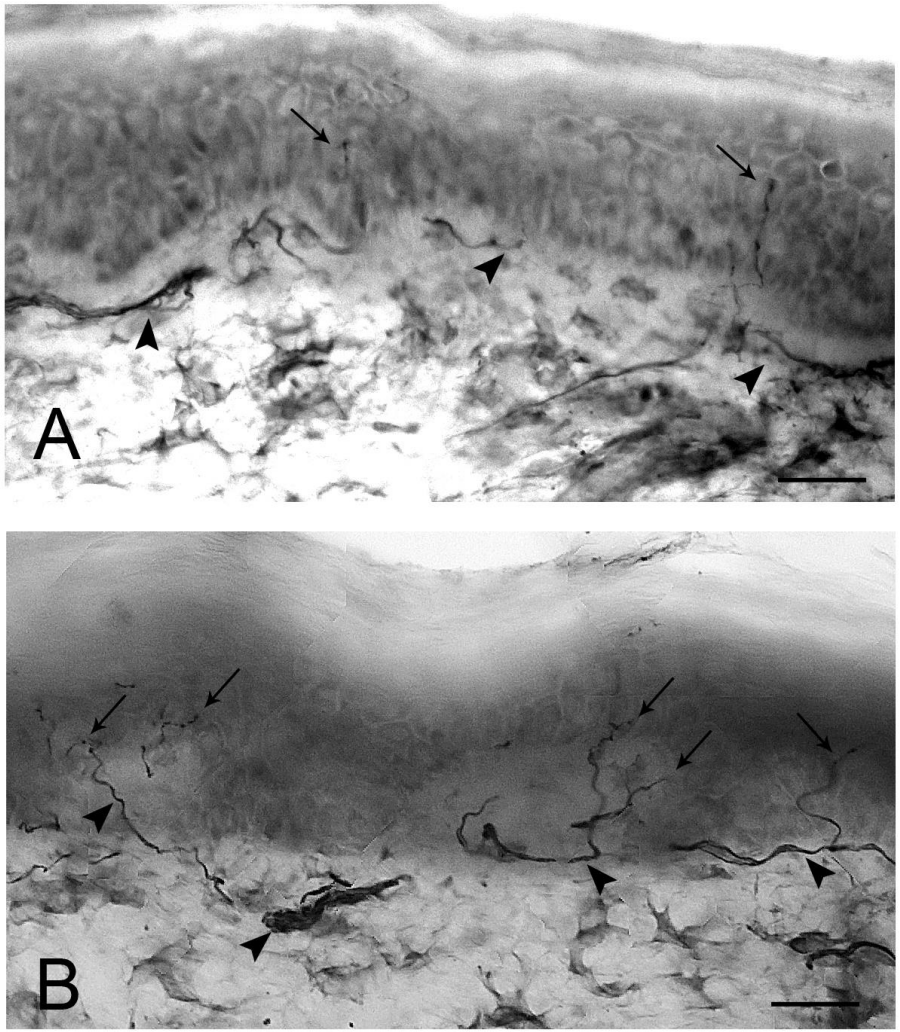
MUSCLE & NERVE April 2003





Painful neuropathy in subclinical hypothyroidism: pain and neuropathological recovery after hormone replacement therapy

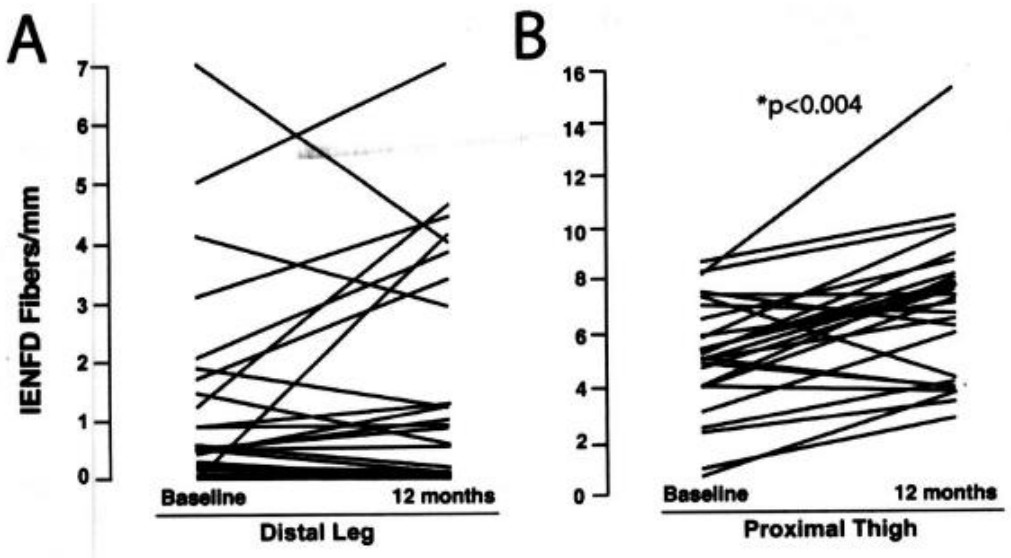
Neurol Sci (2009) 30:149–151

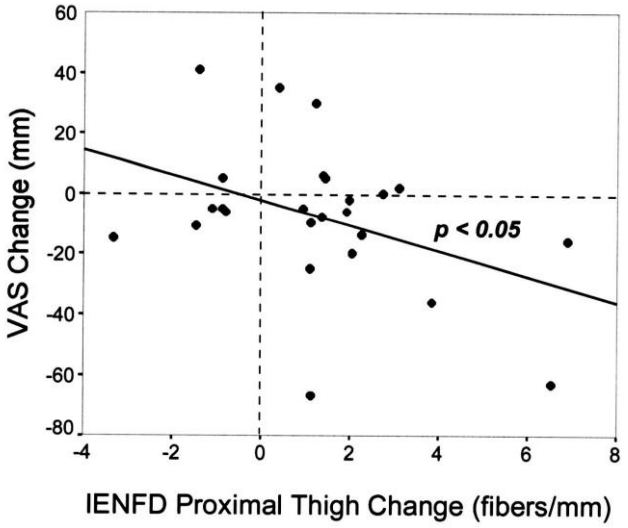


Lifestyle Intervention for Pre-Diabetic Neuropathy

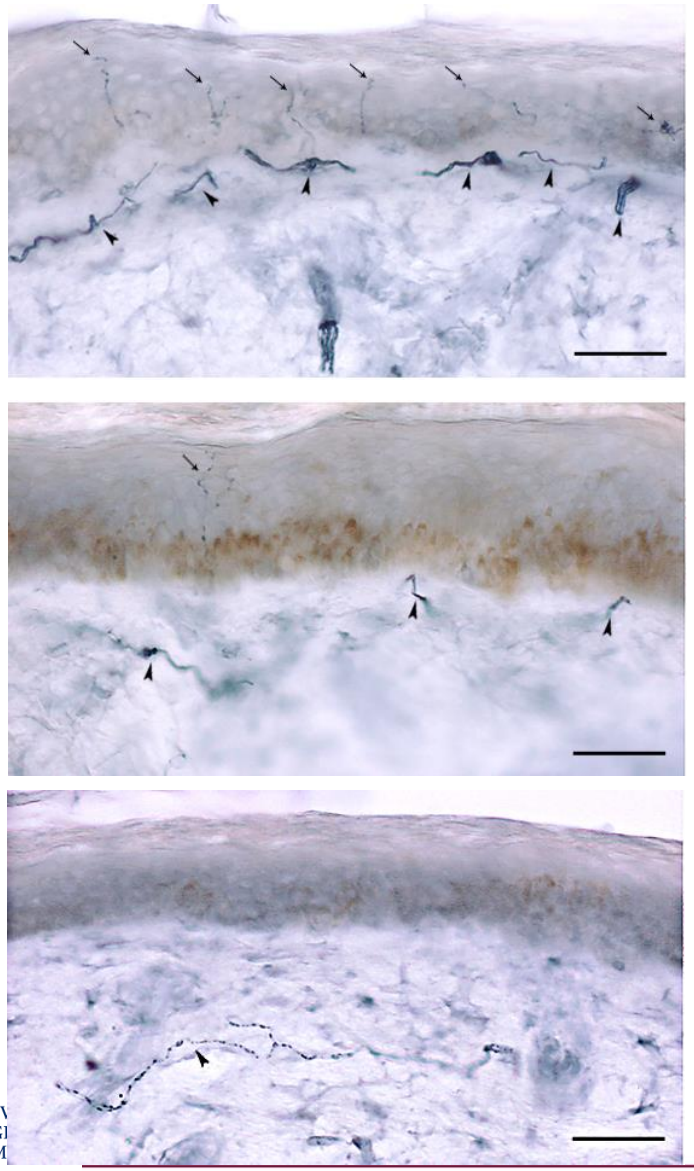
Diabetes Care; Jun 2006; 29, 6

A Gordon Smith: James Russell: Eva L Feldman: Jonathan Goldstein: et al

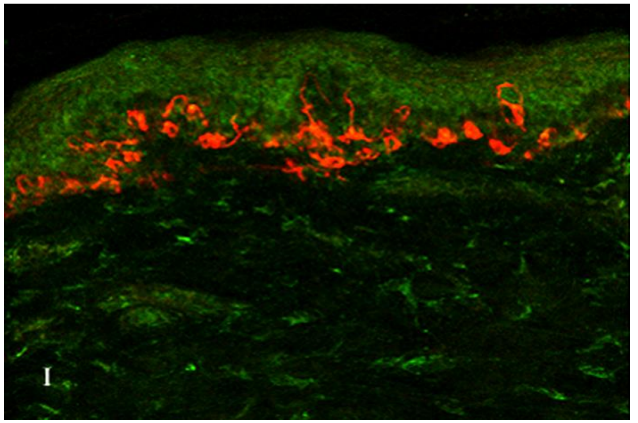




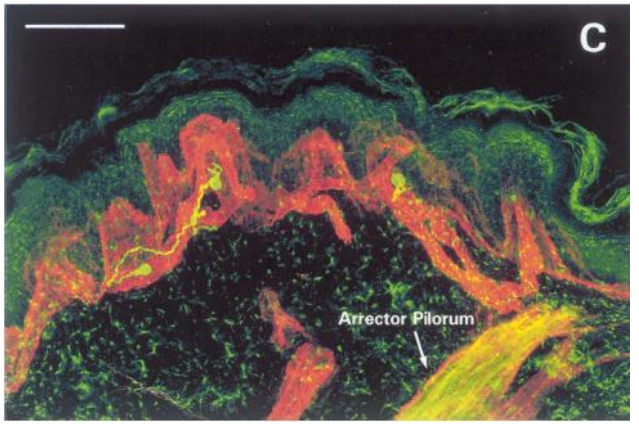
IENFD and risk of neuropathic pain



Painful neuropathy



HSAN IV



Nolano et al., 2000

Painless small fiber pathology

Sensory deficit in Parkinson's disease: evidence of a cutaneous denervation

Maria Nolano,[1] Vincenzo Provitera,[1] Anna Estraneo,[1] Mona M. Selim,[2] Giuseppe Caporaso,[1] Annamaria Stancanelli,[1] Anna Maria Saltalamacchia,[1] Bernardo Lanzillo[1] and Lucio Santoro[3]

Brain (2008), **131**, 1903–1911

Small-fiber neuropathy in patients with ALS

Neurology® 2011;76:2024-202

J. Weis, MD*
I. Katona, MD*
G. Müller-Newen, PhI
C. Sommer, MD
G. Necula
C. Hendrich, MD
A.C. Ludolph, MD
A.-D. Sperfeld, MD

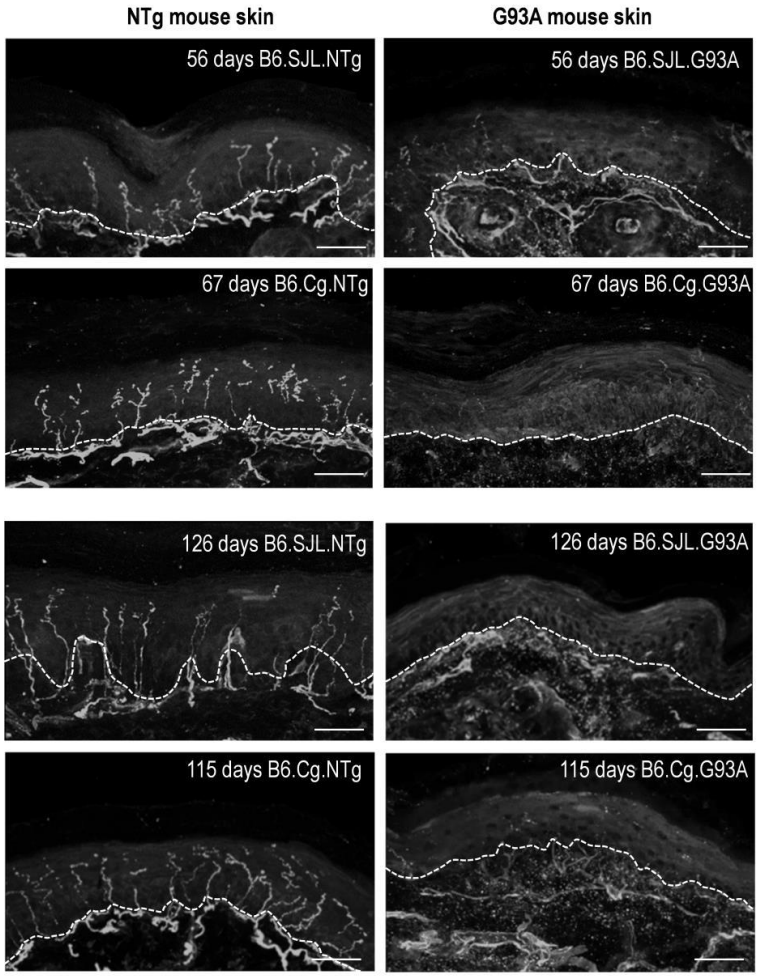
Amyotrophic lateral sclerosis causes small fiber pathology

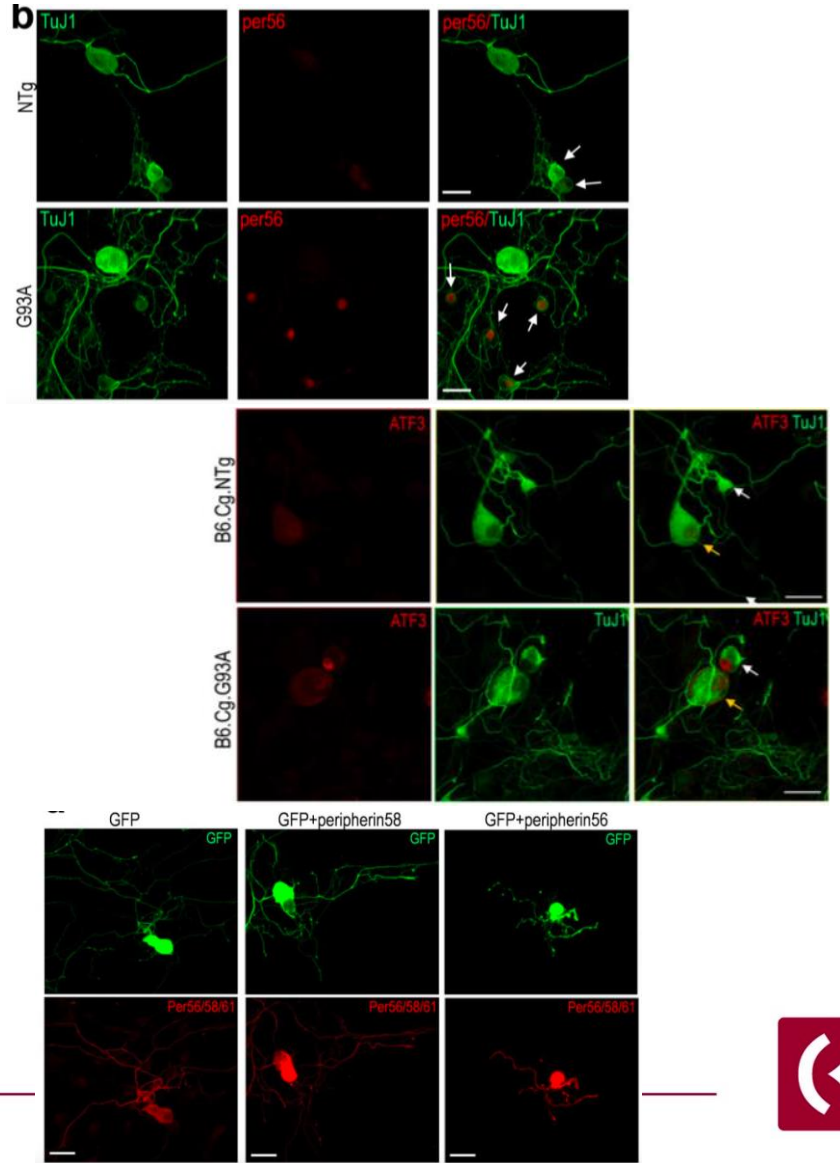
European Journal of Neurology 2016, **23:** 416–420

- 51 ALS and 6 FOSMN
- Reduced IENF density in 75.4% of ALS and 50% of FOSMN patients
- No correlation with genotype, disease features, disease duration and severity

ALS mouse model SOD1^{G93A} displays early pathology of sensory small fibers associated to accumulation of a neurotoxic splice variant of peripherin

Human Molecular Genetics, 2016, Vol. 25, No. 8 1588–1599





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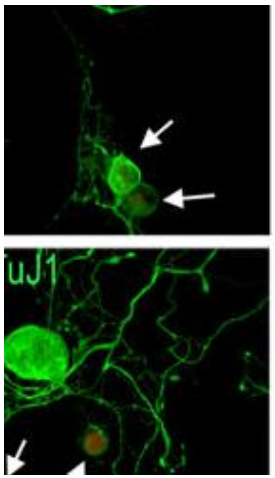
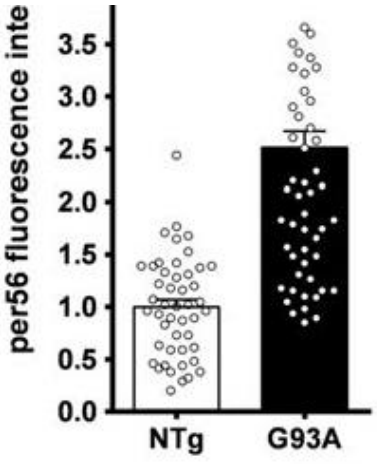
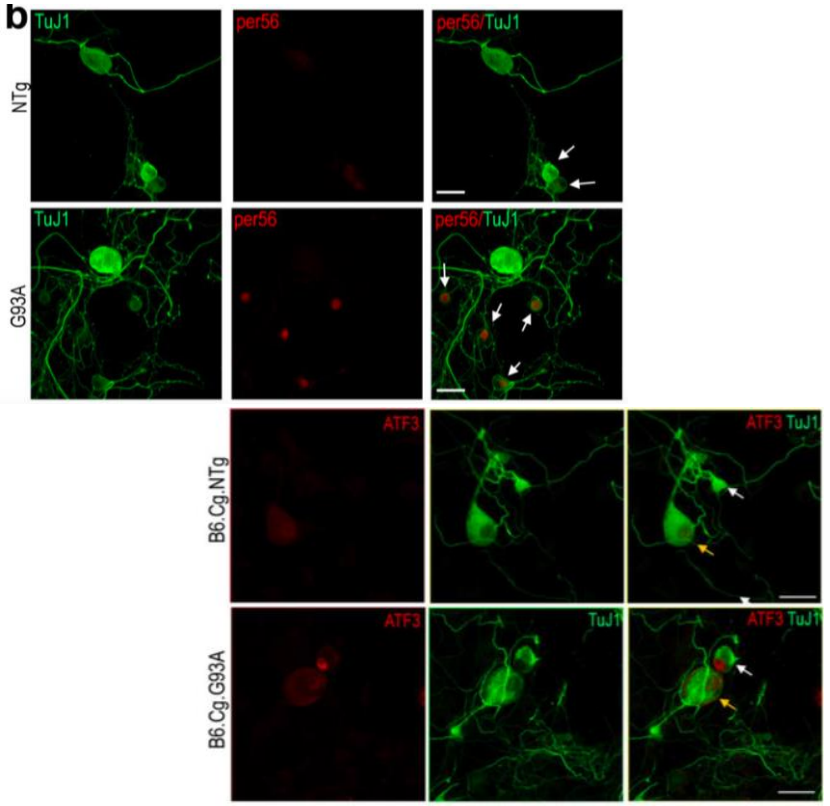
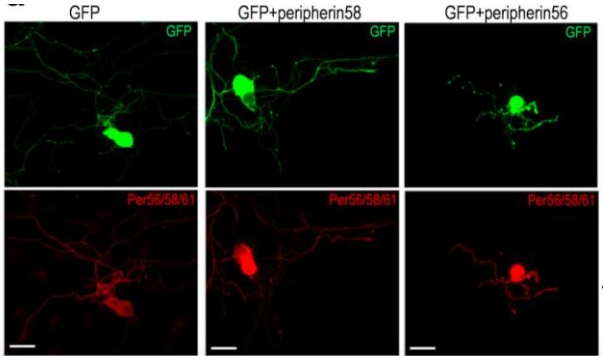
ALS mouse model SOD1^{G93A} displays early pathology of sensory small fibers associated to accumulation of a neurotoxic splice variant of peripherin

Human Molecular Genetics, 2016, Vol. 25, No. 8 1588–1599





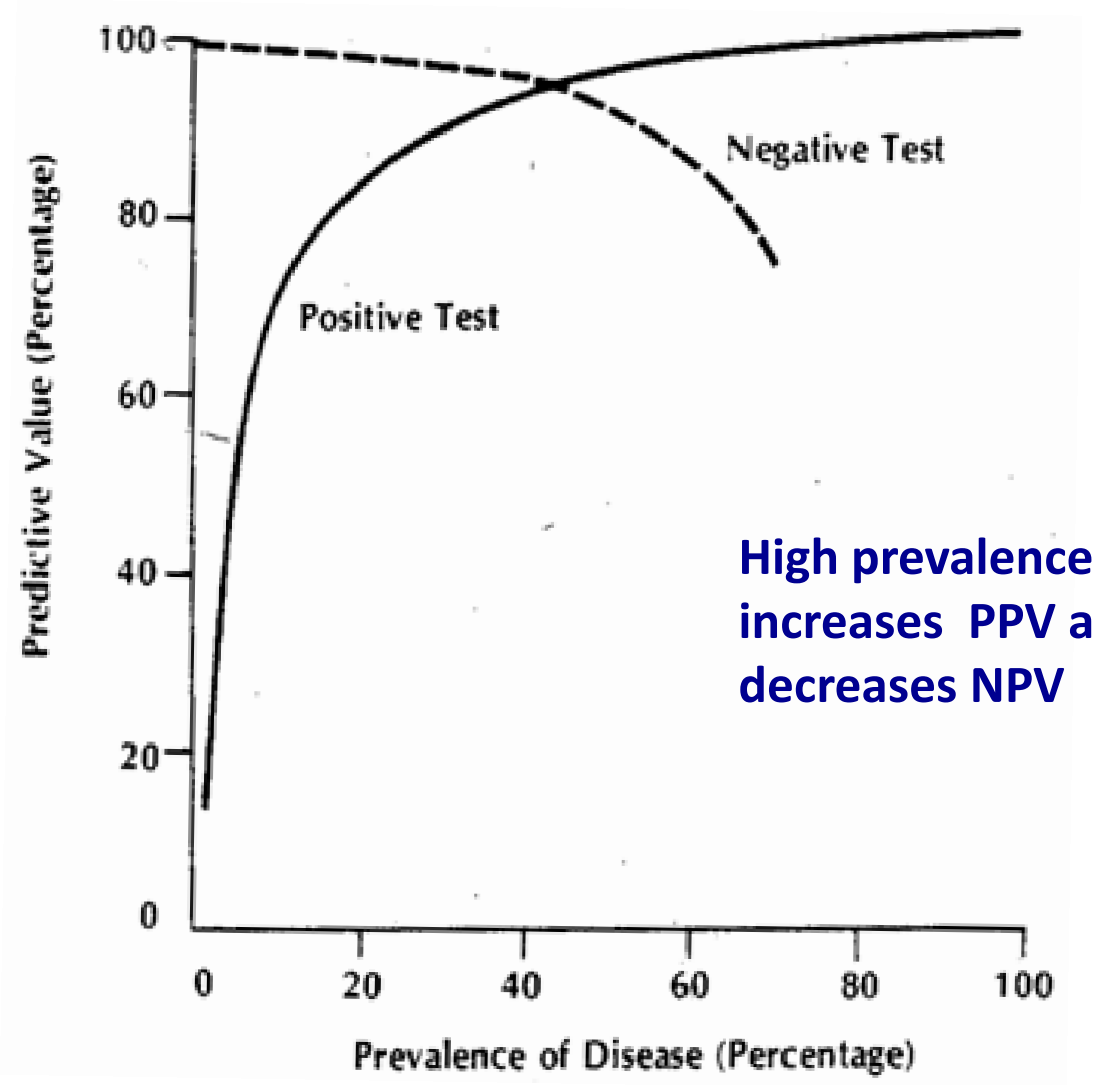




Point #6

- Biopsy results blind to the clinical phenotype is little informative
- The availability of cut-off values based on the 5th percentile made skin biopsy a tool with fixed high (95%) specificity (*% true negative*)
- Intrinsic impossibility to test the sensitivity (*% true positive)* of the method
- Impossible definition of PPV (*% below cut-off who truly have neuropathy*) and NPV (*% above cut-off who truly do not have neuropathy*), which also vary with the prevalence of the disease within a population.

Relationship between disease prevalence and predictive values



High prevalence increases PPV and decreases NPV

Mausner JS, Kramer S: Mausner and Bahn Epidemiology: An Introductory Text. Philadelphia, WB Saunders, 1985

Incidence and prevalence of small-fiber neuropathy

Neurology® 2013;81:1356-1360

A survey in the Netherlands

Martine J.H. Peters, MD*
Mayienne Bakkers, MD*
Ingemar S.J. Merkies, MD, PhD
Janneke G.J. Hoeijmakers, MD
Elisabeth P.M. van Raak, MD, PhD
Catharina G. Faber, MD, PhD

	All adults	Males	Females
Incidence: cases/100,000/y (95% CI)			
Overall (n = 39; 25 males, 14 females)	11.73 (7.12-18.22)	15.56 (8.17-26.88)	8.15 (3.28-16.80)
20-65 y old (n = 28; 18 males, 10 females)	11.10 (6.07-18.63)	14.40 (6.59-27.34)	7.86 (2.55-18.34)
65 y or older (n = 11; 7 males, 4 females)	13.71 (4.77-30.82)	19.59 (4.78-53.21)	8.99 (1.09-32.47)
Prevalence: cases/100,000 (95% CI)			
Overall (n = 88; 49 males, 39 females)	52.95 (42.47-65.23)	60.98 (45.12-80.62)	45.43 (32.30-62.09)
20-65 y old (n = 65; 38 males, 27 females)	51.55 (39.79-65.70)	60.81 (43.04-83.46)	42.45 (27.98-61.76)
65 y or older (n = 23; 11 males, 12 females)	57.34 (36.35-86.02)	61.58 (30.75-110.16)	53.93 (27.87-94.19)

Conclusions on skin biopsy

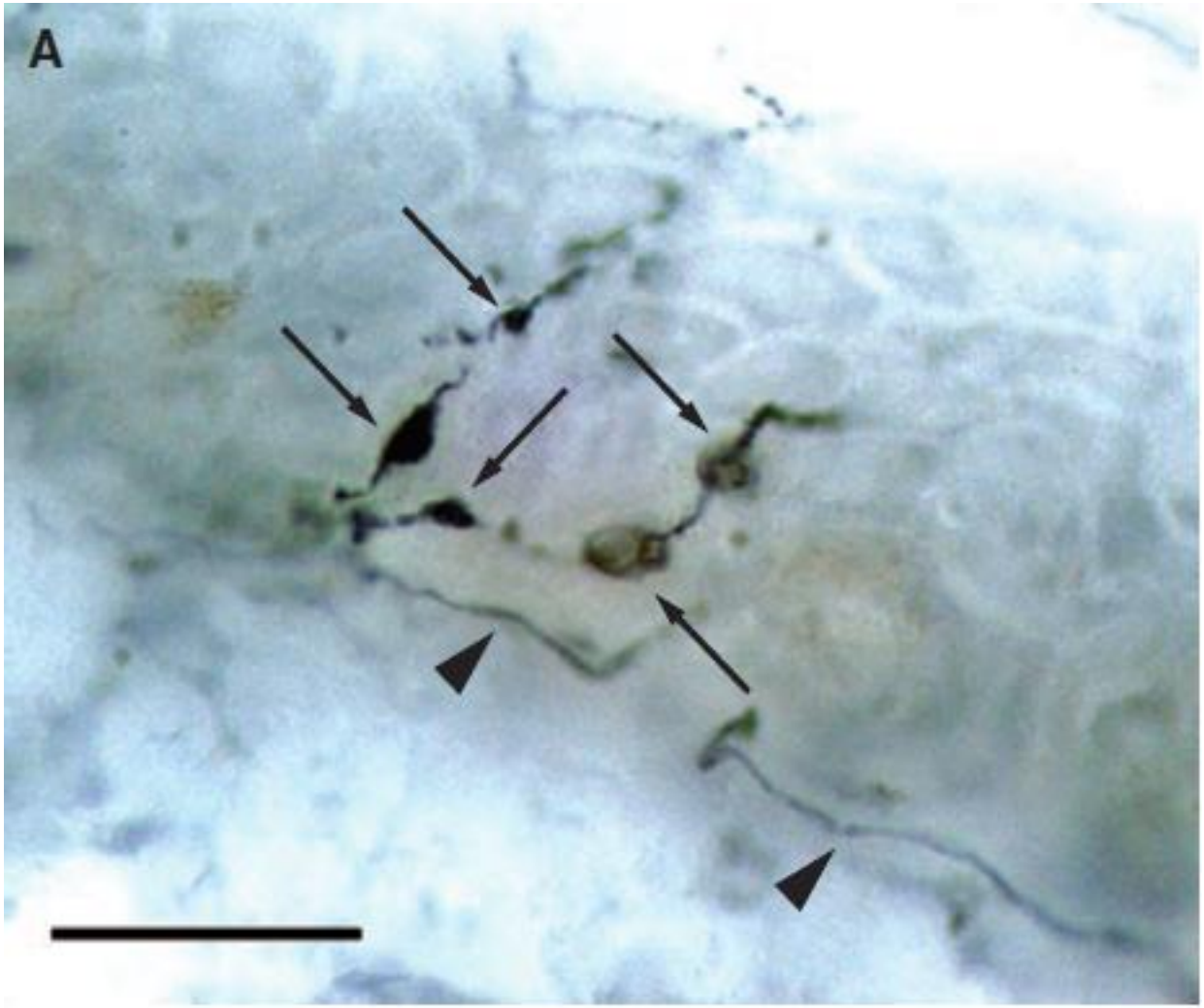
Strengths

- Sex and age-adjusted normative values → tailored on patients
- Agreement between BF and IF methods
- Reliable and feasible in animal models of neuropathy
- High reliability between sites at DL and at 3-w follow-up
- High specificity → reliable confirmatory tool in candidate patients (RCT)

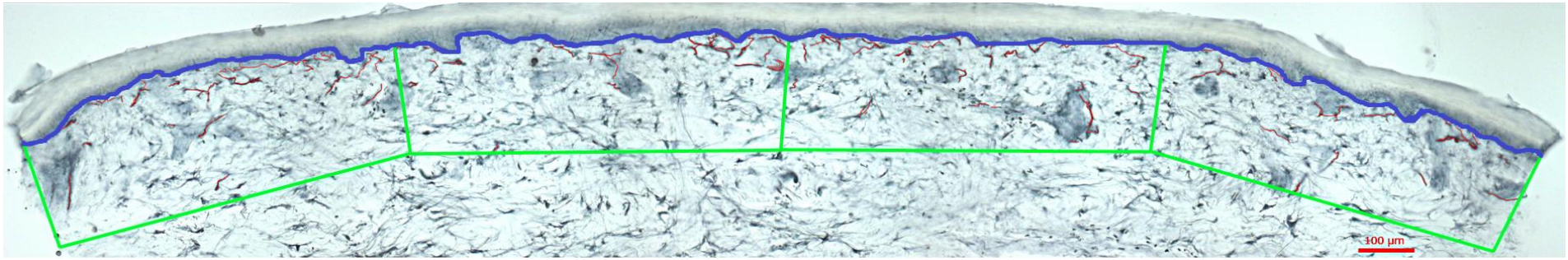
Limitations

- Poor interlab agreement without quality program → mandatory for multicentre studies
- Unknown sensitivity, PPV and NPV → useless as screening tool
- Not to be used as unique tool to determine patient disease subgroups

Furthermore…



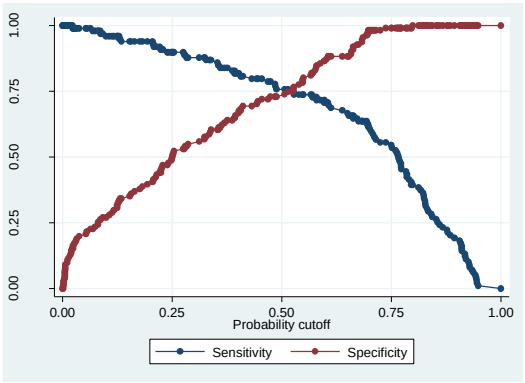
Measurement of dermal nerve fibre length

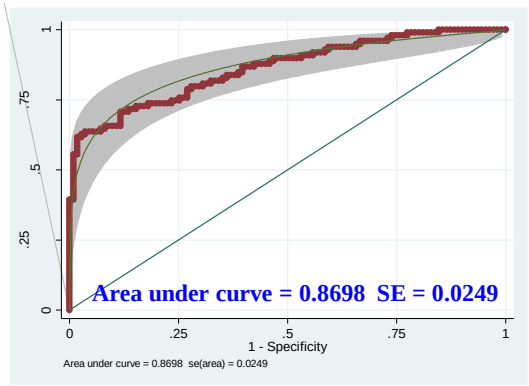


- 3-mm length biopsy section in which the dermal area of interest is divided into four equal portions (green lines).
- The dermal-epidermal junction is highlighted in blue.
- The dermal nerves are highlighted in red.

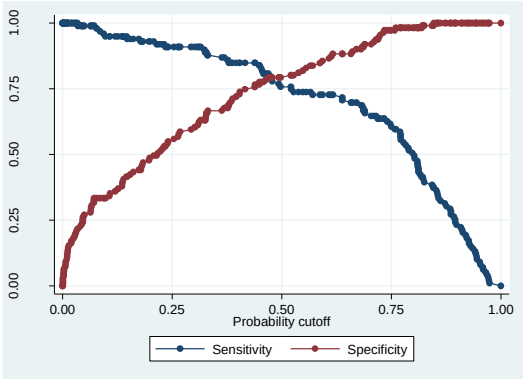
Neurology® 2011;77:242-249

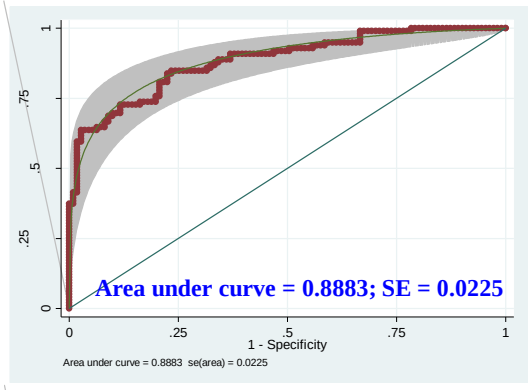
Dermal nerve length discriminates SFN from healthy individuals



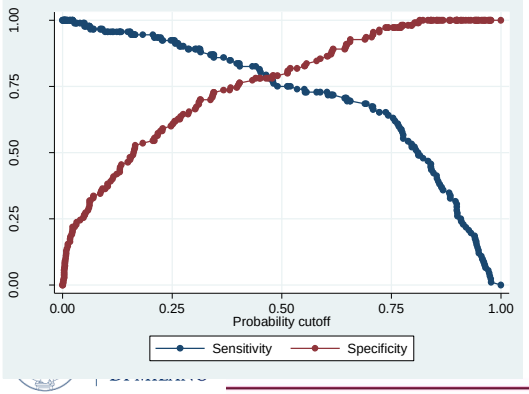


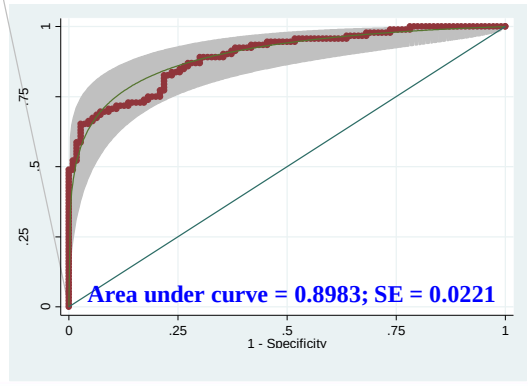
DNFL





DNFL/mm^2





DNFL/mm

Criteria:

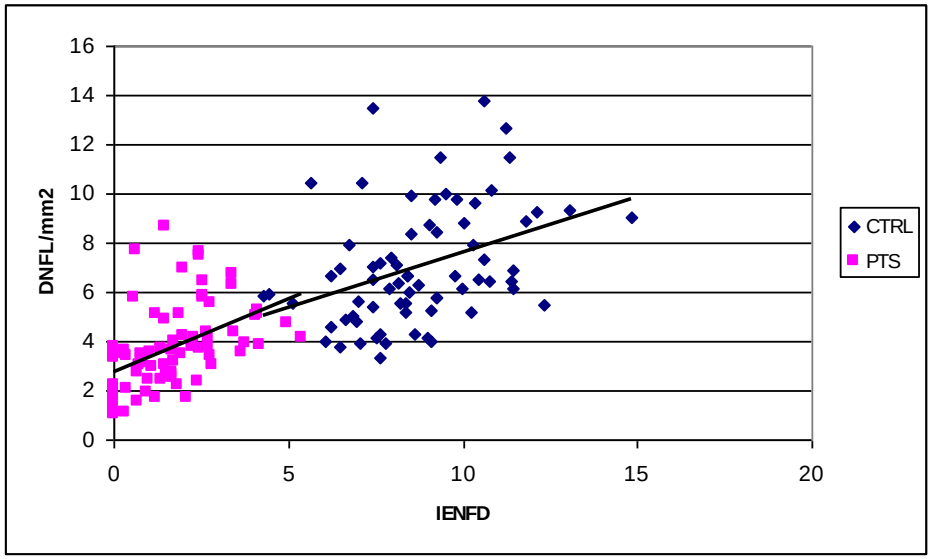
- ≤0.5 no discrimination
- 0.7-0.8 acceptable
- 0.8-0.9 excellent
- ≥ 0.9 outstanding

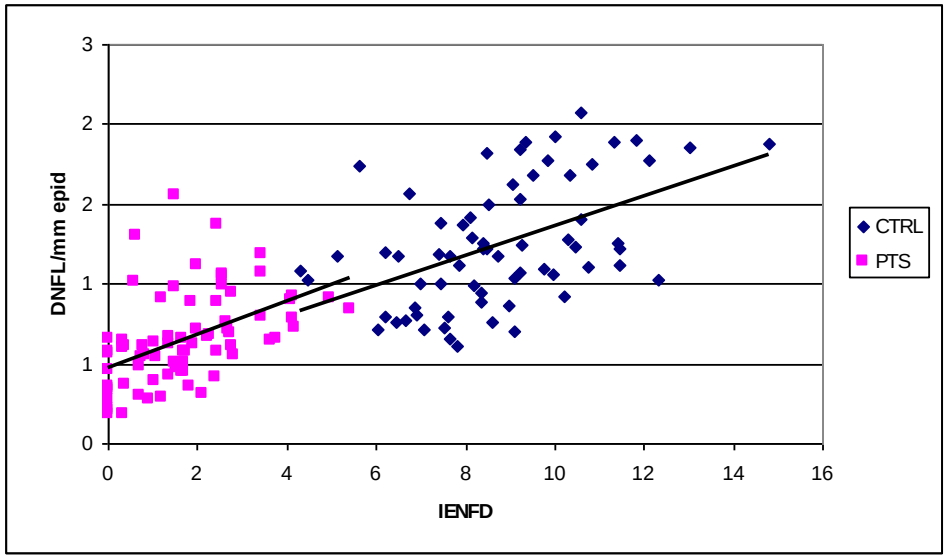
LA STATALE

Neurology® 2011;77:242-249

FONDAZIONE I.R.C.C.S. ISTITUTO NEUROLOGICO CARLO BESTA

IENF – dermal length correlation





- Spearman's Rank correlation coefficient [ρ] between IENFD and DNFL/mm2 or DNFL/mm was ρ=0.72 and ρ=0.73, respectively ($p<0.0001$).