Epidermal Nerve Fiber Density to Predict and Characterize Peripheral Neuropathy

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What is Epidermal Nerve Fiber Density Testing?

An objective method of documenting small fiber peripheral neuropathy by quantifying the terminal branches of peripheral nerves within the epidermis

That absence of subjectivity is a principle advantage of ENFD

Sometimes we exhibit a tendency toward an absolute reliance on subjective information

Why might this be inappropriate?
ENFD Testing

- Categorization of nerves
- What is small fiber peripheral neuropathy?
- The Theory Behind ENFD Testing
- Limitations of Current Testing Methods
- Test Efficiency & Reference Ranges
- Biopsy technique and handling

Categorization of Nerves

- Nerve diameter and/or conduction velocity
  - Medium myelinated (A-gamma): motor
  - Small myelinated (A-delta): temp, pain, autonomic
  - Small unmyelinated (C): temp, pain, autonomic
A-delta and C-nerve fibers are tiny nerve fibers that course into the epidermis toward the skin surface, eg, “epidermal nerves”

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A peripheral nerve abnormality which preferentially affects the A-delta and C fibers (the small fibers)
Usually manifesting in a length dependant pattern (stocking and glove)

Small Fiber Peripheral Neuropathy

- Symptoms
  - "Positive" sensory symptoms (more common)
    - Pain and paresthesias (burning, pricking, shooting)
  - "Negative" sensory symptoms
    - Numbness, tightness, coldness
  - "Autonomic" sensory symptoms

- Common causes
  - Diabetes, Types I & II
  - HIV
  - Amyloidosis / monoclonal gammopathy
  - ETOH abuse
  - Pharmacologic toxins (metronidazole)
  - Solvent exposure
  - IDIOPATHIC / familial (once thought to represent as much as half of all cases)
Small Fiber Peripheral Neuropathy (in summary)

- Inclusion criteria:
  - Preferentially affects A-delta and C fibers
  - Small myelinated and unmyelinated fibers within the skin
  - Pain, temperature, and autonomic function
  - Involves limbs with a distal to proximal pattern

- Exclusion criteria include:
  - Loss of vibratory sensation above ankles
  - Loss of proprioception in toes
  - Muscle wasting
  - Areflexia
  - Abnormal electromyography or nerve conduction

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Nerve degeneration in Small Fiber Neuropathy is a length dependant (distal to proximal) phenomenon.

In keeping with this fact, the most distal terminal nerve branches first begin to degenerate, and then are entirely lost.

At any point in time, these intra-epidermal nerve fibers can be counted and expressed in terms of their density (number / per unit area).

A decrease in the intra-epidermal nerve density parallels evolving small fiber neuropathy.
Small Fiber Neuropathy

In addition, the degenerative changes, which serve as a prelude to nerve fiber loss, may be visualized.

The utility of skin biopsy for prediction of progression in suspected small fiber neuropathy

Axonal swellings

- Degenerative phenomenon
  - Related to cytoskeleton damage
  - May be related to damage to transport systems
- Proceeds, and then parallels declining ENFD

The science behind the test
ENFD became possible with the introduction of Immunohistochemistry in the early 1990’s

What is Immunohistochemistry?

Immunohistochemistry

The Role of ENFD Testing
In Summary

- **Objectively** document the presence of small fiber neuropathy
  - Prior to therapy
  - Prior to palliative care
- **Objectively** predict the onset of small fiber neuropathy
  - ?Preemptive therapy?
- Provide an **Objective** baseline to monitor therapy
The Pharmacological Management of Diabetic Small Fiber Neuropathy Utilizing L-methyl folate as a Neurotrophic Agent

- 11 patients symptomatic DPN patients
- Baseline / 6 month skin biopsies (n=22)
- L-methyl folate B.I.D. for 6 months demonstrated 97% ↑ ENFD

Clinical Case Outcome I

Baseline

6 months

Clinical Case Outcome II

Baseline

6 months

An increase in epidermal nerve fiber density has been shown to be associated with diminished symptoms of anesthesia, paresthesia, or dysesthesia.

Note:
The technique used for counting may vary between labs.
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Traditional methods to gauge neuropathy

• Electromyography (EMG)
• Nerve conduction studies (NCS)
• Quantitative sensory testing
• Laser-evoked potentials
• Sympathetic skin response
• Quantitative sudomotor axon test
• Sural nerve biopsy

1) Subjective
2) Not useful for small fiber peripheral neuropathy
3) Requires a major academic center

Epidermal nerve fiber density and sural nerve morphometry in peripheral neuropathies

Abstract

Objectives: To study epidermal nerve fiber (ENFD) density in hands and feet in patients with various peripheral neuropathies, and normal volunteers, as assessed using morphometry. Methods: We included 12 patients with unselected peripheral neuropathies. ENFD density in hands and feet was assessed using high-magnification epifluorescent microscopy. Results: We observed no significant differences in ENFD density between the two sites in any group of patients but found significant reduction in ENFD density in the hand in patients with autoimmune polyneuropathy (APN, n = 6), in foot in APN (n = 6), and in foot in vasculitis (n = 2). Conclusion: ENFD density in the hand and foot is reduced in APN but not in vasculitis. This study provides new insights into the pathophysiology of peripheral neuropathy.

Figure 3. Qualitative relationships between intraneuronal and sural nerve small myelinated fiber densities.
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For a nerve density to have significance, a standardized curve must be created which is corrected for any potential variables

Variables:
Age
Gender
Anatomic location

Influence of Age on ENFD

Epidermal Nerve Fiber Density
Normative Reference Range and Diagnostic Efficiency
Justin C. McArthur, MBBS, MPH, P. Adalberto Starks, MS; Peter Hauer, BS; David B. Gombash, MD; John W. Griffin, MD
Gender also has no significance regarding ENFD

<table>
<thead>
<tr>
<th>Base group</th>
<th>Mean (95% CI)</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>Median (95% CI)</td>
<td>20.2 (13.8, 26.6)</td>
<td>20.1 (13.6, 26.6)</td>
<td>20.2 (13.7, 26.6)</td>
<td>20.1 (13.6, 26.6)</td>
<td>20.2 (13.7, 26.6)</td>
<td>20.1 (13.6, 26.6)</td>
<td>20.2 (13.7, 26.6)</td>
<td>20.1 (13.6, 26.6)</td>
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<tr>
<td>Range (95% CI)</td>
<td>18.9 (11.5, 26.2)</td>
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For this reason, a normative curve must be created for each anatomic location.

However, the "normal" density of intra-epidermal nerves varies dramatically depending on anatomic location.

The most studied location is 10cm proximal to the lateral malleolus followed by 10cm distal to the femoral greater trochanter.

Some clinicians will do both to demonstrate length dependence.
We recommend bilateral biopsies when possible

Small fiber neuropathy is not always a symmetrical process

Studies are ongoing to establish and publish normal density curves in the foot

Potential sites are:
1) level of the talar dome
2) dorsal midfoot (near base of fourth metatarsal)

However, clinicians should bare in mind:
1) these are subpar sites for statistical analysis
2) there is NO proven utility for both a lower leg and a dorsal foot biopsy

ENFD Test Efficiency
ENFD Test Efficiency

- Review all 9 published studies of symptomatic patients with normal NCS (Rosenberg et al.)
  - Sensitivity 69% (>90% in selected patients)
  - Specificity 97%

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How is the test performed?

- Contact lab to request delivery of ENFD kit
- Receive kit / place cool-pack in freezer for return shipping
- Mark biopsy site with sharpy
- Infiltrate lidocaine with epinephrine in a “V” pattern with apex proximal
- Perform 3mm punch biopsy
- Use appropriate fixation techniques
- Return to lab express shipping

Measure and Mark Biopsy Site
Infiltrate anesthesia in a “V” pattern (apex proximal)

Performing the Punch Biopsy
Fixation and Shipping
ENFD specimens CANNOT be placed in formalin!

Tissue must be placed in special fixative (Zamboni’s or PLP fixative)

PLP (short shelf life)

Zamboni’s (long shelf life)

Zamboni’s Fixative Rinse
- Place in Zamboni’s fixative overnight
- Pour off fixative, leaving specimen in original vial
- Fill the original vial with buffer rinse and then pour off
- Repeat
- Fill original vial (containing biopsy) with cryoprotectant (stable for 2 weeks)
- Mail back
Summary

- Small Fiber Neuropathy is a distinct form of peripheral neuropathy
- Traditional testing methods are of limited value when assessing SFN
- ENFD can allow objective documentation of SFN early in its evolution
- ENFD can be used to monitor therapy
- ENFD can be useful to predict SFN
- Requires a 3mm punch fixed in PLP or Zamboni’s fixative