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The reliability of skin biopsy with measurement of intraepidermal nerve fiber density☆

A. Gordon Smith[a,b,*], James R. Howard[a], Randi Kroll[a], Pam Ramachandran[b], Peter Hauer[c], J. Robinson Singleton[a], Justin McArthur[c,d]

[a]*University of Utah, Department of Neurology, United States*
[b]*University of Utah, Department of Pathology, United States*
[c]*Johns Hopkins University, Department of Neurology, United States*
[d]*Johns Hopkins University, Department of Epidemiology, United States*

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Abstract

Intraepidermal nerve fiber density (IENFD) is a sensitive measure of small fiber injury, and holds promise as a clinical trial endpoint measure. A total of 48 punch biopsies were obtained from 22 patients. Tissue was sectioned and stained with PGP9.5. The relative intertrial variability (RIV) of IENFD measurements for each section and punch made by two different observers was determined (interobserver variability). Intraobserver variability (same observer measuring twice) was determined for 50% of the sections and punches. Sections from 12 punch biopsies were also stained at a second laboratory. The effect of the number of sections counted and processing site on reproducibility was investigated. A total of 223 sections were analyzed. The mean IENFD was 6.7 fibers/mm. Mean (±standard deviation) interobserver variability was 9.6%±9.4 for each biopsy site and 10.2%±11.9 for individual sections. Mean intraobserver variability was 9.6%±8.9 for biopsies, and 8.8%±9.0 for sections. There was no significant difference in IENFD for tissue stained at different laboratories. Intraclass correlation coefficients were greater than 0.98 for each comparison. There was no relationship between absolute IENFD and reproducibility. Reproducibility was highest when four sections were counted. IENFD measurement is highly reproducible. At least four sections should be analyzed. Reliability does not vary with severity of disease. These findings suggest IENFD may be a useful endpoint measure in future neuropathy treatment trials.

Keywords: Skin biopsy; Reproducibility; Neuropathy; Small fiber

1. Introduction

Peripheral neuropathy with prominent small nerve injury is a common clinical problem, occurring in diabetes, prediabetes, drug and toxin exposures, and idiopathic neuropathies [1–3]. Traditional measures of peripheral neuropathy severity, such as nerve conduction studies, primarily reflect large fiber function, limiting their utility in the evaluation of small fiber neuropathy. In contrast, skin punch biopsy with measurement of intraepidermal nerve fiber density (IENFD) directly measures small fiber integrity and is more sensitive than sural nerve morphometry [2,4]. Skin biopsy is now an established means of diagnosing small fiber neuropathy.

IENFD also provides a continuously variable measure of small fiber nerve loss that can be followed through time to evaluate progression of disease and treatment efficacy. As such, it holds promise as an endpoint measure for clinical trials in which small fiber loss is prominent [5]. Before IENFD can be used for this purpose, several issues must be resolved. The sensitivity of IENFD as a measure of

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* Corresponding author. University of Utah, Department of Neurology, 30 North 1900 East, Room 3R152 SOM, Salt Lake City, UT 84132, United States. Tel.: +1 801 581 6873; fax: +1 801 585 2054.

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neuropathy progression must be demonstrated and its correlation with other validated neuropathy endpoint measures confirmed. A high degree of reproducibility for IENFD measures must also be confirmed. This study addresses the intraobserver and interobserver variability of IENFD measurements, the variability of IENFD measurement for tissue processed at two different laboratories, and the effect of absolute IENFD and the number of sections analyzed on reliability.

2. Materials and methods

A total of 18 subjects participating in a study of diabetic and prediabetic neuropathy at the University of Utah had a total of thirty six 6-mm punch biopsies performed. An additional four patients at Johns Hopkins University had a total of 12 punch biopsies performed; two patients had neuropathic pain but normal epidermal fiber density, one patient had idiopathic small fiber neuropathy, and one had a small fiber neuropathy associated with a monoclonal gammopathy of undetermined significance. Tissue was sectioned into 50-μm-thick sections. Sections were stained with PGP 9.5 using previously described methods [6]. The sections to be analyzed were chosen randomly using a random number generating table. Sections from the biopsies performed at Johns Hopkins were stained there and the slides shipped to the University of Utah for analysis. Several free floating sections from the same biopsies were sent in cryoprotectant solution to the University of Utah where they were both stained and analyzed. The number of intra-epidermal nerve fibers was counted following established criteria as summarized below. For each section on each slide, the number of fibers crossing the dermal–epidermal junction was counted. If the fiber subsequently branched within the epidermis, it was only counted as one fiber. If a dermal fiber branched immediately beneath the dermal epidermal junction, and both fibers entered the epidermis, both fibers were counted. Distinct individual fibers observed in the epidermis without crossing the dermal epidermal junction were also counted. Epidermal length was measured using digital image analysis software (Image Pro Plus) in order to calculate the fiber density. For most biopsies, four sections were counted to arrive at a mean IENFD for each punch biopsy site. Because of the difficulty in shipping free floating 50-μm-thick sections, only two or three sections from several of the punch biopsies performed at Johns Hopkins University were available for staining at the University of Utah.

Each section was analyzed by two different observers to determine the interobserver variability. Half were analyzed twice by the same observer in a blinded fashion in order to determine the intraobserver variability. Variability was assessed by calculating the relative inter-trial variability (RIV). RIV is calculated by subtracting the values for the two measurements and dividing this number by the mean of the two measurements, and multiplying by 100 in order to express the result as a percentage. RIV values of less than 10% indicate a high degree of reproducibility. Variability was correlated with absolute IENFD values. Analysis of variance and intraclass correlation coefficients were also used to assess for significant differences between measurement groups [7].

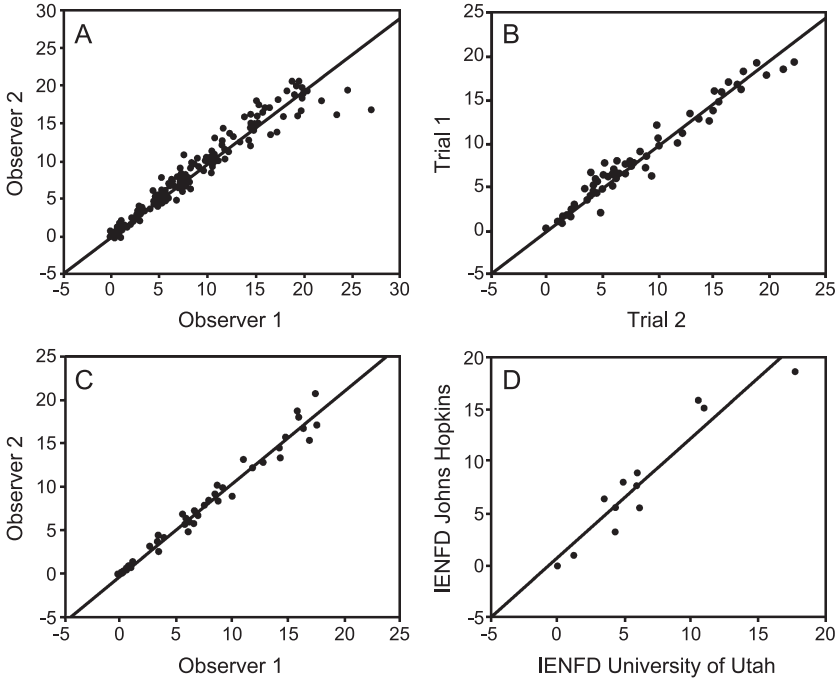

Fig. 1. IENFD reproducibility for (A) inter-observer counts of all sections (ICC 0.98), (B) intra-observer counts of all sections (ICC 0.98), (C) inter-observer counts for each punch biopsy (ICC 0.98), and (D) analysis (by one individual) of tissue from the same biopsy processed at different laboratories (ICC 0.98).