Administrative Information

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If there is a prior, current, or planned submission to other regulatory agencies, list the agencies and dates as appropriate.

None

Context of Use

Proposed Context of Use (COU) (limited to 500 characters)

Diagnostic biomarker to be used with clinical indicators to identify small-fiber polyneuropathy END is a diagnostic biomarker to be used with clinical indicators to identify small-fiber polyneuropathy. END measurement from punch skin biopsies taken 10 cm above the outer ankle, less in children, is the gold-standard objective test for diagnosing SFPN for clinical use and enrollment in clinical trials.

Drug Development Need

Describe the drug development need that the biomarker is intended to address, including (if applicable) the proposed benefit over currently used biomarkers for similar COUs (limited to 1,500 characters).

Small-fiber polyneuropathy (SFPN), aka small fiber neuropathy is the most common widespread peripheral nerve disorder.[1] Most polyneuropathies, including from diabetes, HIV and cancer chemotherapy, begin by and often remain mostly affecting the small fibers. SFPN's cardinal

symptom is widespread chronic neuropathic pain, but it also causes other sensory symptoms including neuropathic itch and numbness and tingling. As the small-fibers include the sympathetic axons that innervate and regulate the body systems, SFPN patients often have other symptoms such as lightheadedness and fainting, rapid heart rate, and gastrointestinal dysmotility symptoms. Most have multiple subjective symptoms, so diagnosis is difficult and often delayed.

Because of this, skin biopsy is considered the gold-standard for clinical diagnosis, and it is increasingly required for trials as well. Use is diagnostic—to include patients and to define study groups (SFPN vs. unaffected). Skin biopsies will be recommended as mandatory for inclusion into SFPN clinical trials by a 2018 ACTTION Committee that the FDA (and Dr. Oaklander) participated in. We see 26 trials currently listed for small-fiber neuropathy on ClinTrials.gov, with many specifying skin biopsy proven diagnosis, and we request qualification to improve analytic performance. Currently same-site biopsies from the same patient and even the same biopsy slides from the same patient sent for interpretation to different accredited labs can yield divergent diagnoses ("normal" in one, "SFPN" in another) because there are no standards for fixation, section thickness, denominator used to convert epidermal neurite measurements to densities, and normative datasets used for comparison.

We (Drs. Oaklander and Klein) are academic SFPN researchers submitting a U01 application (PAR-18-534) to NINDS to prepare to conduct clinical trials for SFPN, and Biomarker Qualification is suggested to accompany these applications. Given that 26 trials for SFPN are currently listed on ClinicalTrials.gov, qualification could improve development of multiple drugs and influence decisions about drug repurposing.

Biomarker Information

Biomarker name and description. If composite, please list the biomarker components.

Name:
Epidermal neurite density (END)

Description:

END measurements quantitate degeneration of the farthest ends of small-fiber axons. 2 or 3 mm full-thickness punch skin biopsies are removed from 10 cm above the lateral malleolus after subcutaneous injection of 2% lidocaine with epinephrine. Punches are fixed overnight in 2% Zamboni's fixative and cryo-protected. Each is serially cryo-sectioned vertically (usually to 50 micron thickness), and selected sections are immunolabeled (antiPGP9.5 diluted 1:1200) using stereological methods (random start, constant inter-section interval). A skilled morphometrist then counts individual neurites (aka nerve fibers, axon twigs) that ascend into the epidermis by established rules.[2]

Only one metric is analyzed, the neurite density per skin surface area, often per mm^2 skin surface area. The diagnostic decision (normal/abnormal) is made by comparing the measured END with predicted norms from a within-lab dataset. END ≤ 5th centile of the norm are universally considered diagnostic but there are no recommendations on whether labs can just used published norms, and how big and representative the normative database should be, and what statistical analysis should be applied. A 2^{nd} biopsy may be removed, often from the proximal thigh 20 cm below the iliac crest, to add sensitivity, particularly for proximal (non-length dependent) neuropathies,[3] but no other biopsy sites are currently endorsed due to insufficient norms and evaluation.

Older names used in the literature to refer to the END biomarker include epidermal nerve fiber density (ENFD), intraepidermal nerve fiber density (IENFD), intraepidermal neurite density

(IND). These interchangeable terms refer to the same biomarker discussed here using simpler terminology.

Type(s) of Biomarker

☐ Molecular
☑ Histologic
☐ Radiologic / Imaging
☐ Physiologic characteristic
☐ Other (please describe)

Biomarker Information

For molecular biomarkers, please provide a unique ID.

Scheme: Please select one: UniProt (http://uniprot.org/)
HUGO Gene Nomenclature Committee (http://genenames.org)
Protein Data Bank (http://rcsb.org/pdb/home/home.do)
Enzyme Commission (http://enzyme.expasy.org)

ID: NA

Matrix (e.g., blood) or modality (e.g., MRI): Full thickness skin punch

Primary biomarker category (see BEST Glossary):

Please select one:	*Susceptibility/Risk*
X	*Diagnosis*
	Monitoring
	Prognostic
	Predictive
	Pharmacodynamic/Response

Describe the mechanistic rationale or biologic plausibility to support the biomarker and its associated COU (limited to 1,500 characters).

Small-fiber polyneuropathy (SFPN) is a type of general peripheral neuropathy that exclusively or predominantly affects the small-diameter unmyelinated and thinly myelinated axons that transmit sensations and mediate autonomic and trophic functions. SNPN has many medical causes include diabetes, autoimmune conditions, toxins, and genetic mutations.[4] Anything that impairs the ability of cell bodies to maintain their long thin axons will cause dysfunction, then degeneration, of small-fiber axons.

Objective diagnostic testing for SFPN requires neuropathological study to demonstrate the small-fiber axonal losses that define it. The biological basis of the skin biopsy test is that the axon bundles coursing through the dermis spread out from each other in the epidermis so they can be individually counted there. Plus, virtually all epidermal neurites are small-fibers, which conveys specificity. The PGP9.5 immunohistochemical reaction product makes these tiny EN visible by light microscopy. Neurodiagnostic skin biopsy has been validated for clinical diagnosis, evaluation of disease progression, and in clinical trials, and found more sensitive than sural nerve biopsy.[5]

Most skin biopsies come from standard locations, the lower leg 10 cm above the lateral malleolus, or the proximal thigh 20 cm below the iliac crest. These sites allow detection of both the ~80% of SFPN that start distally (“length-dependent”) as well as others that are patchy or

non-length dependent (known as ganglionopathies).[3] These are associated with autoimmune conditions that attack the neuronal cell body itself, for instance in Sjögren's syndrome.[6]

If biomarker is an index/scoring system, please provide information on how the index is derived (e.g., algorithm), the biologic rationale for inclusion of each of the components, the rationale for any differential weighting of the elements, and the meaning/interpretation of the index/score (limited to 1,500 characters).

The steps between measuring EN and diagnostic interpretation are ill-defined and vary between labs. There are standards for counting ENF,[2] but not for analysis, so different labs make different interpretations from the same slides.

Some labs just use visual inspection to define normal vs. SFPN, which only captures the most severe cases. Others measure END then compare to published thresholds. However, among the 105 biopsies from patients under 40 we signed out as diagnostic for SFPN in 2012-2013, the most common published "cutoff" (76 ENF/mm^2) would have generated 75% fewer SFPN diagnoses. The difference is not only that our lab is the only one in the world with pediatric norms, but also that we used our data from > 400 normal controls (age 8-92y) to generate a predictive multivariate regression that incorporates the marked age dependence of normal skin innervation, as well as race and sex. We calculate a predicted normal distribution individually for each patient biopsied and apply the 5^{th} centile cutoff using these individualized predictions.[7]

Different labs' normative data sets vary in quality and applicability to other labs, and there are no standards for normative data sets (minimum size, data to be collected, representativeness, inclusion/exclusion criteria and screening). Our lab accepts only demographically characterized subjects screened by history, symptoms, exam, and blood tests (including 2 hour glucose tolerance tests) to exclude undiagnosed neuropathy and common causes such as diabetes and prediabetes. Insofar as we know, we provide perhaps the most accurate pathological diagnosis of SFPN in the world. We seek FDA guidance to help improve the diagnostic quality yet further for our lab and all others.

Biomarker Measurement Information

Provide a general description of what aspect of the biomarker is being measured and by what methodology (e.g., radiologic findings such as lesion number, specific measure of organ size, serum level of an analyte, change in the biomarker level relative to a reference such as baseline) (limited to 1,500 characters).

The metric is the density (number/volume) of individual axon endings visible by light microscopy that immunohistochemically label with Protein Gene Product 9.5 (PGP9.5). This reacts with ubiquitin hydrolase, a lysosomal enzyme present in the cytosol of all human neurons.[8] Being neuron restricted, it is the label of choice for visualizing neuronal processes coursing through other body tissues. The antibody and secondary also expand the width of tiny axons, making them visible by light microscopy whereas electron microscopy was needed before.

The standard location for biopsy (10 cm proximal to the lateral malleolus) has been specified, and punch biopsy size standardized to 3 mm. We use 2 mm punches for children. One of several fixatives are used (usually PLP or Zamboni's); there are no standards for time or temperature. Punches are cryprotected and serially sectioned to varying thicknesses, with most labs, including ours, using 50 µm sections. 3 mm punches yield 55-60 sections.

A morphometrist then counts the neurites penetrating the hard-to-see dermal-epidermal junction in sections selected for analysis. Although rules define what constitutes a countable epidermal neurite to exclude fragments,[2] there are no standards for selecting which sections to immunolabel, and how many should be labeled. The resulting measured number of EN is then

normalized by an undefined volume or area (our lab uses mm^2 skin surface area) but most labs simply use linear density, which doesn't account for section thickness.

The resultant END is then compared to the distribution of normal densities predicted for a given age, gender, race, and ethnicity, and densities less than the 5^{th} centile of predictions from normative datasets are considered diagnostic for SFPN.

Is the biomarker test/assay currently available for public use? No

Indicate whether the biomarker test/assay is one or more of the following:

Laboratory Developed Test (LDT) No
Research Use Only (RUO) No
FDA Cleared/Approved. Provide 510(k)/PMA Number: No

If the biomarker is qualified, will the test/assay be performed in a Clinical Laboratory Improvement Amendments (CLIA)–certified laboratory?

X Yes ○ No

Is the biomarker test currently under review by the Center for Devices and Radiological Health or the Center for Biologics Evaluation and Research?

○ Yes
X No
○ Don't Know

Is there a standard operating procedure (SOP) for sample collection and storage?

X Yes ○ No

Is there a laboratory SOP for the test/assay methodology?

X Yes ○ No

The Mass General Nerve Unit skin biopsy lab is CLIA and hospital certified and has established SOP for all aspects of this biomarker test, but there are no consensus standards or recommendations for SOP or laboratory quality controls so different labs follow different procedures and policies.

Biomarker Measurement Information

Describe the extent of analytical validation that has been performed (e.g., sensitivity, specificity, accuracy, and/or precision of the assay or method) (limited to 1,500 characters)

Methods were developed in academic labs and have had limited validation. The central problem is that skin biopsy, the gold-standard test, has no comparator. Thus specificity is high, but diagnostic accuracy uncertain. Abnormal biopsies have high positive predictive value, but we reported diagnostic END in 41% of patients with fibromyalgia.[9] so skin-biopsy defined SFPN may be so prevalent (~1%) that the positive predictive value needs scrutiny. Negative predictive value is less, given that END ≤ 5^{th} centile of predicted are required for interpretation as "abnormal/diagnostic". Taking additional biopsies at the same or different times and/or sites can improve sensitivity,[3] but there are no guidelines for use.

Diagnostic algorithms used for interpretation are neither standardized nor validated. One study compared 3 statistical methods in 45 SFPN patients and 134 healthy controls: using Z-

scores from multiple regression for age- and gender-specific cut-offs, using 5th percentile cutoffs; and receiver operating characteristic (ROC) analysis cut-offs.[10] Z-scores and 5th percentile had higher specificity (98%, 95%) but lower sensitivity (31% and 35%) compared to ROC analysis (64% specificity, 78% sensitivity). Given the uncertainty, our lab and others label END between the 5th and 15th centile as "borderline" for SFPN.

Another unmet need is for pediatric use, which our lab may be the only one addressing. We have norms down to age 8 so far, with other norms from above age 21 only.[11] We use a separate multivariate algorithm for everyone below age 23, due to the 4x higher END in youth than senescence.[12] but there are no guidelines for pediatric use.

Does data currently exist to support the proposed cut point(s), if imaging results are not reported as a continuous variable? Yes

Provide the name and version of the software package to be used for image acquisition and analysis (limited to 500 characters).

Not applicable

Supporting Information

Please summarize existing preclinical or clinical data to support the biomarker in its COU (e.g., summaries of literature findings, previously conducted studies) (limited to 2,000 characters).

Skin biopsy diagnosis of SFPN was developed in the 1990's, mostly in the Griffin lab at Hopkins where Dr. Oaklander trained, as a less invasive alternative to surgical biopsy of the sural nerve with ultrastructural visualization and quantification of the 1 µm wide small-fiber axons within.[2,13,14] The lower-leg site corresponds to sural-nerve innervated skin, and biopsies there were established as more sensitive than nerve biopsies as they sampled the terminal axon that degenerates first.[5] ENF counting rules were defined, and how to define the reference space between the dermal-epidermal junction and the top of the skin surface.[15] The Hopkins lab published the first normative data series, but they studied only 98 normals, included fragments, and did not identify the big decline with aging or specify age-adjusted norms.[16] Some labs still apply these rudimentary Hopkins norms even today.

Skin biopsy grew to become the defining biomarker of SFPN, even for identification of subclinical, asymptomatic, or presymptomatic cases.[17,18] In the 2000's diagnostic use was assessed in various conditions,[19-27] and a few performance studies were conducted.[28,29] In 2009-10 the American and European Academies of Neurology endorsed use and issued guidelines for clinical use.[30,31] We know of 6 published normative datasets,[16,24,32-34] culminating with 2010 publication of pooled normative data from 550 normals studied in 8 European, U.S., and Asian labs.[11] Reviews appeared in leading neurology journals.[29,35]

Single-case and small-case series suggest that END can improve with time or treatment,[36-38] but this needs systematic study as we propose in our NIH application. Since 2010, studies have begun to compare skin biopsy to other biomarkers and outcomes, [39,40] and to more systematically evaluate performance.[41] and to use skin biopsies for other applications.[42] Clinical trial improvement organizations are beginning to address the use of skin biopsies and to recommend more systematic study,[43] hence our request for Qualification.

Secondary applications not to be considered here include responsiveness to potentially disease-modifying drugs under development (use as a pharmacodynamic biomarker).

Please summarize any planned studies to support the biomarker and COU. How will these studies address any current knowledge gaps? (Limited to 2,000 characters.)

This request comes in the context of applying for NIH research grant RFP NINDS_PAR-16-020 “Clinical Trial Readiness for Rare Neurological and Neuromuscular Diseases (U01)”. The Research Plan includes an Aim to improve the skin biopsy test for SFPN for clinical trial use.

The absolute sensitivity, specificity, positive and negative predictive value of skin biopsy for SFPN diagnosis cannot be determined, since pathology it is itself current gold-standard for diagnosis. However we continue to evaluate the sensitivity and specificity with regards to secondary biomarkers such as quantitative autonomic testing to evaluate convergent validity, and to compare the results of multiple biopsies from the same person at the same time (cross sectional reproducibility) and at different times (longitudinal variability). These will help establish the best context of use. We also continue to acquire normative data from young children, which are essential for accurate diagnosis in children. We also continue to refine the multivariate regression we use to interpret skin biopsy, by adding anthropometric data identified by population based epidemiological study (eg height, weight, smoking status) that could improve diagnostic performance.[44]

Previous Regulatory Interactions

None

Letter of Support (LOS) issued for this biomarker on date:

Discussed in a Critical Path Innovation Meeting (CPIM) on date:

Previous FDA Qualification given to this biomarker with DDT Tracking Record Number:

Attachments

Please provide a list of publications relevant to this biomarker development proposal.

See reference list below.

Optional* – If this biomarker development effort is part of a longer-term goal, please summarize your long-term objectives.*

This biomarker development effort is not tied to any drug development efforts or current clinical trials. It arises from almost 20 years experience at Mass General’s clinical and research neurological skin biopsy lab during which we have tried to improve the quality of this diagnostic test. This request comes in the context of applying for NIH research grant RFP NINDS PAR-18-534 “Clinical Trial Readiness for Rare Neurological and Neuromuscular Diseases (U01)”. This requires submission of “Guidance documents provided by the FDA regarding qualification of the proposed biomarker(s) or COA measure(s)”. The application deadline is August 17, 2018.

Optional* – If you have other supporting information you would like to provide, please submit as attachment(s). *Optional information will not be posted publicly.

Please refer to the Biomarker Qualification Contacts and Submitting Procedures for the mailing address and other important submission-related instructions. If you have any questions about submission procedures, please contact CDERBiomarkerQualificationProgram@fda.hhs.gov.

References

1. Cazzato D, Lauria G. Small fibre neuropathy. Curr Opin Neurol 2017;30:490-9.
2. McCarthy BG, Hsieh ST, Stocks A, et al. Cutaneous innervation in sensory neuropathies: evaluation by skin biopsy. Neurology 1995;45:1848-55.
3. Provitera V, Gibbons CH, Wendelschafer-Crabb G, et al. The role of skin biopsy in differentiating small-fiber neuropathy from ganglionopathy. Eur J Neurol 2018;25:848-53.
4. Lang M, Treister R, Oaklander AL. Diagnostic value of blood tests for occult causes of initially idiopathic small-fiber polyneuropathy. J Neurol 2016;263:2515-27.
5. Herrmann DN, Griffin JW, Hauer P, Cornblath DR, McArthur JC. Epidermal nerve fiber density and sural nerve morphometry in peripheral neuropathies. Neurology 1999;53:1634-40.
6. Lefaucheur JP, Sène D, Oaklander AL. Primary Sjögren's syndrome. New England Journal of Medicine 2018;in press.
7. Klein MM, Downs HM, Oaklander AL. Diagnostic performance of a multivariate model of normal epidermal nerve fiber (enf) density for skin-biopsy diagnosis of small-fiber polyneuropathy (sfpn). Journal of the Neurological Sciences 2015;357:e337-e8.
8. Wilkinson KD, Lee KM, Deshpande S, Duerksen-Hughes P, Boss JM, Pohl J. The neuron-specific protein PGP 9.5 is a ubiquitin carboxyl-terminal hydrolase. Science 1989;246:670-3.
9. Oaklander AL, Herzog ZD, Downs HM, Klein MM. Objective evidence that small-fiber polyneuropathy underlies some illnesses currently labeled as fibromyalgia. Pain 2013;154:2310-6.
10. Nebuchennykh M, Loseth S, Lindal S, Mellgren SI. The value of skin biopsy with recording of intraepidermal nerve fiber density and quantitative sensory testing in the assessment of small fiber involvement in patients with different causes of polyneuropathy. J Neurol 2009;256:1067-75.
11. Lauria G, Bakkers M, Schmitz C, et al. Intraepidermal nerve fiber density at the distal leg: a worldwide normative reference study. J Periph Nerv Syst 2010;15:202-7.
12. Klein MM, Downs H, O'Neil K, Oaklander AL. Skin biopsy of normal children demonstrates inverse correlation between age and epidermal nerve fiber density, meaning age-specific norms are needed. Annals of Neurology 2014;76:S69.
13. Kennedy WR, Wendelschafer-Crabb G, Johnson T. Quantitation of epidermal nerves in diabetic neuropathy. Neurology 1996;47:1042-8.
14. Holland NR, Stocks A, Hauer P, Cornblath DR, Griffin JW, McArthur JC. Intraepidermal nerve fiber density in patients with painful sensory neuropathy. Neurology 1997;48:708-11.
15. Stocks EA, McArthur JC, Griffin JW, Mouton PR. An unbiased method for estimation of total epidermal nerve fibre length. J Neurocytol 1996;25:637-44.
16. McArthur JC, Stocks EA, Hauer P, Cornblath DR, Griffin JW. Epidermal nerve fiber density: normative reference range and diagnostic efficiency. Arch Neurol 1998;55:1513-20.
17. Magri F, Buonocore M, Oliviero A, et al. Intraepidermal nerve fiber density reduction as a marker of preclinical asymptomatic small-fiber sensory neuropathy in hypothyroid patients. European Journal of Endocrinology 2010;163:279-84.
18. Richardson PG, Chanan-Khan A, Schlossman RL, et al. Phase II trial of single agent bortezomib (VELCADE®) in patients with previously untreated multiple myeloma (MM). Blood 2004;104:336-.
19. Polydefkis M, Hauer P, Griffin JW, McArthur JC. Skin biopsy as a tool to assess distal small fiber innervation in diabetic neuropathy. Diabetes TechnolTher 2001;3:23-8.
20. Polydefkis M, Hauer P, Sheth S, Sirdofsky M, Griffin JW, McArthur JC. The time course of epidermal nerve fibre regeneration: studies in normal controls and in people with diabetes, with and without neuropathy. Brain 2004;127:1606-15.
21. Polydefkis M, Sirdofsky M, Hauer P, Petty BG, Murinson B, McArthur JC. Factors influencing nerve regeneration in a trial of timcodar dimesylate. Neurology 2006;66:259-61.

22. Schiffmann R, Hauer P, Freeman B, et al. Enzyme replacement therapy and intraepidermal innervation density in Fabry disease. Muscle & Nerve 2006;34:53-6.
23. Hahn K, Triolo A, Hauer P, McArthur JC, Polydefkis M. Impaired reinnervation in HIV infection following experimental denervation. Neurology 2007;68:1251-6.
24. Bakkers M, Merkies IS, Lauria G, et al. Intraepidermal nerve fiber density and its application in sarcoidosis. Neurology 2009;73:1142-8.
25. Smith AG, Howard JR, Kroll R, et al. The reliability of skin biopsy with measurement of intraepidermal nerve fiber density. Journal of the Neurological Sciences 2005;228:65-9.
26. Scott LJ, Griffin JW, Luciano C, et al. Quantitative analysis of epidermal innervation in Fabry disease. Neurology 1999;52:1249-54.
27. Gøransson, LG, Herigstad A, et al. Peripheral neuropathy in primary Sjogren syndrome: a population-based study. Arch Neurol 2006;63:1612-5.
28. Lauria G, Morbin M, Lombardi R, et al. Axonal swellings predict the degeneration of epidermal nerve fibers in painful neuropathies. Neurology 2003;61:631-6.
29. Lauria G, Devigili G. Skin biopsy as a diagnostic tool in peripheral neuropathy. NatClinPractNeurol 2007;3:546-57.
30. Lauria G, Hsieh ST, Johansson O, et al. 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. Eur J Neurol 2010;17:903-12, e44-9.
31. England JD, Gronseth GS, Franklin G, et al. 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;72:177-84.
32. Pan CL, Lin YH, Lin WM, Tai TY, Hsieh ST. Degeneration of nociceptive nerve terminals in human peripheral neuropathy. Neuroreport 2001;12:787-92.
33. Umapathi T, Tan WL, Tan NC, Chan YH. Determinants of epidermal nerve fiber density in normal individuals. Muscle & Nerve 2006;33:742-6.
34. Gøransson, LG, Mellgren SI, Lindal S, Omdal R. The effect of age and gender on epidermal nerve fiber density. Neurology 2004;62:774-7.
35. Sommer C, Lauria G. Skin biopsy in the management of peripheral neuropathy. The Lancet Neurology 2007;6:632-42.
36. Nodera H, Barbano RL, Henderson D, Herrmann DN. Epidermal reinnervation concomitant with symptomatic improvement in a sensory neuropathy. Muscle Nerve 2003;27:507-9.
37. Oaklander AL, Klein MM. Evidence of small-fiber polyneuropathy in unexplained, juvenile-onset, widespread pain syndromes. Pediatrics 2013;131:e1091-e100.
38. Gibbons CH, Griffin JW, Polydefkis M, et al. The utility of skin biopsy for prediction of progression in suspected small fiber neuropathy. Neurology 2006;66:256-8.
39. Truini A, Biasiotta A, Di Stefano G, et al. Does the epidermal nerve fibre density measured by skin biopsy in patients with peripheral neuropathies correlate with neuropathic pain? Pain 2014;155:828-32.
40. Abuzinadah AR, Kluding P, Wright D, et al. Less is more in diabetic neuropathy diagnosis: Comparison of quantitative sudomotor axon reflex and skin biopsy. Journal of clinical neuromuscular disease 2017;19:5-11.
41. Lauria G, Dacci P, Lombardi R, et al. Side and time variability of intraepidermal nerve fiber density. Neurology 2015;84:2368-71.
42. Brulin P, Godfraind C, Leteurtre E, Ruchoux MM. Morphometric analysis of ultrastructural vascular changes in CADASIL: analysis of 50 skin biopsy specimens and pathogenic implications. Acta Neuropathol (Berl) 2002;104:241-8.

43. Smith SM, Dworkin RH, Turk DC, et al. The potential role of sensory testing, skin biopsy, and functional brain imaging as biomarkers in chronic pain clinical trials: IMMPACT considerations. J Pain 2017;18:757-77.
44. Zirpoli G, Rice MS, Huang T, Tworoger SS, Oaklander AL. Anthropometric, lifestyle, and dietary risk factors for peripheral neuropathy in the Nurses' Health Study II. Journal of the Peripheral Nerve Society 2018;in press.