Journal of
CLINICAL
NEUROMUSCULAR
DISEASE

Volume 20, Number 1
September 2018

OPEN

Low Sensitivity of Skin Biopsy in Diagnosing Small Fiber Neuropathy in Chinese Americans

Peter Jin, MD, Ling Cheng, MD, Ming Chen, BS, and Lan Zhou, MD, PhD

Abstract

Objectives:
To compare skin biopsy sensitivity for diagnosing small fiber sensory neuropathy in Chinese American and non-Chinese American patients.

Methods:
We screened our skin biopsy database and performed chart review to identify Chinese and non-Chinese American patients with a high clinical suspicion for a distal small fiber sensory neuropathy, and compared the skin biopsy sensitivity.

Results:
Twenty-three Chinese American and 32 non-Chinese American patients with the presence of distal small fiber sensory symptoms and signs were studied. Intraepidermal nerve fiber density (IENFD) (fibers/mm) at the distal leg was higher (7.1 ± 3.9), and the diagnostic sensitivity using the worldwide normative reference values of IENFD at the distal leg was lower (26.1%) in the Chinese American group than in the non-Chinese American group (5.1 ± 3.0, $P < 0.05$; 62.5%, $P < 0.05$).

Conclusions:
There may be ethnic differences in IENFD at the distal leg, and a different set of IENFD normative values may be developed for ethnic Chinese to improve the skin biopsy sensitivity.

Key Words: small fiber sensory neuropathy, skin biopsy, intraepidermal nerve fiber density, normative values, ethnic differences

(*J Clin Neuromusc Dis* 2018;20:1–6)

INTRODUCTION

Small fiber sensory neuropathy (SFSN) is a common type of peripheral neuropathy that can be associated with diabetes mellitus, connective tissue diseases, paraproteinemia, B12 deficiency, thyroid dysfunction, HIV infection, hereditary causes, and others. It can also be idiopathic. Patients with SFSN can present with pain, burning, tingling, and numbness, which is often in a length-dependent manner more affecting the feet. Examination can be unremarkable but often shows allodynia, hyperalgesia, or reduced pinprick and temperature sensation in the symptomatic areas. Motor strength, proprioception, and deep tendon reflexes are usually preserved. Routine nerve conduction study and electromyography (NCS/EMG) is typically unrevealing.[1–5] The current standard diagnostic test for SFSN is skin biopsy with intraepidermal nerve fiber density (IENFD) evaluation to compare patients' IENFD at the distal leg with the worldwide normative reference values (fifth quantile IENFD cut-offs) established in 2010.[6] These normative values are adjusted for age and sex. If the IENFD is reduced, a skin biopsy diagnosis of SFSN can be made. The sensitivity of using these normative values for diagnosing SFSN in general population or in different ethnic groups, however, has not been well studied. In our clinical practice, we perceived a low sensitivity in Chinese American patients who otherwise had a high clinical suspicion for a distal SFSN based on the presence of both small fiber sensory symptoms and signs with a distal-to-proximal gradient. We thus hypothesized that the current established worldwide normative reference values of IENFD at the distal leg might not be as sensitive for diagnosing SFSN in Chinese American patients, and that these patients in general might have higher baseline IENFD compared with non-Chinese American

From the Department of Neurology, Icahn School of Medicine at Mount Sinai, New York, NY. Mr. M. Chen and Dr. L. Zhou is now with the Departments of Neurology & Neurotherapeutics and Pathology, University of Texas Southwestern Medical Center, Dallas, TX.
The authors report no conflicts of interest.

Reprints: Lan Zhou, MD, PhD, Departments of Neurology & Neurotherapeutics and Pathology, UT Southwestern Cutaneous Nerve Laboratory, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390-8813 (email: lan.zhou@UTSouthwestern.edu).

patients. In this study, we compared the diagnostic sensitivity of skin biopsy using the worldwide normative reference values in Chinese American and non-Chinese American patients with a high clinical suspicion for a pure distal SFSN to address whether the sensitivity is reduced in the Chinese American patients.

MATERIALS AND METHODS

We screened our Mount Sinai Cutaneous Nerve Laboratory database for subjects who underwent skin biopsies with IENFD evaluation in our neuromuscular center between 2013 and 2017. These subjects underwent 3-mm punch skin biopsies at the distal leg (10 cm above the lateral malleolus), distal thigh (10 cm above the lateral knee), and proximal thigh (10 cm below the greater trochanter). IENFD analysis was performed by our Mount Sinai Cutaneous Nerve Laboratory using the bright-field immunohistochemistry protocol initially developed by the Johns Hopkins' Cutaneous Nerve Laboratory.[7–9] We followed the same published guidelines[10] as the other centers who studied and developed the worldwide normative reference values.[6] Briefly, 3-mm punch skin biopsy specimens were fixed in Zamboni's solution, cut into 50 μm sections, and immunostained with the PGP 9.5 antibody. The number of epidermal nerve fibers, including fibers crossing or originating from the dermal–epidermal junction, was counted in 4 sections per specimen using the established counting rules.[6,8,11] IENFD was calculated and expressed as an average number of intraepidermal fibers per millimeter length of epidermis. The diagnosis of SFSN was made when the IENFD at the distal leg was lower than the worldwide normative reference values (fifth quantile IENFD cutoffs) stratified by age deciles and sex.[6]

We identified 107 subjects who had skin biopsy and clinical evaluations by our neuromuscular specialists at the Mount Sinai Neuromuscular Center. All the Chinese American subjects were evaluated by the corresponding author L.Z. at the Mount Sinai Hospital, Manhattan Chinatown Clinic. L.Z. is a Chinese American herself, who speaks fluent Chinese. There is no language barrier between her and her Chinese American patients. We performed a retrospective chart review of these subjects for their demographics, clinical symptoms and signs, laboratory test results, NCS/EMG and imaging data, and skin biopsy findings. We included the subjects who had a high clinical suspicion for a distal SFSN, which was defined as the presence of both small fiber sensory symptoms (pain, burning, tingling, and/or numbness) and signs (reduced pinprick sensation or hyperalgesia to pinprick) bilaterally in a length-dependent manner. We excluded the subjects who had evidence of large fiber involvement, such as motor weakness, diminished deep tendon reflexes, impaired joint proprioception, or NCS/EMG findings suggestive of a large fiber polyneuropathy because we would address the sensitivity of skin biopsy for diagnosing a pure distal SFSN. After using the selection criteria, we included 23 Chinese American subjects and 32 non-Chinese American subjects for the final study. All these study subjects had NCS/EMG that showed normal antidromic sural nerve action potential amplitudes ($\geq$6 μV for age under 60 years and $\geq$ 4 μV for age at or above 60 years) and conduction velocities ($\geq$40 m/s). All these subjects had completed neuropathy etiology evaluation. We compared demographics, clinical features, and skin biopsy results between the 2 study groups. The Fisher exact test and the 2-tailed Student t test were used for statistical analysis. The difference was considered significant when $P < 0.05$. This study was approved by the institutional review board of Icahn School of Medicine at Mount Sinai.

RESULTS

The demographics of 23 Chinese American and 32 non-Chinese American study subjects were presented in Table 1. Briefly, the 2 cohorts had comparable female predominance with 16/23 (69.6%) women in the Chinese American group and 20/32

Journal of
CLINICAL
NEUROMUSCULAR
DISEASE

Volume 20, Number 1
September 2018

TABLE 1. Demographics of Study Subjects

Demographics	Chinese Americans (n = 23)	Non-Chinese Americans (n = 32)	*P*
Sex (n, %, male/female)	7 (30.4)/16 (69.6)	12 (37.5)/20 (62.5)	0.8
Age at biopsy (years, mean ± SD)	66.0 ± 12.9	58.6 ± 13.4	<0.001
Age decile (years) (n, %)			
30–39	2 (8.7)	2 (6.3)	1
40–49	0	7 (21.9)	0.03
50–59	4 (17.4)	9 (28.1)	0.5
60–69	8 (34.8)	6 (18.7)	0.2
70–79	6 (27.8)	7 (21.9)	0.7
80–89	3 (13.0)	1 (3.1)	0.3
Ethnicity (n, %)			
Chinese	23 (100)	0	—
White		25 (78.1)	—
Black		3 (9.4)	—
Hispanic		3 (9.4)	—
Asian Indian		1 (3.1)	—
BMI at biopsy (kg/m^2) (mean ± SD)	23.1 ± 5.1	28.4 ± 5.8	0.002

(62.5%) in the non-Chinese American group. The age (years) at skin biopsy ranged from 35 to 85 years in the Chinese American group and 34 to 87 years in the non-Chinese American group, and the age was older in the Chinese American group (mean ± SD: 66.0 ± 12.9) than in the non-Chinese American group (58.6 ± 13.4, $P < 0.001$). There were 7 patients in the age group 40–49 years in the non-Chinese American cohort, but none was in this age group in the Chinese American cohort. The percentage of patients in the other age groups was not different between the 2 cohorts. The body mass index (BMI) (kg/m^2) was lower in the Chinese American cohort (mean ± SD: 23.1 ± 5.1) than in the non-Chinese American cohort (28.4 ± 5.8, $P < 0.05$). Most of the patients in the non-Chinese American cohort were whites (25/32, 78.1%).

Two groups had similar symptom durations (mean ± SD), 30.6 ± 20.6 months in the Chinese American group and 27.5 ± 28.9 months in the non-Chinese American group. All patients had both length-dependent symptoms and signs of SFSN, mostly involving the feet and distal legs. Although sharp pain and numbness (reduced sensation) were more common in the Chinese American group, burning pain and tingling (pins and needles sensation) were more common in the non-Chinese American group (Table 2). All the study subjects, except for 2 in the Chinese American group, had decreased pinprick sensation detected by examination. A few subjects, including the 2 without reduced pinprick sensation, showed hyperesthesia to pinprick (Table 2). Seven (30.4%) subjects in the Chinese American group and 4 (12.5%) in the non-Chinese American group reported mild nonradiating low back pain, and their lumbosacral spine magnetic resonance imaging showed no significant spinal or neural foraminal stenosis.

Skin biopsies in 6 subjects in the Chinese American group and 20 in the non-Chinese American group showed reduced IENFD at the distal leg as compared to the worldwide normative reference values adjusted for age and sex.[6] The diagnostic sensitivity was significantly lower in the Chinese American group (26.1%) than in the non-Chinese American group (62.5%, $P = 0.01$). The IENFD (fibers/mm) at all 3 biopsy sites was higher in the Chinese American group (distal leg: 7.1 ± 3.9; distal thigh: 9.8 ± 5.1;

TABLE 2. Clinical Features of Study Subjects

Features	Chinese Americans (n = 23)	Non-Chinese Americans (n = 32)	*P*
Symptoms (n, %)			
Symptom duration (months)	30.6 ± 20.6	27.5 ± 28.9	0.7
Sharp pain	10 (43.5)	5 (15.6)	0.03
Burning pain	3 (13.0)	13 (40.6)	0.04
Tingling	3 (13.0)	19 (59.4)	<0.001
Numbness	20 (87.0)	19 (59.4)	0.04
Signs (n, %)			
Hyperesthesia	3 (13.0)	2 (6.3)	0.6
Decreased pinprick sensation	21 (91.3)	32 (100)	0.2
Associated conditions (n, %)			
Diabetes mellitus	11 (47.8)	10 (31.3)	0.3
B12 deficiency	3 (13.0)	1 (3.1)	0.3
Thyroid disease	5 (21.7)	5 (15.6)	0.7
Paraproteinemia	0	2 (6.3)	0.5
Connective tissue disease	1 (4.3)	5 (15.6)	0.4
Neurotoxic chemotherapy	3 (13.0)	2 (6.3)	0.6
B6 toxicity	1 (4.3)	1 (3.1)	1
None	5 (21.7)	11 (34.4)	0.4

and proximal thigh: 11.7 ± 4.1) than in the non-Chinese American group (distal leg: 5.1 ± 3.0, $P < 0.05$; distal thigh: 7.6 ± 2.4, $P < 0.05$; and proximal thigh: 9.8 ± 2.8, $P < 0.05$) (Fig. 1).

Neuropathy-associated conditions were present in 18/23 (78.3%) subjects of the Chinese American group and in 21/32 (65.6%) subjects of the non-Chinese American group, with diabetes mellitus being the most common, followed by thyroid disease, connective tissue disease, and B12 deficiency, among others. The prevalence of these associated conditions was not different between the 2 study groups (Table 2).

DISCUSSION

Skin biopsy with IENFD evaluation has become a standard test for diagnosing a distal SFSN[1,6,10,12] with a high diagnostic efficiency.[11,13] The guidelines for using this test[10] and the age- and sex-adjusted normative reference values of IENFD at the distal leg have been established for both bright-field immunohistochemistry and immunofluorescence techniques.[6,14] The diagnostic sensitivity of using these normative values for a distal SFSN, however, has not been well studied. By following the skin biopsy and IENFD evaluation guidelines[6,10] to study Chinese American and non-Chinese American patients with a high clinical suspicion for a pure distal SFSN, we found that the diagnostic sensitivity of skin biopsy using the bright-field immunohistochemistry protocol and the worldwide normative reference values of IENFD at the distal leg[6] was low in the Chinese American patients. Although the diagnostic sensitivity was 62.5% in the non-Chinese American patients, it was only 26.1% in the Chinese American patients. Therefore, the worldwide normative values appear insensitive for diagnosing a pure distal SFSN in Chinese Americans.

It has been shown that the normative values of IENFD at the distal leg are significantly influenced by age and sex but not by height, weight, or BMI.[6] There is an inverse correlation between age and IENFD at the distal leg. When people get older, the IENFD at the distal leg becomes lower. Although the

Journal of
CLINICAL
NEUROMUSCULAR
DISEASE

Volume 20, Number 1
September 2018

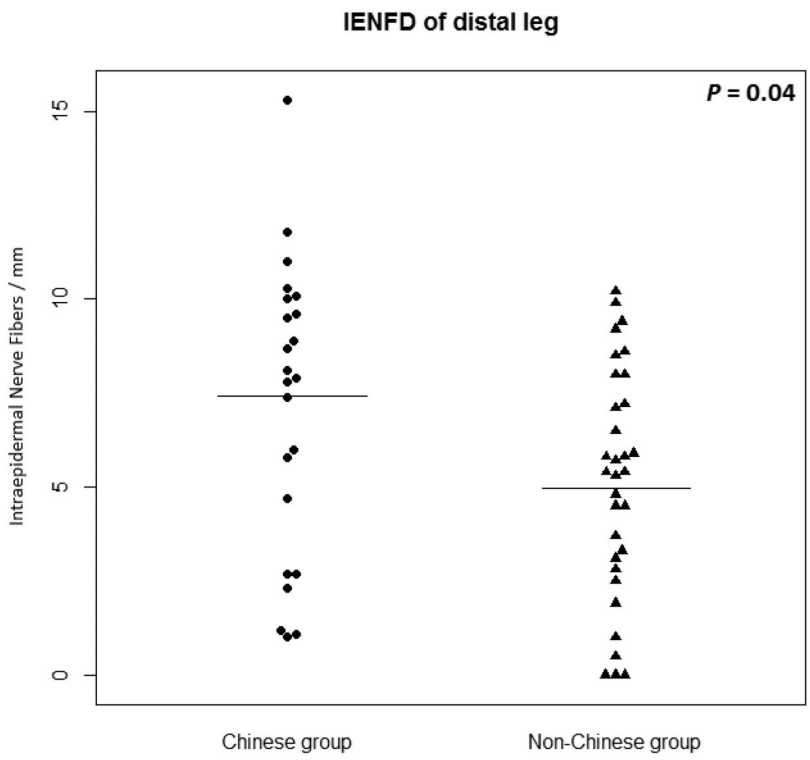

FIGURE 1. Comparison of IENFD at the distal leg showing a significantly higher IENFD in the Chinese American group than that in the non-Chinese American group.

mean age at skin biopsy is older in the Chinese American group than in the non-Chinese American group, the IENFD at all 3 biopsy sites is higher in the Chinese American group. The finding suggests that the low diagnostic sensitivity in the Chinese American group is unlikely to be caused by the age difference. The finding also suggests that the baseline IENFD at the distal leg in Chinese American patients may be significantly higher than those in non-Chinese American patients, especially whites as the majority of the subjects in our non-Chinese American group. There is no difference in sex between the Chinese and non-Chinese American groups. BMI is lower in the Chinese American group, which may contribute, in part, to the higher IENFD in this group, but BMI does not significantly influence the fifth percentile IENFD normative values in men or women based on the findings from the multicenter IENFD normative reference study.[6] The differences in SFSN symptoms in 2 groups are detected. The negative symptoms (numbness) are more frequently reported by the Chinese American patients, whereas the positive symptoms (pain and tingling) are more frequently reported by the non-Chinese American patients. Among the positive symptoms, the Chinese American patients more report sharp pain than burning pain and tingling. The cause of these differences is unclear. It is unlikely to be caused by a language barrier, as all the Chinese American patients are evaluated by the neuromuscular specialist who is a Chinese American herself and who speaks Chinese.

The studies of ethnic differences in IENFD at the distal leg are very limited. One study showed that IENFD at the distal leg in healthy ethnic Thais was 2–3 times higher than those in healthy subjects in the United States.[15] Another study[14] showed no differences of immunofluorescent IENFD at the distal leg between white and non-white healthy subjects; however, only 34/528 (6.4%) study subjects were non-whites and 8/528 (1.5%) were Asians, and no definitive conclusion

could be drawn. Although the worldwide normative reference values of IENFD at the distal leg was developed by a multinational study,[6] the potential differences in different ethnic groups were not mentioned. The findings from our present study suggest that the IENFD normative values at the distal leg are probably influenced by ethnicity in addition to age and sex, and different normative reference values may need to be developed for different ethnic populations to improve the diagnostic sensitivity. The worldwide normative values may not be representative of all centers, especially those with a skewed ethnic population. These centers may need to develop their own normative values.

Although our study is retrospective, all the study subjects were evaluated by the experienced neuromuscular specialists in our Mount Sinai neuromuscular center, and all the Chinese American subjects were evaluated by the neuromuscular specialist who is a Chinese American herself and who speaks fluent Chinese with no language or cultural barrier. In addition, all the skin biopsies and IENFD evaluation were performed by our Mount Sinai skin biopsy service and Mount Sinai Cutaneous Nerve Laboratory following the published guidelines.[10] Therefore, the data are reliable. We also used strict patient selection to only involve those who had both distal SFSN symptoms and signs to avoid underestimation of the skin biopsy sensitivity in diagnosing a pure distal SFSN. The main weakness is the relative small sample size. We cannot make a definitive conclusion. Future large-scale studies are warranted. In the future, it is also important to address the hypothesis that ethnic Chinese may have higher fifth percentile IENFD normative values at the distal leg than the worldwide normative values. If the hypothesis is correct, a new set of IENFD normative values at the distal leg should be used for ethnic Chinese to improve the diagnostic sensitivity.

REFERENCES

1. Cazzato D, Lauria G. Small fibre neuropathy. *Curr Opin Neurol.* 2017;30:490–499.
2. Chan AC, Wilder-Smith EP. Small fiber neuropathy: getting bigger! *Muscle Nerve.* 2016;53:671–682.
3. Tavee J, Zhou L. Small fiber neuropathy: a burning problem. *Cleve Clin J Med.* 2009;76:297–305.
4. Terkelsen AJ, Karlsson P, Lauria G, et al. The diagnostic challenge of small fibre neuropathy: clinical presentations, evaluations, and causes. *Lancet Neurol.* 2017;16:934–944.
5. Lacomis D. Small-fiber neuropathy. *Muscle Nerve.* 2002;26:173–188.
6. Lauria G, Bakkers M, Schmitz C, et al. Intraepidermal nerve fiber density at the distal leg: a worldwide normative reference study. *J Peripher Nerv Syst.* 2010; 15:202–207.
7. Holland NR, Stocks A, Hauer P, et al. Intraepidermal nerve fiber density in patients with painful sensory neuropathy. *Neurology.* 1997;48:708–711.
8. McCarthy BG, Hsieh ST, Stocks A, et al. Cutaneous innervation in sensory neuropathies: evaluation by skin biopsy. *Neurology.* 1995;45:1848–1855.
9. Zhou L, Kitch DW, Evans SR, et al. Correlates of epidermal nerve fiber densities in HIV-associated distal sensory polyneuropathy. *Neurology.* 2007;68: 2113–2119.
10. Lauria G, Cornblath DR, Johansson O, et al. EFNS guidelines on the use of skin biopsy in the diagnosis of peripheral neuropathy. *Eur J Neurol.* 2005;12: 747–758.
11. McArthur JC, Stocks EA, Hauer P, et al. Epidermal nerve fiber density: normative reference range and diagnostic efficiency. *Arch Neurol.* 1998;55:1513–1520.
12. Lauria G, Lombardi R. Small fiber neuropathy: is skin biopsy the holy grail? *Curr Diab Rep.* 2012;12:384–392.
13. Devigili G, Tugnoli V, Penza P, et al. The diagnostic criteria for small fibre neuropathy: from symptoms to neuropathology. *Brain J Neurol.* 2008;131:1912–1925.
14. Provitera V, Gibbons CH, Wendelschafer-Crabb G, et al. A multi-center, multinational age- and gender-adjusted normative dataset for immunofluorescent intraepidermal nerve fiber density at the distal leg. *Eur J Neurol.* 2016;23:333–338.
15. Shikuma CM, McArthur JC, Ebenezer GJ, et al. Ethnic differences in epidermal nerve fiber density. *Muscle Nerve.* 2013;48:462–464.