Original article

Difference in normal limit values of nerve conduction parameters between Westerner and Japanese people may need to be considered when diagnosing diabetic polyneuropathy using a Point-of-Care Sural Nerve Conduction Device（NC-stat®/DPNCheck™）

Kazuhiro Hirayasu, Hideyuki Sasaki, Shohei Kishimoto, Seigo Kurisu, Koji Noda, Kenichi Ogawa, Hiroto Tanaka, Yumiko Sakakibara, Shohei Matsuno, Hiroto Furuta, Mikio Arita, Keigo Naka, Kishio Nanjo

Department of Medicine, Kihoku Hospital, Wakayama Medical University, Wakayama, Japan.

Corresponding Author：Hideyuki Sasaki,

Department of Medicine, Kihoku Hospital, Wakayama Medical University

219, Myoji, Katsuragi-Cho, Ito-Gun, Wakayama-Ken, 649-7113, JAPAN

E-mail: sasaki-h@wakayama-med.ac.jp

Phone: +81-736-22-0066, Fax: +81-736-22-2579

Running title: Japanese Sural Amp differ from Westerner

ABSTRACT

Aim/Introduction

Studies on a novel point-of-care device for nerve conduction study called DPNCheck have been limited to Westerners. We aimed to clarify Japanese normal limits of nerve action potential (Amp)

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/jdi.12818

and conduction velocity (CV) by DPNCheck (Investigation-I), and validity of DPNCheck to identify diabetic symmetric sensorimotor polyneuropathy (DSPN) (Investigation-II).

Materials and Methods

Investigation-I: 463 non-neuropathic Japanese subjects underwent DPNCheck examinations. Regression formulas calculating normal limits of Amp and CV (Japanese regression formulas: JRF) were determined by quantile regression and then compared with those of US subjects (USRF).

Investigation-II: In 92 Japanese diabetic patients, 'Probable DSPN' was diagnosed and nerve conduction abnormalities (NCA1: one or more abnormalities and NCA2: two abnormalities in Amp and CV) were determined. Validity of NCAs to identify 'Probable DSPN' was evaluated by determining sensitivity, specificity, reproducibility (kappa-coefficient) and area under the curve (AUC) of receiver operating characteristic (ROC) curves.

Results

Investigation-I: JRF was different from USRF, and normal limits by JRF were higher than that of USRF. Prevalence of Amp abnormality calculated by JRF was significantly higher than that of USRF.

Investigation-II: Sensitivity, specificity and reproducibility of NCA1 and NCA2 judged from JRF were 85%, 86%, 0.57 and 43%, 100%, 0.56, respectively. These values of JRF were higher than those of USRF. AUC of JRF (0.89) was larger than USRF (0.82).

Conclusions

Significant difference in normal limits of nerve conduction parameters by DPNCheck between Japanese and US subjects was suggested. Validity to identify DSPN of NCAs may improve by changing judgement criteria from USRF to JRF.

Keywords: 1. Clinical
2. Diagnosis and pathophysiology
3. Complication I – Nerve

Optical Keywords: 1. Nerve conduction study
2. Diabetic polyneuropathy
3. Point-of care device

Introduction

Diabetic symmetric sensorimotor polyneuropathy (DSPN) is the most common disorder of heterogeneous diabetic neuropathies[1]. Early and accurate diagnosis of DSPN is necessary to prevent its progression through appropriate management. Although the presence of DSPN is primarily confirmed by a Nerve Conduction Study[1], clinics where nerve conduction studies can be practiced have been limited to only specialized clinics due to the need for skilled laboratory technicians and expensive equipment. A novel point-of-care device (POCD) for nerve conduction study called NC-stat®/DPNCheck™ (DPNCheck, Neurometrix Inc, Waltham, MA, USA) has recently been developed. Using DPNCheck, the nerve action potential amplitude (Amp) and conduction velocity (CV) of the sural nerve can be semi-automatically measured quickly and reliably at the bedside without the need of skilled physician or laboratory technician. Two reports have evaluated the accuracy of the results by DPNCheck compared to conventional nerve conduction method [2,3]. Although acceptable accuracy in clinical use was indicated in both reports, overestimation of CV [2] and underestimation of Amp [3] were reported compared to conventional methods. Therefore, the results measured by DPNCheck should not be evaluated by the normal limit values obtained by conventional method. For this reason, DPNCheck result sheet displays measurement values of Amp and CV with the normal limits adjusted by age and height estimated from the normative database[4] by the attached software. The database is also published on the Neurometrix website[4]. Normal limit values displayed by DPNCheck are based on data from a US population, mainly Westerners. There may be significant differences due to the differences in physique between Japanese and US people. Therefore, it is necessary to determine the normal limit values of Amp, CV in Japanese subjects and compare it with the value of the US one.

Firstly we aim to clarify the normal limit values of Amp and CV in Japanese and compare them with the US values (Investigation- I). Secondly, we aimed to compare the validity of diagnosing DSPN when using Japanese normal limit values compared with using the US value (Investigation- II).

Materials and Methods

Ethics Statement

These protocol and consent procedures were conducted in accordance with the World Medical Association's Helsinki Declaration and were approved by the Ethics Board of the Wakayama Medical University (Approval number 92, 468). All participants provided written informed consent.

Investigation- Ⅰ. Determinations of normal limit values in Japanese

Research Design and Subjects

This study was designed to determine the Japanese normal limit values of Amp, CV measured by DPNCheck. The subjects were restricted to those among the residential examinees of the regional health examination considered to clinically have no peripheral neuropathy. Eventually, the Japanese normal limit values were compared to the values of the US subjects described in the DPNCheck detabase[4].

We selected 463 subjects who were clinically diagnosed as having no peripheral neuropathy from 626 community medical screening program examinees. Absence of clinical peripheral neuropathy was confirmed by the following exclusion criteria; (i) positive history for clinical cerebral infarction sequelae, renal failure, diabetes, hypothyroidism or alcoholism, (ii) positive symptoms of numbness, pricking sensation, pain on the legs/feet/toes, (iii) bilaterally abnormal Achilles tendon reflex.

Demographics data (gender, age, height, weight and body mass index: BMI) of the Japanese subjects were obtained by physical examination. Demographic data of the US subjects described in the database of the DPNCheck were provided with permission.

Evaluation of neurological functions

Subjective symptoms of peripheral neuropathy were determined by asking whether there were any positive symptoms (numbness, pricking sensation, pain) on legs/feet/toes by interview. Achilles tendon reflexes on both sides were examined at the knee standing position.

Amp and CV of the bilateral sural nerve were measured by DPNCheck according to the test manual[5] by clinical laboratory technicians trained in advance. The average values of the left and

right were used for statistical analyses. Seven subjects refused the examination of both sides because of a feeling of discomfort while being tested. In these cases, measured values on the tested side were represented as averages.

In order to verify the signs of symmetric decrease of distal sensation at lower limbs, quantitative vibration threshold (VT) at 125Hz was assessed of both big toe tips using a vibratory sensation meter (AU-02B^{TM}, RION Inc, Tokyo, Japan). The method of VT measurement is described previously[6].

Statistical methods

In this study, we used the same statistical methods as the DPNCheck normative database[4]. The cut-off value of the normal limit of Amp/ CV and VT were defined as the 5th- and 95th percentile values, respectively. To determine the cut-off values, the multivariate quantile regression analysis (quantile regression analysis) was used. In this method, the normal limit cut-off value is expressed as a linear function with demographic variables as follows: Normal Limit = $K + C_1V_1 + C_2V_2 + ... + C_nV_n$. In this regression formula, K is a constant, V_i is the i^{th} demographic variable, and C_i is the coefficient related to the i^{th} demographic variable. Firstly, demographic variables on which nerve functions (Amp, CV and VT) depend significantly ($p < 0.05$) were selected from gender, age, height, weight, and BMI by forward stepwise analysis. Then, the first quantile regression analysis using selected variables was performed to determine the variables on which normal limit values of nerve functions significantly depend. Finally, the determined variables were adopted to the second quantile regression analysis, and the constant (K) and the coefficient (C) of the regression formula showing the normal limit value for each of the examinations (Amp, CV and VT) were calculated. The precision of the resulting normal limits was assessed by calculating both point estimates and 95% confidence intervals (95% CI) for representative demographics using the bootstrap method with 100,000 random samples.

The obtained regression formulas showing the cut-off value for the normal limit of nerve function tests in Japanese subjects (Japanese regression formulas: JRF) were compared with the regression formulas of US subjects (USRF) described in the DPNCheck database[4].

Statistical analyses were carried out with the JMP (SAS Institute, Cary, NC, USA).

Investigation-Ⅱ. Validity of nerve conduction abnormality (NCA) detection using DPNCheck for diagnosing DSPN.

Research Design and Subjects

Nerve conduction abnormality (NCA) was defined based on Amp and CV measurements by DPNCheck. In Japanese diabetic patients, the validity of NCA judged by JRF to detect clinical DSPN was examined. Likewise, the validity of NCA determined by USRF to detect clinical DSPN was also examined. After that, we determined whether the JRF or the USRF were superior in reproducibility, sensitivity and specificity.

Ninety-two diabetic outpatients, who received medical interviews, physical examinations and nerve function tests (Amp, CV and VT) were used as subjects. The patients who have a positive history for clinical cerebral infarction sequelae, renal failure, hypothyroidism or alcoholism were excluded. Of the 92 diabetic subjects, 62 were chosen from the examinees under diabetes therapy received from the regional medical examination of the Investigation I and 30 were selected from outpatients of Wakayama Medical University Hospital.

Diagnosis of clinical DSPN

DSPN was diagnosed according to the latest international consensus on diabetic neuropathies as reported by the Toronto diabetic neuropathy expert group (Toronto Consensus)[1)]. In the consensus, three characteristic clinical symptoms/signs for DSPN were proposed as follow; i) positive neuropathic sensory symptoms (e.g., “asleep numbness,” prickling or stabbing, burning or aching pain in the toes, feet, or legs), ii) sign of symmetric decrease of distal sensation and iii) unequivocally decreased or absent ankle reflexes. Then, DSPN was categorized into four stepwise criteria according to the presence of above symptoms/signs; ‘Possible DSPN’: one symptom/sign, ‘Probable DSPN’: two or more symptoms/signs, ‘Confirmed DSPN’: one or more symptoms/signs with nerve conduction abnormality (abnormal small fiber neuropathy: SFN can be substituted), and ‘Subclinical DSPN’: nerve conduction abnormality or SFN without any symptoms/signs. The consensus has recommended that ‘Probable DSPN’ definitions be used for clinical practice.

In this study, we diagnosed DSPN based on the modified criterion of ‘Probable DSPN’. Modifications were made to increase accuracy and objectivity. Specifically, the bilateral abnormal vibratory perception threshold (VT) judged by the regression formula for the normal limit of Investigation I were adopted as a sign of symmetric decrease of distal sensation in the lower extremities.

Comparison of the validity of diagnosing 'Probable DSPN' between when using Japanese normal limit values compared with using US values.

The validity of NCA for diagnosing 'Probable DSPN' was evaluated by determining the sensitivity, specificity and reproducibility to predict the presence or absence of 'Probable DSPN'. We used two NCA criteria (one or more abnormal value of Amp and CV: NCA1, two abnormal values of both: NCA2), and those NCAs were judged from two regression formulas (JRF and USRF). Sensitivity, specificity and reproducibility of each of the NCAs were compared to assess the superiority in diagnosis of DSPN when compared to each other. Furthermore, the values of sensitivity, 1-specificity of NCA1 and NCA2 judged by JRF and USRF were plotted in receiver operating characteristic (ROC) curves, and the areas under the curves (AUC) were calculated.

Reproducibility was assessed by the Cohen's kappa coefficient. Statistical analyses were performed by using the statistical software Excel statistics （Ekuseru-Toukei 2010； Social Survey Research Information Co., Ltd. Tokyo, Japan).

Results

Investigation-Ⅰ. Determinations of normal limit values in Japanese

Characteristics of the subjects

Demographics data (gender, age, height, and weight and body mass index: BMI) of the Japanese and US subjects are shown in table 1. Japanese subjects were significantly older, of a shorter height and less obese compared to the US subjects.

Demographic variables on which the normal limit values of Amp, CV, VT depend

The results of the first quantile regression analyses after forward stepwise analyses are shown in Table 2a. Bold characters indicate statistically significant values. The demographic variable on which normal limit values of Amp depend significantly was age. Demographic variables on which normal limit values of CV depend significantly were age and height. Similarly, the demographic variable on which normal limit values of VT depend significantly was age. There was no difference in the variables showing significant dependency between the data of this study and the US database.

Determination of normal limit of Amp/CV and VT

The constants and coefficients of the regression formulas representing the cut-off value of normal limit of Amp, CV and VT which were calculated by the second (final) quantile regression method are shown in Table 2b. Bold characters indicate statistically significant values. The regression formulas themselves are also shown in Table 2b. As results, regression formulas representing the cut-off value of the normal limit of each nerve function test were as follows, Amp = 12.62 - 0.103 x age (years), CV = 94.88 - 0.148 x Age (years) - 0.231 x Height (cm), VT = 4.615 + 0.385 × Age (years). On the other hand, the regression formulas Amp and CV of US subjects described in the DPNCheck database[4)] were as follows; Amp = 11.2 - 0.099 × Age (years), CV = 88.5 - 0.13 x Age (years) - 0.20 x Height (cm). There were differences between the regression formulas of Japanese (Japanese regression formula: JRF) and that of US subjects (US regression formula: USRF).

Figure 1a shows the relationship between Amp and age. Solid and broken lines show the age dependent normal limit calculated by JRF and USRF, respectively. The regression line of JRF is clearly higher than that of USRF. Figure 1b shows the relationship between CV and age. Solid and dotted lines show the age dependent normal limit at the height of 155 cm calculated by JRF and USRF, respectively. In the same way, double line and broken lines show the age dependent normal limit at the height of 165 cm calculated by JFR and USRF, respectively. The regression lines at the height of 155 cm are clearly higher than that of 165 cm. Especially at a younger age, the regression lines by JRF are somewhat higher than those by USRF.

The prevalence of Amp abnormal values in the 463 Japanese subjects (919 limbs) judged by JRF and USFR were 63/919 (6.6%) and 48/919 (3.8%), respectively. Similarly, the prevalence of CV abnormal values in the 463 Japanese subjects (919 limbs) judged by JRF and USFR were 74/919 (8.1%) and 65/919 (7.1%), respectively. Although the prevalence of Amp abnormality calculated in JRF was significantly higher than that in USRF (χ^2 = 8.46, P = 0.0036), the prevalence of CV in JRF was not different compared to that of USRF (χ^2 = 0.63, P = 0.4272).

Investigation-Ⅱ. Validity of nerve conduction abnormality (NCA) detection using DPNCheck for diagnosing 'Probable DSPN'.

Clinical characteristics and averaged nerve function data is shown in table 3a. The diabetic patients were relatively elderly and well glycemic controlled (average age 65.7 years old, average HbA1c 7.04 %). The Amp, CV and VT of diabetic patients were significantly worse than those of

Japanese normal subjects in table 1. We used unpaired t-test for statistical analysis (actual data is not shown).

Prevalence of 'Probable DSPN' and NCAs (table 3b, 3c)

The prevalence of positive neuropathic sensory symptoms, unequivocally decreased/absent ankle reflexes and symmetric decrease of distal sensation (Abnormal VT) were 20.7%, 34.8% and 10.9%, respectively. 'Probable DSPN' (two or more symptoms/signs) was observed in 14/92 (15.2%). Asymptotic DSPN was 4/14 (28.5%).

The prevalence of NCA1 (one or more abnormal value of Amp and CV) judged by JRF and USRF were 25.0% and 19.6%, respectively. Similarly, the prevalence of NCA2 (two abnormal values of Amp and CV) determined by JRF and USRF were 6.50% and 4.35%, respectively.

Validity for diagnosing 'Probable DSPN' by NCAs judged from JRF and USRF (table 3d).

Sensitivity, specificity and Cohen's kappa coefficient (reproducibility) to identify 'Probable DSPN' of NCA1 judged from JRF or USRF were 85%, 86%, 0.57 and 71%, 90%, 0.55, respectively. In the same way, those values of NCA2 judged from JRF or USRF were 43%, 100%, 0.56 and 29%, 100%, 0.40, respectively. All values of sensitivity and kappa coefficient of both of NCA1 and NCA2 judged by JRF were higher than those values judged by USRF.

ROC curve showing the validity of NCAs assessed by DPNCheck to diagnose 'Probable DSPN' is shown in figure 2. The solid line plot the sensitivity points- '1-specificity' coordinate points of NCA1 (*) and NCA2 (#) determined by JRF. Similarly, the broken line plot the sensitivity points- '1-specificity' coordinate points of NCA1 (&) and NCA2 ($) determined by USRF. The AUC of JRF (0.89) was larger than AUC of USRF (0.82).

Discussion

Investigation- Ⅰ was performed on Japanese subjects who were supposed to have no peripheral neuropathy. The associated demographic factors with the Amp and CV measured by DPNCheck and a normal limit of them were determined. These were compared with the US values. Two major findings were observed.

First, among demographic factors (age, sex, height, weight, BMI), age showed a significant association with both AMP and CV, and height showed a significant association with CV. The associated demographic factors with nerve conduction parameters of Japanese subjects did not differ from the database[4)] of US subjects. These findings confirm the deteriorating effect of aging on nerve conduction functions (Amp and CV) which is generally well known. Several papers reported the significant relationship between nerve conduction velocity and height in normal[7)] and diabetic[8)] Caucasian subjects.

As nutritional metabolites to maintain axonal fibers are delivered from neuron through axonal transport, supply of nutritive metabolites to the terminal of the longest fibers is most challenging. Thus impairment of metabolic processes essential to maintain axonal integrity is thought to explain the length-dependency of distal dominant nerve dysfunction. Mild impairment of metabolic processes is supposed to be caused by not only hyperglycemia, but also ischemia (arteriosclerosis), hypothermia and aging. Since the taller the stature, the longer the axonal fiber length, the tall persons may be more susceptible to distal axonal dysfunction such as nerve conduction delay than short stature persons. In our search scope, this paper is the first report to demonstrate the relationship between conduction velocity and height in Japanese (East Asian subjects).

Second, the normal limit of Amp calculated by Japanese regression formulas (JRF) was higher than that calculated by US regression formula (USRF), and the prevalence of abnormal Amp values judged by JRF was significantly higher than that by USRF. On the other hand, the normal limit of CV calculated by JRF was slightly higher than that calculated by USRF, but there was no significant difference between the prevalence of abnormal CV values judged by JRF and that by USRF. These findings suggest that the normal limit of nerve conduction parameters, especially Amp, of Japanese subjects determined by DPNCheck may be different from that of US ones. The difference of Amp normal limit between Japanese and US subjects is supposed to be due to the degree of obesity. In obese people, the subcutaneous tissue is thicker than the thin tissue, the distance between the electrode of the nerve conduction device and the nerve beneath it becomes longer, and as a result, the action potential amplitude (Amp) is considered to decay. Although it is a children's study, the sural nerve SNAP in obesity and insulin resistant group has been reported to be significantly lower than the normal control group [9)].

These findings suggest that there are unignorable differences in the normal limits of nerve conduction parameters measured by DPNCheck between in the US subjects and Japanese. Therefore, it is considered necessary to compare the validity of diagnosing peripheral neuropathy

using DPNCheck between the two regression formulas that calculate the normal lower limit of Japanese and US subjects (JRF and USRF).

In Investigation-Ⅱ, we evaluated the validity of predicting 'Probable DSPN' defined by Toronto consensus[1)] according to the detection of nerve conduction abnormalities (NCAs) by DPNCheck in Japanese diabetic patients. This investigation aimed to clarify whether the validity improves when changing the regression formula that decides NCAs from USRF to JRF. The validity of the predictability of diagnosing 'Probable DSPN' by NCAs was assessed by the sensitivity, specificity and Cohen's kappa coefficient (reproducibility). Two major findings were observed.

First, all values of sensitivity and Cohen's kappa coefficient (reproducibility) to predict for diagnosing 'Probable DSPN' of both of NCA1 and NCA2 judged from JRF were higher than those values judged from USRF. These findings may suggest that the validity to predict 'Probable DSPN' by NCAs judged by JRF was superior to that judged by USRF. Especially, sensitivity (85%), specificity (86%) and kappa coefficient (0.57) to predict 'Probable DSPN' by NCA1 judged from JRF were very well. Previous papers have also reported well sensitivity and specificity for DPNCheck to identify diabetic polyneuropathy. Lee JA et al[2)] reported the sensitivity and specificity were 95% and 71%, respectively. Perkins BA et al[10)] reported the sensitivity and specificity were 88% and 82%, respectively.

Second, from the ROC curve analysis, the AUC of JRF (0.89) was larger than the AUC of USRF (0.82). This finding supports the validity of NCA1 judged by JRF. Lee JA et al[2)] also reported results similar to this study. In their paper, the AUC calculated by the same method as our study was 0.88 Therefore, NCA assessment using DPNCheck is thought to have excellent reliability and acceptable accuracy to identify diabetic polyneuropathy. The usefulness of NCA assessment using DPNCheck has been reported in peripheral neuropathy other than diabetic polyneuropathy, such as chemotherapy-induced peripheral neuropathy[11)] and carpal tunnel syndrome[12)]. Thus, this POCD for nerve conduction study might be expected to be widely used in many fields of clinical practice. Our study, showing the racial difference in the normal limits of this POCD, is considered important for accurate clinical diagnosis of peripheral neuropathy.

From all of our investigations, we could find a difference in the normal limit of nerve conduction parameters, especially Amp, between Japanese and US subjects. And we could propose a Japanese formula calculating age and/or height adjusted normal limits of the nerve conduction parameters determined by the POCD (DPNCheck). We also would like to emphasize that the validity of identifying diabetic polyneuropathy may improve by changing the judgement criteria of nerve

conduction abnormalities from US to Japanese normal limits, that will lead to excellent reliability. The findings obtained in this study may be applicatable to East Asians other than Japanese.

The advantage of our studies is that we set normal limits by an appropriate method called quantile regression method[13] using a sufficient number of non-neuropathic subjects confirmed by ATR. Another advantage is that we modified the criteria of 'Probable DSPN' by exchanging the sign of symmetric decrease of distal sensation at legs to quantitative vibration sensation abnormality in order to improve objectivity.

One limitation of our studies is that the number of subjected diabetic patients was not so large. Therefore, in order to confirm the improved accuracy to identify diabetic polyneuropathy using the POCD for nerve conduction study by using Japanese normal limits instead of US normal limits, a larger scaled study is needed.

Acknowledgment

We thank Prof. Toshio Shimokawa for excellent advice on statistical analysis. We also thank the staff of the Divisions of Health and Welfare of Katsuragi town for their cooperation. This work was supported by JSPS KAKENHI Grant Number JP15K01723.

Disclosure

We have no conflict of interest in this work.

References

1. Tesfaye S, Malik RA, Boulton AJ et al. Diabetic Neuropathies: Update on Definitions, diagnostic criteria, estimation of severity, and treatments. Diabetes Care. 2010; 33: 2285-2293.

2. Lee JA, Halpern EM, Lovblom LE, et al. Reliability and validity of a point-of-care sural nerve conduction device for identification of diabetic neuropathy. PLoS One. 2014;9:e86515

3. Perkins BA, Ngo M, Grewel J, et al. Validation of a novel point-of-care nerve conduction device for the detection of diabetic sensorimotor polyneuropathy. Diabetes Care. 2006;29: 2023–2027

4. Neurometrix . NC-stat® DPNCheck™ Normative Database: Collection, Analysis And Recommended Normal Limits. http://www.dpncheck.com/resources/Resources/nc-stat_dpncheck_normative_data_monograph_for_software_version_2_0_pn2203866_rev_a.pdf

5. Neurometrix . NC-stat® DPNCheck™ User Manual. (2013) http:// http://www.dpncheck.com/resources/Resources/nc-stat_dpncheck_user_manual_pn2203282_rev_g.pdf

6. Matsuno S, Sasaki H, Yamasaki H, et al. Pro198Leu missense polymorphism of the glutathione peroxidase 1 gene might be a common genetic predisposition of distal symmetric polyneuropathy and macrovascular disease in Japanese type 2 diabetic patients. J Diabetes Invest. 2011; 2: 457-482.

7. Trojaborg WT, Moon A, Andersen BB, et al. Sural nerve conduction parameters in normal subjects related to age, gender, temperature, and height: a reappraisal. Muscle Nerve. 1992;15: 666-671.

8. Gadia MT, Natori N, Ramos LB, et al. Influence of height on quantitative sensory, nerve-conduction, and clinical indices of diabetic peripheral neuropathy. Diabetes Care 1987; 10: 613-616.

9. Akın O, Eker İ, Arslan M, et al. Association of nerve conduction impairment and insulin resistance in children with obesity. Childs Nerv Syst. 2016; 32: 2219-2224.

10. Perkins BA, Ng E, Orszag A, et al. Multi-Site Testing With a point-of-Care Nerve Conduction Device Can Be Used in an Algorithm to Diagnose Diabetic Sensorimotor Polyneuropathy. Diabetes Care 2008; 31:522–524.

11. Matsuoka A, Mitsuma A, Maeda O, et al. Quantitative assessment of chemotherapy-induced peripheral neurotoxicity using a point-of-care nerve conduction device. Cancer Sci 2016; 107: 1453–1457

12. Shepherd MM. Clinical Outcomes of Electrodiagnostic Testing Conducted in Primary Care. Am Board Fam Med 2010; 23: 584–590.

13. Peng L, Wuu J, Benatar M. Developing reference data for nerve conduction studies: an application of quantile regression. Muscle Nerve 2009; 40: 763-771.

Figure legends

Figure 1. The relationships between Amp and age, and CV and age are shown in figure 1a and 1b, respectively. Detailes are descrbed in the main text. JRF: Japanese regression formula; USRF: US regression formula.

Figure 2. ROC curve showing the validity of NCAs assessed by DPNCheck to diagnose 'Probable DSPN'. The solid line is a line connecting the sensitivity – '1-specificity' coordinate points of NCA1 (*) and NCA2 (#) determined by JRF. Similarly, the broken line is a line connecting the sensitivity – '1-specificity' coordinate points of NCA1 (&) and NCA2 ($) determined by USRF. The AUC of JRF (0.89) is larger than AUC of USRF (0.82). ROC: receiver operating characteristic, AUC: areas under the curves, NCA: nerve conduction abnormality; DSPN: diabetic symmetric sensorimotor polyneuropathy; JRF: Japanese regression formula; USRF: US regression formula.

Table 1. Comparison of demographics and nerve function data (Amp, CV and VT) between Japanese and US subjects.

	Japanese subjects	US subjects	
	Mean ± SD (N)	Mean ± SD (N)	p values
Demographics data			
Gender (male/female)	186 / 277	257 /270	0.0067
Age (years)	60.8 ± 9.8 (463)	48.3 ± 18.5 (527)	<0.0001
Height (cm)	160.3 ± 8.5 (463)	167 ± 11.5 (527)	<0.0001
Weight (kg)	57.7 ± 10.6 (463)	73.1 ± 16.8 (527)	<0.0001
Body mass index (m/kg^2)	22.3 ± 3.1 (463)	26.0 ± 4.09 (527)	<0.0001

Nerve function data			
Amp (μ V)	15.2 ± 7.04 (463)	16.9 ± 8.62 (527)	<0.0001
CV (m/sec)	54.5 ± 4.23 (463)	53.0 ± 5.17 (523)	<0.0001
VT (dB)	17.3 ± 7.8 (463)	NE	

Data of US subjects were provided from DPNCheck normative database (reference 4) with manufacturer's permission.

Unpaired t-test and chi-squared test were used for statistical analysis. NE : not examined.

Table 2. Statistical data obtained by of the first and second multivariate quantile regression analyses (quantile regression analyses).

a. Results of the first quantile regression analyses after forward stepwise analyses verifying dependency of Amp, CV, VT on demographic variables.

		Demographic variables				
		Gender (female)	Age	Height		
Weight	Body mass index					
	Constant	Regression coefficient	Regression coefficient	Regression coefficient	Regression coefficient	Regression coefficient
		p value	p value	p value	p value	p value
Nerve function tests		[95%CI]	[95%CI]	[95%CI]	[95%CI]	[95%CI]
Amplitude of action potential of sural nerve (Amp)						
	15.87	-	**-0.095**	-	-	-0.158
			<0.001			0.097
			[-0.156,-0.038]			[-0.344,0.028]
Conduction velocity of sural nerve (CV)						
	85.90	1.146	**-0.112**	**-0.224**	0.064	-

		0.389	**0.020**	**0.007**	
0.244					
		[-1.459,3.751]	**[-0.206,-0.017]**	**[-0.390,-0.059]**	
[-0.044,0.173]					
Vibratory perception threshold (VT)					
	-35.25	-	**0.487**	0.208	-
-					
			<0.001	0.059	
			[0.350,0.625]	[0.050,0.365]	

b. The results of the second quantile regression analysis, and the regression formulas representing the cut-off value of normal limit of nerve function tests.

		Age	Height	
	Constant	Regression coefficient	Regression coefficient	
		p value	p value	
Nerve function tests		[95%CI]	[95%CI]	Regression formulas representing the normal limit
Amplitude of action potential of sural nerve (Amp)				
	12.62	**-0.103**		Amp = 12.62 - 0.103 x Age (years)
		<0.001		
		[-0.162, -0.044]		
Conduction velocity of sural nerve (CV)				

	94.88	**-0.148**	**-0.231**	CV = 94.88 - 0.148 x Age (years) - 0.231 x Height (cm)
		0.016	**0.001**	
		[-0.269, -0.027]	**[-0.369, -0.092]**	
Vibratory perception threshold (VT)				
	4.615	**0.385**		VT = 4.615 + 0.385 × Age (years)
		<0.001		
		[0.224, 0.545]		

-: Excluded by forward stepwise analyses. Bold characters indicate the statistically significant values.

Table 3. Clinical characteristics, nerve function data and statistical data in 92 Japanese diabetic subjects.

a.	Clinical characteristics and averaged nerve function data	Mean ± SD
	Gender (male/female)	59 /33
	Age (years)	65.7 ± 7.0
	Height (cm)	161.7 ± 9.15
	Weight (kg)	63.8 ± 13.8
	Body mass index (m/kg^2)	24.2 ± 3.97
	HbA1c (NGSP) (%)	7.04 ± 1.13
	Amp (μ V)	10.8 ± 5.46
	CV (m/sec)	50.8 ± 5.29
	VT (dB)	22.0 ± 8.6

b.	Prevalence of neurological symptoms and signs, 'Probable DSPN'.	
	Positive neuropathic sensory symptoms	19/92 (20.7%)
	Unequivocally decreased or absent ankle reflexes	32/92 (34.8%)
	Symmetric decrease of distal sensation (Abnormal VT)	10/92 (10.9%)
	Probable diabetic symmetrical sensorimotor polyneuropathy ('Probable DSPN')	14/92 (15.2%)

c. Prevalence of nerve conduction abnormalities (NCA1, NCA2) judged by JRF or USRF.

	JRF	USRF	p values
Bilaterally abnormal Amp	14/92 (15.2%)	8/92 (8.70%)	0.1728
Bilaterally abnormal CV	15/92 (16.3%)	14/92 (15.2%)	0.8397
NCA1 (one or more abnormal value of Amp and CV)	23/92 (25.0%)	18/92 (19.6%)	0.3757
NCA2 (two abnormal values of Amp and CV	6/92 (6.50%)	4/92 (4.35%)	0.5154

d. Sensitivity, specificity and reproducibility of the predictively of diagnosing 'Probable DSPN' by the each NCAs based on JRF or USRF.

	JRF			USRF		
	Sensitivity	Specificity	Kappa coefficient (p values)	Sensitivity	Specificity	Kappa coefficient (p values)
NCA1	85%	86%	0.57 (<0.001)	71%	90%	0.55 (<0.001)
NCA2	43%	100%	0.56 (<0.001)	29%	100%	0.40 (<0.001)

Amp: nerve action potential amplitude of the sural nerve; CV :conduction velocity of the sural nerve. Amp and CV were measured by a point-of-care nerve conduction device : DPNCheck®. VT: quantitative vibration threshold at 125Hz at the big toe tips using the vibratory sensation meter: AU-02B®.

JRF : regression formulas representing the cut-off value of normal limit in Japanese subjects calculated by the quantile regression method ;

USRF :regression formulas representing the cut-off value of normal limit in US subjects. Chi-squared test and Cohen's kappa coefficient method were used

for statistical analysis.

Figure 1.

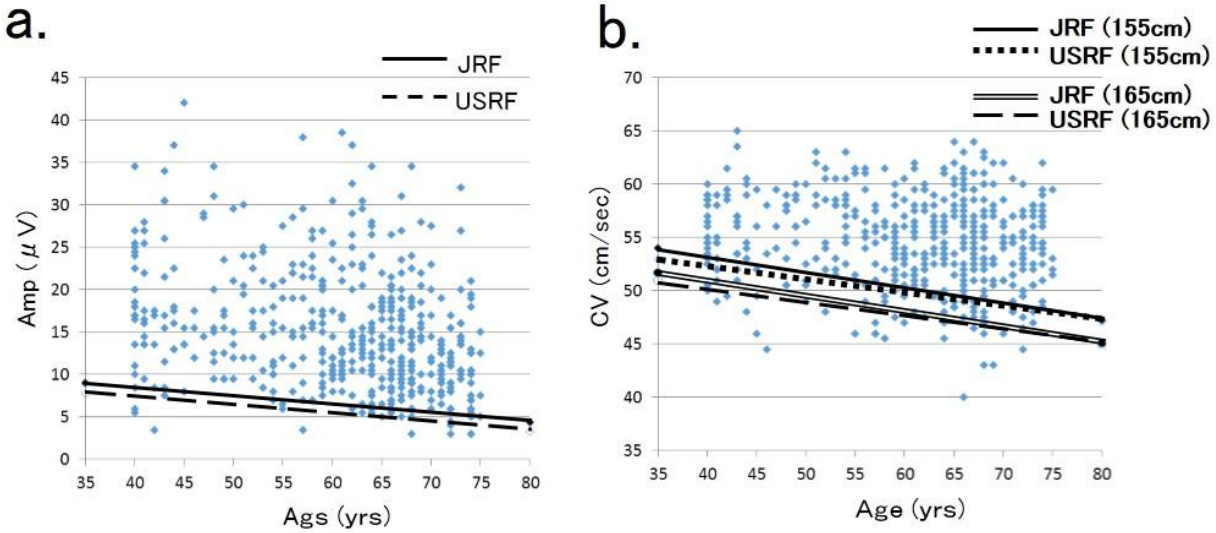

Figure 2.