


Longitudinal effects of one-leg standing time on neuropathy outcomes in association with glycemic control in non-elderly patients with type 2 diabetes

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Keywords

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ABSTRACT

Aims/Introduction: Diabetic neuropathy leads to postural instability. This study compared longitudinal changes in neuropathy outcomes relative to long-term glycemic control in patients aged <60 years with uncontrolled type 2 diabetes with and without a short one-leg standing time (OLST <60 s).

Materials and Methods: In this retrospective study, 58 hospitalized patients with type 2 diabetes (glycated hemoglobin [HbA1c] >7.0%; aged 17–59 years), who underwent re-evaluation of neuropathic sensory symptoms, ankle reflexes and nerve conduction attributes, and cardiac autonomic function (R-R interval), >1 year after discharge were divided into OLST <60 and ≥60 s groups. Patients were followed up every 2–3 months for HbA1c levels for up to 8 years. Neuropathy outcomes relative to OLST and HbA1c levels at baseline and over follow up were compared.

Results: Additional development of sensory symptoms (one patient) and abnormal ankle reflexes (five patients) were identified during follow up, and decreased peripheral and cardiac autonomic function at both baseline and follow up, only in patients with OLST <60 s. Mean HbA1c levels were significantly higher in patients with OLST <60 s versus ≥60 s ($7.8 \pm 0.9\%$ vs $7.2 \pm 1.2\%$; $P = 0.022$). Better glycemic control during follow up was associated with better neuropathy outcomes only in patients with OLST ≥60 s.

Conclusion: Non-elderly type 2 diabetes patients with OLST <60 s and decreased peripheral nerve function at baseline are at increased risk for intractable diabetic neuropathy. Better glycemic control alone might not improve neuropathy outcomes in these patients.

INTRODUCTION

Diabetic neuropathy (DN) is one of the earliest and most common complications of diabetes, developing even in people with prediabetes¹, and affecting at least 50% of patients over time as age advances². The only effective therapeutic option is strict glycemic control, which is known to dramatically reduce the development and progression of DN in type 1 diabetes patients³, although it provides only modest effects in type 2

diabetes patients^{4,5}. The underlying mechanisms for the progressive and intractable nature of DN in type 2 diabetes patients remain elusive, and might involve certain risk factors and/or comorbidities that differ between type 1 and type 2 diabetes patients^{2,4}.

Postural stability has been recognized as being impaired in patients with DN⁶. Recently, we analyzed cross-sectional data from hospitalized patients with uncontrolled type 2 diabetes and showed that a short one-leg standing time (OLST) with eyes open, a simple measure of postural instability⁷, was

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associated with peripheral and cardiac autonomic nerve dysfunction and clinical neuropathy in these patients, regardless of age⁸. Postural stability is also especially impaired in elderly individuals aged >60 years⁹. To date, however, no studies have investigated the longitudinal effects of OLST on neuropathy outcomes and their association with glycemic control in non-elderly patients with type 2 diabetes. Therefore, the present study aimed to explore longitudinal changes in neuropathy outcomes, and compare them with baseline and long-term glycemic control in patients aged <60 years with uncontrolled type 2 diabetes stratified according to OLST at baseline. We hypothesized that a shorter OLST might be associated with worse long-term neuropathy outcomes. This information would help identifying patients at risk of developing intractable DN in routine clinical settings.

MATERIALS AND METHODS

Study patients

A flow diagram of study patients is shown in Figure S1. Elderly patients aged ≥ 60 years were excluded in the present study, as our preliminary analyses showed that they were more likely to have a short (<60 s) OLST (data not shown). Initially, we selected 139 patients with uncontrolled type 2 diabetes (hemoglobin (Hb) A1c >7.0% (53 mmol/mol)) aged between 17 and 59 years who were admitted to Ohta Nishinouchi Hospital, Koriyama, Japan, and were being treated by a diabetologist (KS) between December 2011 and November 2018. The common reason for hospitalization was to improve diabetes self-management practice and glycemic control.

Then, we excluded patients who had newly diagnosed (known diabetes duration of <1 year) or longstanding diabetes (diabetes duration of >20 years), to match the duration of diabetes between patients with OLST <60 s and OLST ≥ 60 s. Additionally, we excluded patients with the following exclusion criteria at admission, which could affect the relationship between OLST and neuropathy outcomes: (i) ketonuria (1+ or more) accompanied by glycosuria; (ii) estimated glomerular filtration rate <30 mL/min/1.73 m²; (3) C-reactive protein of >3.0 mg/dL or erythrocyte sedimentation rate of >100 mm per 2 h (these upper limits were chosen to adjust for the cohort size and the potential inclusion of patients with underlying diseases, such as infection, collagen vascular diseases, hematological disorders and malignant tumors); and (iv) a history of coronary heart disease, stroke, peripheral artery disease or psychiatric disorders.

After excluding 54 patients who met the above-mentioned exclusion criteria, 85 patients remained as candidates for this longitudinal follow-up study. All patients were able to participate in the supervised exercise programs (stretching/balance/resistance training), and underwent an OLST test and neurological examinations during hospitalization, as described further in this section.

Of these candidates, 58 patients who underwent re-evaluation of neuropathic sensory symptoms, ankle reflexes and

nerve conduction (NC) attributes >1 year after discharge until June 2020 were finally identified as being eligible for study participation. During hospitalization and after discharge, diabetes self-management education/support for lifestyle modification and glucose-lowering therapy were continued to achieve the best possible glycemic control without hypoglycemia and to meet individualized needs, based on the consensus statements of the American Diabetes Association and the European Association for the Study of Diabetes. Patients visited the outpatient clinic of this center typically every 2–3 months and were followed for up to 8 years after discharge.

Available laboratory data, including levels of plasma glucose, HbA1c, serum creatinine, lipids and C-peptide, obtained from fasting blood samples on the second or third day after admission and from either fasting or non-fasting blood samples at every visit after discharge, were retrieved from electronic medical records and analyzed anonymously. HbA1c data after discharge were selected only if they were obtained more than 2 months after the previous measurement.

Body composition

We assessed body composition at the beginning of hospitalization. A multifrequency bioelectrical impedance analyzer (InBody 720; Biospace, Tokyo, Japan) was used to assess body composition (fat mass and skeletal muscle mass). The skeletal muscle index was calculated as appendicular skeletal muscle mass in kilograms divided by the square of the height in meters.

OLST test

One-leg standing time with eyes open was measured for both legs, up to a maximum of 60 s, to assess functional balance during hospitalization. The study protocol allowed each patient up to three attempts to reach the 60 s goal. If reached, a time of 60 s was recorded. The values for both legs were averaged and used for analysis, because the bilateral difference was nearly zero (data not shown). As more than half of the OLSTs were ≥ 60 s, patients were dichotomized into two groups: 0–<60 and ≥ 60 s. Additionally, to confirm the repeatability of the OLST, the OLST was re-evaluated more than 100 days after discharge in 25 patients (mean follow-up period 877 days [standard deviation (SD) 531 days]), and was observed as showing no difference between that during hospitalization and that at follow up (46 [SD 19] vs 51 [SD17] s).

Ankle brachial index

Bilateral ankle brachial index in the supine position was measured using an oscillometric device (Form PWV/ABI BP-203RPE; Omron Colin, Tokyo, Japan) during hospitalization, and the values for the two sides were averaged for use in statistical analysis.

Neurological examinations

Neurological examinations were carried out during hospitalization and within 3 months before the latest NC studies after

discharge. Positive neuropathic sensory symptoms included numbness, prickling or tingling, burning, freezing, aching, or lancinating pain in both toes, feet or legs¹⁰. Ankle reflexes were deemed abnormal if they were unequivocally decreased or absent despite reinforcement.

Current perception threshold detection

On the same day as the performance of the NC studies, as described below, current perception threshold (CPT) was measured in the right great toe using the Neurometer NS3000 (Neurotron Inc., Baltimore, MD, USA) to selectively assess the excitability of large myelinated A β fibers, small myelinated A δ fibers and small unmyelinated C fibers, by using an electrical stimulus with a sinusoidal alternating current at three different impulse frequencies of 2,000, 250 and 5 Hz, respectively. This device uses a patient-directed double-blind procedure that is automatically cycled (typically three to five times) with increasing electrical stimuli from 0 to 10 mA, until a minimum and constant threshold is obtained. The intra- and inter-rater reliability of the device has been previously reported¹¹.

Nerve conduction study

Peripheral motor nerve function was assessed for the median and tibial nerves, and peripheral sensory nerve function was assessed for the median and sural nerves during hospitalization and at follow up >1 year after discharge. NC studies were carried out by the standard method, as described elsewhere¹², with digital electromyographic equipment using surface electrodes for stimulation and recording (Viking Quest or EDX, Viking Electrodiagnostic Software Version 22.1; CareFusion Japan, Tokyo, Japan). If NC studies were repeatedly carried out after discharge, we analyzed data from the latest follow up.

Coefficient of variation of R-R interval

Coefficient of variation of R-R interval (CVRR) at rest and during deep breathing (6 breaths per min) was calculated from 100 R-R intervals recorded on an electrocardiogram (Cardio Star FCP-7541; Fukuda Denshi, Tokyo, Japan). This device can identify supraventricular arrhythmia as a narrow (≤ 120 ms) QRS complex and irregular adjacent R-R interval ($\geq 20\%$ shorter than the preceding R-R interval and the median value of all R-R intervals recorded), and automatically remove it from the CVRR analysis. Other electrocardiographic abnormalities, including premature atrial contraction with an abnormal P wave showing a different morphology and axis from sinus P waves, were detected by visual inspection on the R-R trend graphs and electrocardiographic waveforms of leads II or V5. We also calculated the absolute change in CVRR from rest to deep breathing to estimate the degree of enhancement of cardiac parasympathetic activity during deep breathing. CVRRs were measured during hospitalization and on the day when the latest NC studies were carried out after discharge.

Statistical analysis

The information obtained during hospitalization served as the baseline value for exploring the longitudinal effects of OLST on neuropathy outcomes and their association with the following three HbA1c values: (i) baseline HbA1c at admission; (ii) mean HbA1c over the follow-up period of >1 year after discharge (excluding baseline HbA1c); and (iii) absolute difference in HbA1c between admission (baseline) and final follow up, at the time when the latest NC studies were carried out. Continuous variables were evaluated as the mean (SD) or median (range) according to their distribution, whereas categorical variables were evaluated using counts and proportions. Differences in clinical variables between patients with OLST <60 s and those with OLST ≥ 60 s were assessed using the *t*-test or the Wilcoxon rank sum test (two-sided) for continuous variables, and Fisher's exact test for categorical variables. Differences in continuous and categorical variables between baseline and follow up were assessed using the Wilcoxon signed-rank sum test (two-sided) and the McNemar test, respectively. Pearson's correlation coefficient (*r*) was used to evaluate the correlation between the three HbA1c values and absolute differences between baseline and follow-up values of the parameters used to assess neuropathy outcomes. All statistical analyses were carried out by a qualified and experienced statistician (TS) using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Baseline characteristics

Baseline clinical and anthropometric characteristics of the entire cohort and of patients stratified according to OLST of <60 s ($n = 26$) and ≥ 60 s ($n = 32$) are shown in Table 1. For all patients, the median age and known diabetes duration was 50 (range 19–59) and 7 years (range 1–20), respectively. Patients with both OLST <60 s and OLST ≥ 60 s showed similar baseline characteristics, including anthropometric data, blood pressure, levels of HbA1c, fasting serum C-peptide and lipids, and microvascular complications.

Antihyperglycemic medications

Antihyperglycemic medications at admission and at follow up in the entire cohort, and in groups stratified according to an OLST of <60 s and OLST of ≥ 60 s are summarized in Table 2. The use of any antihyperglycemic medications at admission and at follow up did not differ between OLST <60 s and OLST ≥ 60 s groups. The use of sodium–glucose cotransporter 2 inhibitors was more frequent, and that of insulin was less frequent, at follow up than at admission, in the entire cohort, and in the OLST <60 s and OLST ≥ 60 s groups. The use of glucagon-like peptide (GLP)-1 receptor agonists (RAs) was more frequent at follow up than at admission only in the entire cohort. The use of injectable medications (i.e., insulins, GLP-1RAs or both) was less frequent at follow up than at admission in the entire cohort and in patients with OLST ≥ 60 s, but not in those with OLST <60 s. There was a trend ($P = 0.070$) toward less

Table 1 | Baseline clinical and anthropometric characteristics in the entire cohort and in patients stratified according to one-leg standing time with eyes open of <60 and ≥60 s

| | All patients (n = 58) | OLST <60 s (n = 26) | OLST ≥60 s (n = 32) | P-value |
|--|---------------------------|-------------------------|--------------------------|---------|
| OLST (s) | 46 ± 19 | 29 ± 17 | 60 | – |
| Men, n (%) | 40 (69.0) | 18 (69.2) | 22 (68.8) | 1.000 |
| Age (years) | 50 [19–59] | 53 [19–59] | 46 [30–58] | 0.168 |
| Diabetes duration (years) | 7 [1–20] | 8 [1–15] | 4 [1–20] | 0.346 |
| Missing (n) | 15 | 4 | 11 | |
| Alcohol use, n (%) | | | | |
| Never/former/current | 33/6/19 (56.9/10.3/32.8) | 18/3/5 (69.2/11.5/19.2) | 15/3/14 (46.9/9.4/43.8) | 0.141 |
| Smoking history, n (%) | | | | |
| Never/former/current | 14/20/24 (24.1/34.5/41.4) | 7/8/11 (26.9/30.8/42.3) | 7/12/13 (21.9/37.5/40.6) | 0.843 |
| Physical activity for at least 10 min/day, n (%) | | | | |
| None/<3 times/week/≥3 times/week | 28/9/10 (59.6/19.2/21.3) | 15/4/4 (65.2/17.4/17.4) | 13/5/6 (54.2/20.8/25.0) | 0.781 |
| Missing data (n) | 11 | 3 | 8 | |
| Body mass index (kg/m ²) | 25.6 ± 5.1 | 26.2 ± 5.5 | 25.2 ± 4.7 | 0.468 |
| Total body fat mass (%) | 27.9 ± 9.4 | 29.8 ± 9.6 | 26.5 ± 9.0 | 0.189 |
| Missing (n) | 1 | 1 | | |
| Total-body skeletal muscle mass (%) | 39.5 ± 5.3 | 38.2 ± 5.5 | 40.4 ± 4.9 | 0.118 |
| Lower limb skeletal muscle mass (%) | 22.5 ± 3.3 | 21.8 ± 3.4 | 23.0 ± 3.1 | 0.158 |
| Missing (n) | 1 | 1 | | |
| Skeletal muscle index (kg/m ²) | 7.6 ± 1.3 | 7.5 ± 1.4 | 7.7 ± 1.1 | 0.738 |
| Missing (n) | 1 | 1 | | |
| Ankle brachial index | 1.12 ± 0.08 | 1.12 ± 0.07 | 1.13 ± 0.08 | 0.832 |
| Systolic blood pressure (mmHg) | 125 ± 18 | 128 ± 23 | 122 ± 14 | 0.295 |
| Diastolic blood pressure (mmHg) | 79 ± 14 | 81 ± 16 | 77 ± 11 | 0.200 |
| HbA1c (%) | 10.9 ± 2.2 | 11.1 ± 2.1 | 10.8 ± 2.3 | 0.611 |
| HbA1c (mmol/mol) | 95.7 ± 24.5 | 97.5 ± 23.4 | 94.2 ± 25.5 | 0.611 |
| Fasting PG (mmol/L) | 9.2 ± 3.3 | 8.9 ± 3.1 | 9.5 ± 3.5 | 0.534 |
| Fasting serum C-peptide (nmol/L) | 0.57 ± 0.33 | 0.57 ± 0.38 | 0.57 ± 0.30 | 0.942 |
| eGFR (mL/min/1.73 m ²) | 87.8 ± 25.8 | 86.5 ± 30.3 | 88.9 ± 21.9 | 0.730 |
| Total cholesterol (mmol/L) | 4.8 ± 1.0 | 4.6 ± 1.0 | 4.9 ± 1.0 | 0.255 |
| Triglycerides (mmol/L) | 1.8 [0.5–7.3] | 1.6 [0.8–3.9] | 1.9 [0.5–7.3] | 0.306 |
| HDL cholesterol (mmol/L) | 1.1 ± 0.3 | 1.1 ± 0.3 | 1.1 ± 0.3 | 0.597 |
| Retinopathy, n (%) | 14 (24.1) | 7 (26.9) | 7 (21.9) | 0.761 |
| Albuminuria, n (%) | | | | |
| Normo/micro/macro | 34/17/3 (63.0/31.5/5.6) | 11/10/2 (47.8/43.5/8.7) | 23/7/1 (74.2/22.6/3.2) | 0.137 |
| Missing (n) | 4 | 3 | 1 | |

Data are presented as the number (%), mean ± standard deviation or median [range]. One-leg standing time with eyes open (OLST) was measured for a maximum of 60 s, and patients were dichotomized based on OLSTs of 0 to <60 and ≥60 s. Skeletal muscle index was calculated as the appendicular skeletal muscle mass in kilograms divided by the height in meters squared. Significant differences between patients with OLST <60 s and those with OLST ≥60 s were assessed using the unpaired *t*-test or the Wilcoxon rank-sum test (two-sided). Fisher's exact test was used for comparisons of the proportions of patients with OLST <60 s versus ≥60 s. Normoalbuminuria was defined as a urine albumin : creatinine ratio of <30 mg/g or <3.4 mg/mmol. Microalbuminuria was defined as a urine albumin : creatinine ratio of 30 to <300 mg/g or 3.4 to <33.9 mg/mmol. Macroalbuminuria was defined as a urine albumin : creatinine ratio of ≥300 mg/g or ≥33.9 mg/mmol. eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; PG, plasma glucose.

frequent use of dipeptidyl peptidase-4 inhibitors and more frequent use of GLP-1RA at follow-up than at admission only in patients with OLST <60 s.

Neurological examination

Results of neurological examinations at baseline and at follow up in the entire patient cohort, and in patients stratified into OLST <60 s and OLST ≥60 s groups are shown in Table 3.

The prevalence of sensory symptoms and abnormal ankle reflexes did not differ between patients in the OLST <60 and ≥60 s groups at baseline. We identified an additional one patient with sensory symptoms, and five patients with abnormal ankle reflexes at follow up, as compared with baseline in the group with OLST <60 s, and the prevalence of abnormal ankle reflexes was significantly higher in patients with OLST >60 s than in those with OLST ≥60 s at follow up.

Table 2 | Antihyperglycemic medications at admission and at follow up in the entire cohort and in the groups stratified according to one-leg standing time with eyes open <60 and ≥60 s

| Antihyperglycemic medications | All patients (n = 58) | OLST <60 s (n = 26) | OLST ≥60 s (n = 32) | P-value [†] (<60 s vs ≥60 s) |
|---|-----------------------|---------------------|---------------------|---------------------------------------|
| Metformin, n (%) | | | | |
| At admission | 48 (83) | 23 (88) | 25 (78) | 0.487 |
| At follow up | 41 (71) | 18 (69) | 23 (72) | 1.000 |
| P-value (at admission vs at follow-up) [‡] | 0.144 | 0.125 | 0.754 | |
| SGLT-2 inhibitors, n (%) | | | | |
| At admission | 7 (12) | 3 (12) | 4 (13) | 1.000 |
| At follow-up | 23 (40) | 10 (38) | 13 (41) | 1.000 |
| P-value (at admission vs at follow up) [‡] | 0.001* | 0.039* | 0.023* | |
| DPP-4 inhibitors, n (%) | | | | |
| At admission | 17 (29) | 11 (42) | 6 (19) | 0.081 |
| At follow up | 10 (17) | 5 (19) | 5 (16) | 0.740 |
| P-value (at admission vs at follow-up) [‡] | 0.119 | 0.070 | 1.000 | |
| Pioglitazone, n (%) | | | | |
| At admission | 6 (10) | 5 (19) | 1 (3) | 0.081 |
| At follow up | 9 (16) | 6 (23) | 3 (9) | 0.274 |
| P-value (at admission vs at follow-up) [‡] | 0.508 | 1.000 | 0.625 | |
| Sulfonylureas, n (%) | | | | |
| At admission | 3 (5) | 2 (8) | 1 (3) | 0.582 |
| At follow up | 1 (2) | 0 (0) | 1 (3) | 1.000 |
| P-value (at admission vs at follow up) [‡] | 0.625 | – | 1.000 | |
| GLP-1RAs, n (%) | | | | |
| At admission | 3 (5) | 2 (8) | 1 (3) | 0.582 |
| At follow up | 13 (22) | 8 (31) | 5 (16) | 0.213 |
| P-value (at admission vs at follow-up) [‡] | 0.013* | 0.070 | 0.219 | |
| Insulins, n (%) | | | | |
| At admission | 29 (50) | 13 (50) | 16 (50) | 1.000 |
| At follow up | 7 (12) | 3 (12) | 4 (13) | 1.000 |
| P-value (at admission vs at follow up) [‡] | <0.001* | 0.006* | 0.004* | |
| Injectable medications, n (%) | | | | |
| At admission | 29 (50) | 13 (50) | 16 (50) | 1.000 |
| At follow up | 16 (28) | 10 (38) | 6 (19) | 0.140 |
| P-value (at admission vs at follow up) [‡] | 0.024* | 0.581 | 0.021* | |

Data are presented as the number (%). *Statistical significance ($P < 0.05$). Injectable medications include insulin and glucagon-like peptide-1 receptor agonists (GLP-1RAs). DPP-4, dipeptidyl peptidase-4; SGLT, sodium–glucose cotransporter. [†]Fisher’s exact test was used for comparisons of the proportions of patients with one-leg standing time with eyes open (OLST) <60 s versus OLST ≥60 s. [‡]Categorical values at baseline and at follow-up were compared using McNemar’s test.

CPT detection

At baseline, there were no significant differences in any of the CPT values at the three frequencies of 2,000, 250 and 5 Hz between patients in the two OLST groups (Table 3). Median values of CPT at 2,000 Hz at follow up significantly increased and decreased compared with those at baseline in patients with OLST <60 s and OLST ≥60 s, respectively, and were significantly higher in patients with OLST <60 s than in those with OLST ≥60 s.

Nerve conduction study

Nerve conduction attributes measured at baseline and at follow up in the entire cohort, and in patients stratified into OLST <60 s and OLST ≥60 s groups are summarized in

Table 4. Mean follow-up periods between baseline and the final follow-ups were $1,204 \pm 685$ days in the entire cohort, and were similar in the OLST <60 s and OLST ≥60 s groups. Patients with OLST <60 s had significantly slower tibial motor NC velocities (MNCVs) and lower tibial compound muscle action potentials at both baseline and follow-up than those with OLST ≥60 s. Tibial MNCVs were significantly faster, and F-wave latencies were significantly shorter at follow up than at baseline in the entire cohort and in patients with OLST ≥60 s, but not in those with OLST <60 s. None of the attributes of sural sensory conduction differed between patients with OLST <60 s and OLST ≥60 s, nor did they differ between baseline and follow up in the entire cohort or in the two groups. Absence of evoked potentials was detected in

Table 3 | Results of neurological examinations and current perception threshold detection at baseline and follow up in the entire cohort and in patients segregated into one-leg standing time with eyes open <60 s and one-leg standing time with eyes open ≥60 s groups

| | All patients (n = 58) | OLST <60 s (n = 26) | OLST ≥60 s (n = 32) | P value (<60 s vs ≥60 s) |
|---------------------------------------|-----------------------|---------------------|---------------------|--------------------------|
| Sensory symptoms, n (%) | | | | |
| At baseline | 13 (22.4) | 8 (30.8) | 5 (15.6) | 0.213 |
| At follow up | 14 (24.1) | 9 (34.6) | 5 (15.6) | 0.126 |
| P-value (at baseline vs at follow up) | 1.000 | 1.000 | 1.000 | |
| Abnormal ankle reflexes, n (%) | | | | |
| At baseline | 36 (62.1) | 17 (65.4) | 19 (59.4) | 0.787 |
| At follow up | 41 (70.7) | 22 (84.6) | 19 (59.4) | 0.046* |
| P-value (at baseline vs at follow up) | 0.227 | 0.125 | 1.000 | |
| CPT (100 = 1 mA) | | | | |
| 2,000 Hz (Aβ fiber function) | | | | |
| At baseline | 260 [10–740] | 260 [10–740] | 280 [140–600] | 0.720 |
| Missing (n) | 5 | 2 | 3 | |
| At follow up | 280 [100–920] | 320 [120–920] | 260 [100–480] | 0.018* |
| Missing (n) | 2 | | 2 | |
| P-value (at baseline vs at follow up) | 0.902 | 0.024* | 0.011* | |
| 250 Hz (Aδ fiber function) | | | | |
| At baseline | 105 [15–300] | 98 [15–285] | 105 [45–300] | 0.399 |
| Missing, n | 5 | 2 | 3 | |
| At follow up | 113 [15–540] | 105 [30–540] | 120 [15–225] | 0.650 |
| Missing (n) | 2 | | 2 | |
| P-value (at baseline vs at follow up) | 0.743 | 0.503 | 0.265 | |
| 5 Hz (C fiber function) | | | | |
| At baseline | 70 [10–240] | 74 [10–240] | 60 [10–160] | 0.964 |
| Missing (n) | 5 | 2 | 3 | |
| At follow up | 50 [10–470] | 40 [10–470] | 65 [10–130] | 0.195 |
| Missing (n) | 2 | | 2 | |
| P-value (at baseline vs at follow up) | 0.116 | 0.156 | 0.456 | |

Data are presented as the number (%) or median [range]. *Statistical significance ($P < 0.05$). Fisher's exact test was used for comparisons of the proportions of patients with one-leg standing time with eyes open (OLST) <60 s versus OLST ≥60 s. Categorical values at baseline and at follow up were compared using McNemar's test. Significant differences in current perception threshold (CPT) values between patients with OLST <60 s and OLST ≥60 s, and between baseline and follow-up values were assessed using the Wilcoxon rank-sum test and Wilcoxon signed rank sum test (two-sided), respectively.

eight and seven patients with OLST <60 s at baseline and follow up, respectively.

CVRR

Coefficient of variation of R-R interval at rest was significantly lower at baseline, whereas CVRR during deep breathing and absolute changes in CVRR from rest to deep breathing were significantly lower at both baseline and follow up in patients with OLST <60 s than in those with OLST ≥60 s (Table 4). There were no significant differences in any measures of CVRR between baseline and follow up in the entire cohort or in the two groups.

Changes in HbA1c

Mean values of HbA1c changed from 11.1 (SD 2.1)% (97.5 [SD 23.4] mmol/mol) at admission to 8.1 (SD 1.6)% (65.4 [SD 17.5] mmol/mol) at follow up in patients with OLST <60 s, and from 10.8 (SD 2.3)% (94.2 [SD 25.5] mmol/mol) at

admission to 7.8 (SD 2.0)% (61.5 [SD 21.9] mmol/mol) at follow up in those with OLST ≥60 s (Figure 1). Absolute differences in HbA1c between baseline and follow up were similar between patients with OLST <60 s (mean -2.9 [SD 2.8]% [-32.1 (SD 30.3) mmol/mol]) and those with OLST ≥60 s (mean -3.0 [SD 3.1]% [-32.7 (SD 33.8) mmol/mol]). In contrast, the mean HbA1c over the follow-up period was significantly higher in patients with OLST <60 s than in those with OLST ≥60 s ($7.8 \pm 0.9\%$ [62.2 ± 10.4 mmol/mol] vs $7.2 \pm 1.2\%$ [54.8 ± 13.1 mmol/mol]; $P = 0.022$; Table 4). The number of measurements used for calculating the mean HbA1c did not differ between the two groups. Among the evaluated HbA1c measurements, values ≥8.0% were seen at least once in 10 patients (38.5%) in the OLST <60 s group and seven patients (21.9%) in the OLST ≥60 s group. Overall, the mean HbA1c tended to increase with a longer duration of follow up. Two patients in the OLST <60 s group and 10 in the OLST ≥60 s group had a mean HbA1c <6.5% over the first 3 years,

Table 4 | Follow-up period, mean glycosylated hemoglobin, nerve conduction attributes and coefficient of variation of the R-R interval measured at baseline and at follow up in the entire cohort and in groups stratified according to one-leg standing time with eyes open <60 and one-leg standing time with eyes open ≥60 s

| | All patients (n = 58) | OLST <60 s (n = 26) | OLST ≥60 s (n = 32) | P-value* (<60 s vs ≥60 s) |
|--|-----------------------|-------------------------|---------------------|---------------------------|
| Follow-up period (days) | 1,204 ± 685 | 1,209 ± 673 | 1,200 ± 706 | 0.963 |
| Mean HbA1c (%) over the follow-up period | 7.5 ± 1.1 | 7.8 ± 0.9 | 7.2 ± 1.2 | 0.022 [§] |
| Mean HbA1c (mmol/mol) over the follow-up period | 58.2 ± 12.4 | 62.2 ± 10.4 | 54.8 ± 13.1 | 0.022 [§] |
| No. HbA1c measurements during follow up | 14.0 ± 8.5 | 14.7 ± 8.2 | 13.4 ± 8.8 | 0.571 |
| No. (%) patients with at least one HbA1c measurement ≥8.0% (64 mmol/mol) | 17 (29.3) | 10 (38.5) | 7 (21.9) | 0.247 |
| Tibial nerve (motor) | | | | |
| MNCV (m/s) | | | | |
| At baseline | 41.0 ± 5.5 | 39.1 ± 5.6 | 42.5 ± 5.0 | 0.017 [§] |
| At follow up | 41.9 ± 5.5 | 39.6 ± 5.3 | 43.8 ± 5.0 | 0.003 [§] |
| P-value (at baseline vs at follow-up)** | 0.008 [§] | 0.260 | 0.015 [§] | |
| F-wave latency (ms) | | | | |
| At baseline | 53.1 ± 7.7 | 54.8 ± 8.4 | 51.4 ± 6.9 | 0.136 |
| Missing (n) | 1 [†] | 1 [†] | | |
| At follow up | 51.1 ± 8.0 | 53.3 ± 9.2 | 49.5 ± 6.7 | 0.082 |
| Missing (n) | 3 [†] | 2 [†] | | |
| P-value (at baseline vs at follow up)** | 0.001 [§] | 0.162 | 0.001 [§] | |
| CMAP, mV (ankle) | | | | |
| At baseline | 9.1 ± 4.4 | 7.1 ± 3.8 | 10.7 ± 4.2 | 0.001 [§] |
| At follow up | 9.2 ± 4.9 | 7.1 ± 5.0 | 11.0 ± 4.2 | 0.002 [§] |
| P-value (at baseline vs at follow up)** | 0.670 | 0.877 | 0.470 | |
| Sural nerve, sensory | | | | |
| SNCV (m/s) | | | | |
| At baseline | 48.9 ± 5.3 | 48.3 ± 5.9 [†] | 49.2 ± 4.9 | 0.568 |
| Missing (n) | 8 [†] | 8 [†] | | |
| At follow up | 49.6 ± 4.9 | 49.2 ± 4.7 | 49.9 ± 5.0 | 0.630 |
| Missing (n) | 7 [†] | 7 [†] | | |
| P-value (at baseline vs at follow up)** | 0.218 | 0.663 | 0.228 | |
| SNAP (µV) | | | | |
| At baseline | 9.6 ± 7.0 | 7.8 ± 7.3 | 11.1 ± 6.6 | 0.072 |
| At follow up | 9.2 ± 6.7 | 7.5 ± 7.5 | 10.6 ± 5.7 | 0.078 |
| P-value (at baseline vs at follow up)** | 0.410 | 0.677 | 0.477 | |
| CVRR | | | | |
| At rest (%) | | | | |
| At baseline | 2.3 ± 1.0 | 1.9 ± 0.6 | 2.6 ± 1.2 | 0.008 [§] |
| Missing (n) | 5 [†] | 2 [†] | 3 [†] | |
| At follow up | 2.3 ± 1.4 | 1.7 ± 0.7 | 2.6 ± 1.7 | 0.058 |
| Missing (n) | 6 [†] | 2 [†] | 4 [†] | |
| P-value (at baseline vs at follow up)** | 0.933 | 0.828 | 0.849 | |
| During deep breathing (%) | | | | |
| At baseline | 6.5 ± 3.2 | 5.1 ± 2.6 | 7.4 ± 3.3 | 0.007 [§] |
| Missing (n) | 4 [†] | 3 [†] | 1 [†] | |

Table 4. (Continued)

| | All patients (n = 58) | OLST <60 s (n = 26) | OLST ≥60 s (n = 32) | P-value* (<60 s vs ≥60 s) |
|---|-----------------------|---------------------|---------------------|---------------------------|
| At follow up | 6.1 ± 3.8 | 4.6 ± 2.3 | 7.2 ± 4.3 | 0.007 [§] |
| Missing (n) | 7 [†] | 2 [†] | 5 [†] | |
| P-value (at baseline vs at follow up)** | 0.251 | 0.236 | 0.565 | |
| Absolute change from rest to deep breathing (%) | | | | |
| At baseline | 4.2 ± 2.8 | 3.2 ± 2.6 | 4.9 ± 2.7 | 0.025 [§] |
| Missing (n) | 6 [†] | 3 [†] | 3 [†] | |
| At follow up | 3.8 ± 2.8 | 2.7 ± 2.1 | 4.7 ± 3.1 | 0.011 [§] |
| Missing (n) | 7 [†] | 2 [†] | 5 [†] | |
| P-value (at baseline vs at follow up)** | 0.297 | 0.290 | 0.643 | |

Data are presented as the number (%) or mean ± standard deviation. [§]Statistical significance ($P < 0.05$). CMAP, compound muscle action potential amplitude; CVRR, coefficient of variation of the R-R interval; HbA1c, glycated hemoglobin; MNCV, motor nerve conduction velocity; SNAP, sensory nerve action potential amplitude; SNCV, sensory nerve conduction velocity. *Significant differences between patients with one-leg standing time with eyes open (OLST) <60 s and OLST ≥60 s were assessed using the unpaired t-test (two-sided) or Fisher's exact test. **Significant differences between baseline and follow-up values were assessed using the paired t-test (two-sided). [†]Missing data due to absent evoked potentials. [‡]Missing data due to a history of arrhythmias or the occurrence of supraventricular arrhythmias or premature atrial contractions.

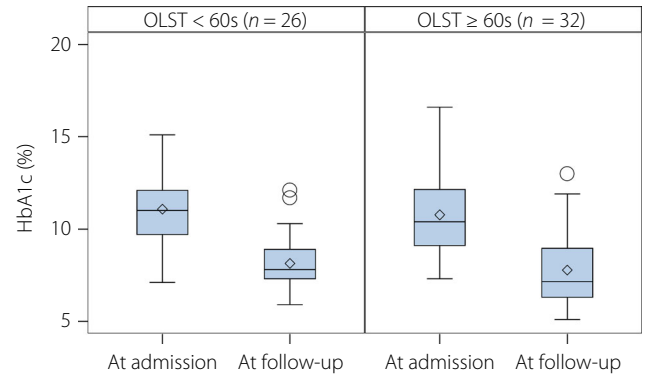


Figure 1 | Box and whisker plots showing the mean (diamonds in the boxes) and median (horizontal lines in the boxes) values of hemoglobin A1c (HbA1c) at admission (baseline) and follow up in patients with one-leg standing time with eyes open (OLST) <60 s and OLST ≥60 s. The boxes represent standard deviations. The whisker lines extend from the minimum to the maximum values, excluding any outliers. The outliers are plotted as individual open circles.

although none of the remaining 46 patients (24 (92.3%) in the OLST <60 s group and 22 (68.8%) in the OLST ≥60 s group) had a mean HbA1c of <6.5% over the follow-up period of up to 8 years.

Correlation between the three HbA1c values and neuropathy outcomes

We calculated Pearson's correlation coefficient (r) to assess the correlation between the three HbA1c values (baseline HbA1c, mean HbA1c during follow-up, and difference between baseline and follow-up values) and absolute differences in multiple measures of neuropathy outcomes between baseline and follow up (Table 5). As hypothesis testing depends on sample size, P -values were not applied when we sought to determine the associations between the variables examined in the present study. Instead, we took note of the association with absolute r ($|r|$) values >0.4.

Baseline HbA1c was positively associated with absolute differences in CPT values at 5 Hz ($r = 0.55$) and sensory nerve action potential amplitude (SNAP; $r = 0.48$) only in patients with OLST ≥60 s. The mean HbA1c over the follow-up period was positively associated with absolute differences in CPT values at 2,000 Hz ($r = 0.42$) and F-wave latency ($r = 0.43$), and was negatively associated with absolute differences in compound muscle action potential amplitude (CMAP; $r = -0.40$), sensory NC velocity (SNCV) ($r = -0.41$) and SNAP ($r = -0.42$) only in patients with OLST ≥60 s. Absolute differences in HbA1c between baseline and follow up were positively associated with absolute differences in CPT values at 2,000 Hz ($r = 0.44$) and F-wave latency ($r = 0.64$), and were negatively associated with absolute differences in CPT values at 5 Hz ($r = -0.45$), MNCV ($r = -0.55$), CMAP ($r = -0.43$) and SNAP ($r = -0.54$) only in patients with OLST ≥60 s (Figure 2).

Table 5 | Pearson's correlation coefficients for the three glycated hemoglobin values with respect to differences in neuropathy outcomes between baseline and follow-up in patients with one-leg standing time with eyes open <60 s and one-leg standing time with eyes open ≥60 s

| Absolute differences in neuropathy outcomes | Coefficient (95% CIs) | | | | | |
|---|-----------------------|----------------------|---------------------|----------------------|-------------------------|----------------------|
| | Baseline HbA1c (%) | | Mean HbA1c (%) | | Difference in HbA1c (%) | |
| | OLST <60 s (n = 26) | OLST ≥60 s (n = 32) | OLST <60 s (n = 26) | OLST ≥60 s (n = 32) | OLST <60 s (n = 26) | OLST ≥60 s (n = 32) |
| CPT (100 = 1 mA) | | | | | | |
| 2000 Hz (Aβ fiber function) | 0.37 (-0.04, 0.67) | -0.22 (-0.55, 0.17) | 0.02 (-0.38, 0.42) | 0.42 (0.04, 0.68) | -0.31 (-0.63, 0.11) | 0.44 (0.07, 0.70) |
| 250 Hz (Aδ fiber function) | -0.06 (-0.45, 0.35) | 0.01 (-0.37, 0.39) | -0.08 (-0.47, 0.34) | 0.30 (-0.10, 0.61) | -0.01 (-0.41, 0.40) | 0.19 (-0.21, 0.53) |
| 5 Hz (C fiber function) | -0.09 (-0.47, 0.33) | 0.55 (0.21, 0.77) | -0.13 (-0.51, 0.29) | -0.05 (-0.42, 0.34) | 0.02 (-0.39, 0.42) | -0.45 (-0.70, -0.07) |
| Tibial nerve (motor) | | | | | | |
| MNCV (m/s) | 0.28 (-0.13, 0.60) | 0.27 (-0.09, 0.56) | 0.22 (-0.19, 0.55) | -0.37 (-0.63, -0.02) | -0.21 (-0.55, 0.20) | -0.55 (-0.75, -0.23) |
| F-wave latency (ms) | -0.21 (-0.57, 0.21) | -0.38 (-0.65, -0.03) | -0.04 (-0.44, 0.37) | 0.43 (0.08, 0.68) | 0.27 (-0.15, 0.61) | 0.64 (0.36, 0.81) |
| CMAP, mV (ankle) | -0.02 (-0.40, 0.37) | 0.22 (-0.14, 0.53) | -0.06 (-0.44, 0.33) | -0.40 (-0.65, -0.06) | 0.12 (-0.28, 0.48) | -0.43 (-0.68, -0.09) |
| Sural nerve (sensory) | | | | | | |
| SNCV (m/s) | 0.32 (-0.21, 0.70) | 0.02 (-0.33, 0.36) | 0.07 (-0.44, 0.55) | -0.41 (-0.66, -0.06) | -0.25 (-0.66, 0.29) | -0.21 (-0.52, 0.16) |
| SNAP (μV) | -0.04 (-0.42, 0.36) | 0.48 (0.15, 0.70) | -0.14 (-0.50, 0.27) | -0.42 (-0.67, -0.07) | -0.03 (-0.41, 0.36) | -0.54 (-0.74, -0.23) |
| CVRR | | | | | | |
| At rest (%) | 0.02 (-0.40, 0.43) | -0.10 (-0.48, 0.31) | -0.17 (-0.50, 0.27) | -0.29 (-0.61, 0.12) | -0.12 (-0.50, 0.31) | -0.10 (-0.48, 0.30) |
| During deep breathing (%) | -0.27 (-0.62, 0.18) | -0.30 (-0.61, 0.09) | -0.31 (-0.64, 0.14) | -0.21 (-0.54, 0.19) | 0.14 (-0.30, 0.53) | 0.10 (-0.30, 0.46) |
| Absolute change from rest to deep breathing (%) | -0.32 (-0.65, 0.13) | -0.33 (-0.64, 0.08) | -0.31 (-0.64, 0.14) | -0.07 (-0.45, 0.33) | 0.18 (-0.26, 0.56) | 0.25 (-0.17, 0.58) |

Ci, confidence interval; CMAP, compound muscle action potential amplitude; CPT, current perception threshold; CVRR, coefficient of variation of the R-R interval; HbA1c, glycated hemoglobin; MNCV, motor nerve conduction velocity; OLST, one-leg standing time with eyes open; SNAP, sensory nerve action potential amplitude; SNCV, sensory nerve conduction velocity. †Absolute values of the coefficient that are >0.4.

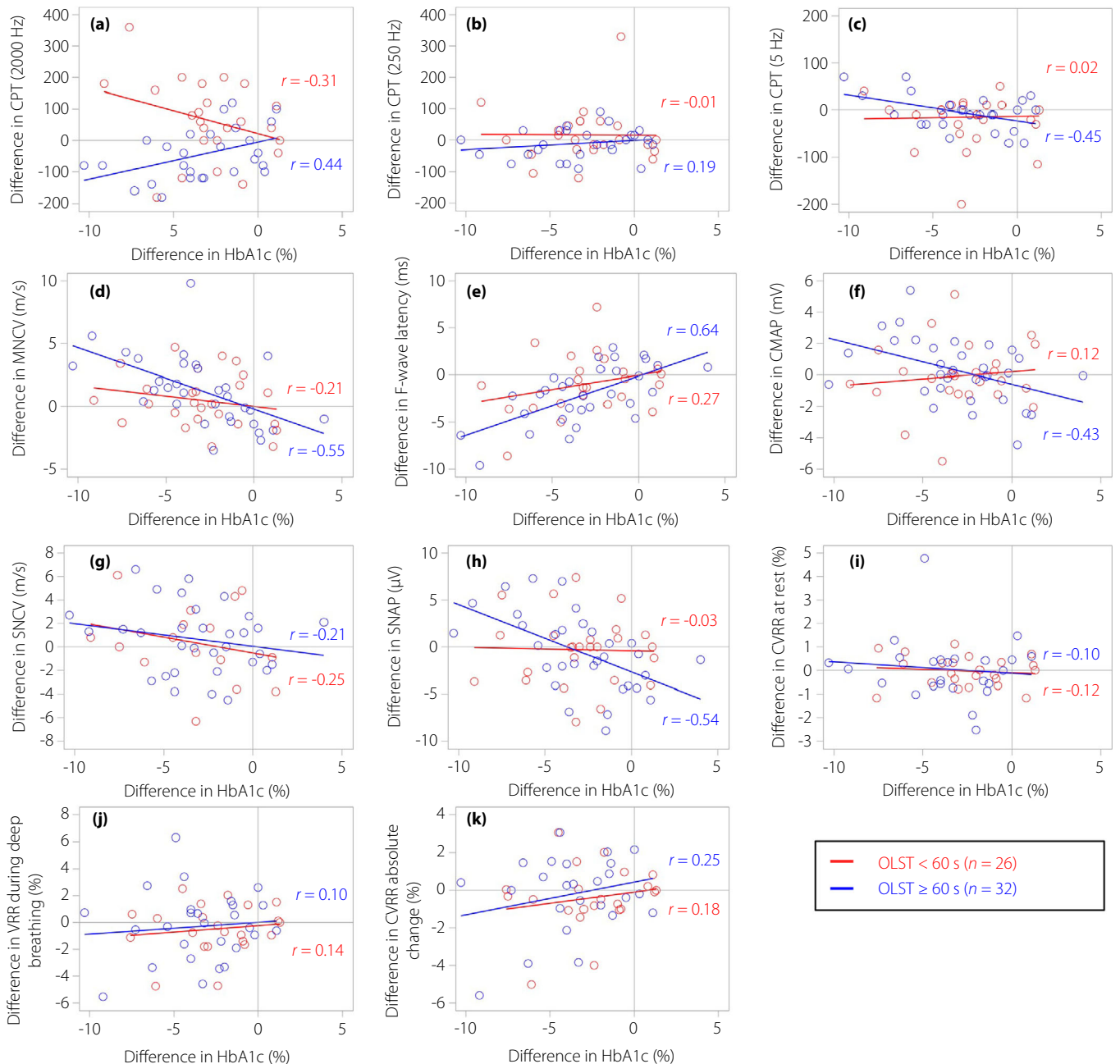


Figure 2 | Linear regression lines for absolute differences in hemoglobin A1c (HbA1c) between baseline and follow up versus absolute differences in current perception threshold (CPT) values at (a) 2000 Hz, (b) 250 Hz and (c) 5 Hz, (d) motor nerve conduction velocity (MNCV), (e) F-wave latency, (f) compound muscle action potential amplitude (CMAP), (g) sensory nerve conduction velocity (SNCV), (h) sensory nerve action potential amplitude (SNAP), (i) CVRR at rest, (j) coefficient of variation of the R-R interval (CVRR) during deep breathing and (k) absolute change in CVRR between baseline and follow up in patients with one-leg standing time with eyes open (OLST) <60 s and OLST ≥60 s. *r*, Pearson's correlation coefficient.

DISCUSSION

In the present retrospective, longitudinal, cohort study, non-elderly patients (aged <60 years) with OLST <60 and ≥60 s shared similar baseline clinical and anthropometric

characteristics, as well as microvascular complications (Table 1), and were treated with similar antihyperglycemic medications (Table 2) at admission. Although HbA1c decreased significantly in both the OLST groups after discharge, an additional one and

five patients developed sensory symptoms and abnormal ankle reflexes, respectively, over the follow-up period of up to 8 years only in the OLST <60 s group (Table 3). Median values of CPT at 2,000 Hz at follow up increased significantly in patients with OLST <60 s, whereas they decreased in those with OLST \geq 60 s compared with the respective values at baseline (Table 3), suggesting higher and lower likelihoods of developing large myelinated A β fiber dysfunction in patients with OLST <60 s and OLST \geq 60 s after discharge, respectively. In contrast, we did not detect any changes in CPT values at 250 or 5 Hz between baseline and follow-up in the two OLST groups, suggesting no significant longitudinal effects of OLST or HbA1c reduction after discharge on small myelinated A δ fiber function or unmyelinated C fiber function, respectively. The reasons for such different outcomes in the different subclasses (large vs small) of sensory fibers remain unknown. Recently, small sensory fiber neuropathy has been linked to metabolic syndrome¹³. As insulin receptors with a higher affinity for insulin are predominantly expressed in small- to medium-sized sensory neurons^{14,15}, we speculate that impaired availability of insulin might preferentially affect small sensory fiber function. This assumption, however, should be tested in future studies.

The present study evaluated objective measurements of peripheral (NC) and cardiac autonomic nerve function (CVRR; Table 4), and showed significant decreases in MNCV, CMAP, and CVRR at rest and during deep breathing, as well as in its absolute change from rest to deep breathing at both baseline and follow up in patients with OLST <60 s compared with those with OLST \geq 60 s. These findings are consistent with our previous study that showed the association between short OLST and peripheral and cardiac autonomic nerve dysfunction⁸. Additionally, a significant increase in MNCV and decrease in F-wave latency at follow up, as compared with baseline, were observed in patients with OLST \geq 60 s, showing that better motor nerve function outcomes can be expected in patients with a longer OLST. In contrast, large sensory nerve functions were well preserved, as shown by SNCV and SNAP, both of which were close to normal (our hospital reference values for SNCV and SNAP developed in 141 healthy volunteers are \geq 50.7 m/s and \geq 8.0 μ V, respectively), and did not differ between the two OLST groups at either baseline or follow up. Therefore, OLST might be more closely related to motor nerve functions rather than large sensory nerve functions, an assumption that also needs to be addressed in future studies.

HbA1c at both baseline and follow up did not differ between patients with OLST <60 and \geq 60 s. Substantial decreases in HbA1c were associated with less frequent use of insulin in both the OLST groups after discharge, which likely reflected the individualized clinical decisions to achieve the best possible glycemic control while minimizing hypoglycemia. In contrast, the mean HbA1c over the follow-up period was significantly higher in patients with OLST <60 s than in those with OLST \geq 60 s, which might be because glycemic control tended to deteriorate sooner after discharge in patients with OLST <60 s than in

those with OLST \geq 60 s. In fact, over the first 3 years, just two patients with OLST <60 s showed mean HbA1c <6.5%, compared with 10 patients in the OLST \geq 60 s group. Suboptimal glycemic control associated with OLST <60 s might trigger the immediate intensification of antihyperglycemic medications, especially with a switch from dipeptidyl peptidase-4 inhibitors to GLP-1RA over the follow-up period (Table 2). As our recent study showed that patients with OLST <60 s were less likely to discontinue injectable medications than those with OLST \geq 60 s during hospitalization¹⁶, the present results suggest that patients with a short OLST might bear an increasing burden of antihyperglycemic medications required to achieve optimal glycemic control.

Cumulative glycemic exposure has been linked to the development of microvascular complications of diabetes, including DN¹⁷. Maintenance of near-normoglycemia from the diagnosis of type 1 diabetes over 24 years completely prevents peripheral and autonomic nerve dysfunction, as well as clinical neuropathy¹⁸. In patients with uncontrolled type 2 diabetes of short duration and DN, reduction in HbA1c to near-normal levels (mean HbA1c 6.1%) over 4.3 years more effectively improves DN than does standard glycemic control (mean HbA1c 7.0%)¹⁹. Nevertheless, achieving near-normoglycemia seems to be less effective in terms of preventing the onset and progression of DN in type 2 diabetes than in type 1 diabetes^{20,21}. In the Rochester Diabetic Neuropathy Study cohort following patients with both type 1 and type 2 diabetes for up to 20 years, cumulative glycemic exposure explained only a minor part of the variability of the severity of complications, indicating the role of other putative mechanisms for DN¹⁷. In the present study, lower mean HbA1c over the follow-up period of up to 8 years was associated ($|r| > 0.4$) with better neuropathy outcomes, as shown by absolute differences in the values of CPT (at 2,000 Hz) and NC attributes (F-wave latency, CMAP, SNCV and SNAP) between baseline and follow up, only in patients with OLST \geq 60 s and not in those with OLST <60 s (Table 5). The reasons for this discrepancy in the impact of better long-term glycemic control on neuropathy outcomes between OLST <60 s and OLST \geq 60 s groups remain unclear, although it is possible that a short OLST associated with decreased peripheral nerve function might be a potential biomarker for identifying intractable DN that is difficult to treat even after achieving better glycemic control.

We found that absolute differences in HbA1c between baseline and follow up had slightly stronger associations with absolute differences in some measures of neuropathy outcomes (such as MNCV, F-wave latency, CMAP and SNAP) than did mean HbA1c over the follow-up period in patients with OLST \geq 60 s (Table 5). This suggests that the degree to which improvements in glycemic control can be achieved has a greater influence on neuropathy outcomes than the long-term maintenance of glycemic control in these patients. Among the three HbA1c values assessed, baseline HbA1c was found to have the weakest association with changes in neuropathy

outcomes (Table 5), underscoring the importance of future improvements in glycemic control to obtain better DN outcomes in patients with uncontrolled type 2 diabetes.

In the present study, we unexpectedly found that none of the three HbA1c measurements had high associations ($|r| > 0.4$) with absolute differences in CVRR measurements, suggesting the involvement of risk factors other than hyperglycemia, such as age, diabetes duration, obesity, hypertension, dyslipidemia and other microvascular complications, in the cardiac autonomic dysfunction of type 2 diabetes^{22,23}.

There were several limitations to the present study. First, as the mean HbA1c over the follow-up period was higher in patients with OLST <60 s, their long-term glycemic control might have been insufficient for improving nerve function. However, as shown in Figure 2, up to a 10% decrease in HbA1c from baseline to follow up was not associated with significant improvements in neuropathy outcomes in these patients, supporting the idea that patients with a shorter OLST might be more likely to have intractable DN. Second, glycemic variability cannot be assessed by measuring HbA1c alone, and is worth further investigation as a possible risk factor for both large²⁴ and small fiber neuropathy¹⁹. Third, as each patient had different follow-up periods, spurious associations between the follow-up period and the variables examined cannot be ruled out. However, inconsistent (positive/inverse/no) associations between the follow-up period and mean HbA1c and changes in neuropathy outcomes between patients with OLST <60 s and OLST ≥ 60 s (data not shown) precluded us from adjusting for the follow-up period in the observed associations. Finally, unexpected associations existed between baseline HbA1c and absolute differences in SNAP, and between absolute differences in HbA1c and absolute differences in CPT values at 5 Hz (Table 5). These findings suggest that hyperglycemia at baseline and its persistence at follow up might result in better outcomes in terms of certain sensory functions in patients with uncontrolled type 2 diabetes. These observations warrant further confirmation in future large prospective studies, especially as they seem contrary to the commonly held clinical beliefs.

In conclusion, the present study results suggest that non-elderly uncontrolled type 2 diabetes patients with OLST <60 s and decreased peripheral nerve function might be at an increased risk for intractable DN and cross a “point of no return”, beyond which better glycemic control alone might not lead to better neuropathy outcomes. Further study is required to confirm these findings and assumptions.

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DISCLOSURE

The authors declare no conflict of interest.

Approval of the research protocol: All analyses were carried out with the approval of the Ethics Committee of Ohta Nishinouchi Hospital (approval no 32; approval date 7 June 2019), and in accordance with the Helsinki Declaration of 1964 and its later amendments, as well as with the ethical guidelines for medical and health research involving human subjects issued by the Ministry of Health, Labor and Welfare of Japan in 2017. Informed consent: N/A.

Registry and the registration no. of the study/trial: N/A.

Animal studies: N/A.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 | Flow diagram of the study participants.