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Nonalcoholic fatty liver disease is associated with an increased prevalence of distal symmetric polyneuropathy in adult patients with type 1 diabetes

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ABSTRACT

Aims: Presently, data on the association between nonalcoholic fatty liver disease (NAFLD) and distal symmetric polyneuropathy in people with diabetes are scarce and conflicting. The aim of this retrospective, cross-sectional study was to examine whether NAFLD was associated with an increased prevalence of distal symmetric polyneuropathy in type 1 diabetic adults.

Methods: We studied all white type 1 diabetic outpatients ($n = 286$, 42.3% male, mean age 43 ± 14 years, median diabetes duration 17 [10–30] years), who participated in a foot screening program at our adult diabetes clinic after excluding those who had excessive alcohol consumption and other known causes of chronic liver disease. NAFLD was diagnosed by ultrasonography. Distal symmetric polyneuropathy was detected using the Michigan Neuropathy Screening Instrument method and the biothesiometer Vibrotest.

Results: Overall, the prevalence rates of NAFLD and distal symmetric polyneuropathy were 52.4% and 35.3%, respectively. Patients with NAFLD had a substantially increased prevalence of distal symmetric polyneuropathy compared to their counterparts without NAFLD (51.0% vs. 17.1%, $p < 0.001$). In univariate analysis, NAFLD was associated with an approximately 5-fold increased risk of prevalent distal symmetric polyneuropathy (odds ratio [OR] 5.32, 95% confidence interval [CI] 3.1–9.3, $p < 0.001$). This association remained significant even after adjustment for age, sex, diabetes duration, hemoglobin A1c, diabetic retinopathy, smoking, metabolic syndrome, chronic kidney disease and carotid artery stenoses ≥ 40% (adjusted-OR 2.23, 95% CI 1.1–4.8, $p < 0.05$).

Conclusions: Our results show that NAFLD, diagnosed by ultrasonography, is strongly associated with an increased risk of distal symmetric polyneuropathy in type 1 diabetic adults, independently of several cardio-metabolic risk factors.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide (occurring in up to 30% of adults in Western countries) and has now become the second most frequent indication for liver transplantation in the United States with the worrying prospect of becoming the first indication in the coming years.[1–3]

The prevalence of NAFLD in people with diabetes is much higher, ranging from 50% to 75% in patients with type 2 diabetes and from 40% to 50% in those with type 1 diabetes.[3,4] In addition, patients with diabetes are more likely to develop the more severe histologic forms of NAFLD, including nonalcoholic steatohepatitis (NASH), advanced fibrosis and cirrhosis.[3,4] To date, convincing evidence indicates that NAFLD is associated not only with considerable liver-related morbidity and mortality, but also with an increased risk of cardiovascular disease (CVD), chronic kidney disease (CKD) and other important extra-hepatic complications both in patients without diabetes and in those with type 1 or type 2 diabetes.[5–9]

In parallel, distal symmetric polyneuropathy, a chronic, nerve-length-dependent, sensory and motor polyneuropathy, is the most common form of diabetic neuropathy that affects at least one third of patients with type 1 or type 2 diabetes.[10–12] Notably, the presence of distal symmetric polyneuropathy not only confers a predisposition to painless foot ulcers and subsequent amputations, but is also associated with an increased risk of all-cause and CVD mortality.[13–15] It is well known that poor glycemic control is the strongest risk factor for the development of diabetic peripheral

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neuropathy, but other important pathological conditions, such as dyslipidemia, hypertension, diabetic retinopathy, nephropathy and smoking, may play important roles.[10,16–18] Given that all these risk factors and pathological conditions are also strongly associated with NAFLD (irrespective of pre-existing diabetes), and that NAFLD is associated with an increased risk of diabetic nephropathy (another chronic microvascular complication of diabetes),[7,8] it is reasonable to assume that there is an association between NAFLD and diabetic distal symmetric polyneuropathy. Currently, however, published studies that have evaluated the existence of such association are very few and have produced conflicting results.[19–21]

Thus, the aim of this retrospective, observational study was to assess whether NAFLD, as diagnosed by ultrasonography, was associated with an increased prevalence of distal symmetric polyneuropathy in type 1 diabetic adults.

2. Methods

2.1. Patients

For the purpose of this study, we have retrospectively analyzed the electronic records of all white outpatients with established type 1 diabetes ($n = 563$), who regularly attended our adult diabetes clinic and accepted to participate in a foot-screening program (over the years 2004–2012). The only exclusion criteria for participating in this screening program were the presence of foot ulcers, gangrene or prior amputations. Type 1 diabetes was diagnosed by the typical presentation of disease, the absolute dependence on insulin treatment for survival, the presence of undetectable fasting C-peptide levels and the presence of anti-islet cell auto-antibodies.[10]

We have subsequently excluded all patients with: (1) missing liver ultrasound data ($n = 184$, 32.7%); (2) a history of end-stage renal disease or malignancy ($n = 6$, 1%); and (3) a documented history of cirrhosis of any etiology or chronic liver disease due to secondary causes, such as excessive alcohol consumption (i.e., defined as alcohol consumption >30 g/day for men and >20 g/day for women, respectively), viral hepatitis or use of steatogenic medications ($n = 87$, 15.4%).

As a consequence of this selection, 286 (50.8%) adult outpatients with established type 1 diabetes (mean age 43 ± 14 years; 42.3% male; median duration of diabetes 17 [10–30] years) were included in the final analysis. Details of the study design are summarized in Supplemental Fig. 1.

No significant differences were found in main demographic/laboratory variables and frequency of distal symmetric polyneuropathy between patients with ($n = 286$) and without ($n = 184$) liver ultrasound examination (data not shown). It is important to note that in our diabetes clinic an ultrasound examination of the liver is almost routinely performed among the outpatients with diabetes.

The local ethics committee approved the study protocol. The informed consent requirement for this study was exempted by the ethics committee, because researchers only accessed retrospectively a de-identified database for analysis purposes.

2.2. Clinical and laboratory data

Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared. Blood pressure was measured in duplicate with a standard mercury manometer after a rest of at least 5 min. Patients were considered as having hypertension if their blood pressure was ≥130/85 mm Hg or if they were taking any anti-hypertensive drugs. Detailed information on smoking status, daily alcohol consumption and current use of medications was obtained from all patients via interviews during medical examinations.

Venous blood was drawn in the morning after an overnight fast. Serum lipids, creatinine (measured using a Jaffe´ rate-blanked and compensated assay), liver enzymes and other biochemical blood measurements were determined by standard laboratory procedures on Siemens Dimension Vista (Siemens, Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA). Normal ranges for serum alanine and aspartate aminotransferase levels (ALT and AST) in our laboratory were 10–40 U/l for both men and women. Normal ranges for serum gamma-glutamyltransferase (GGT) levels were 5–50 units/l for women and 5–55 U/l for men, respectively. LDL-cholesterol was calculated by the Friedewald's equation. No patients had serum triglyceride levels above 4.5 mmol/l. Hemoglobin A1c (HbA1c) was measured by a high-performance liquid chromatography analyzer on Tosoh G7 automated analyzer (Tosoh Bioscience Inc., San Francisco, CA; USA); the upper limit of normal for our laboratory was 5.6% (38 mmol/mol). The estimated glomerular filtration rate ($eGFR_{MDRD}$) was calculated by the 4-variable Modification of Diet in Renal Disease (MDRD) study equation.[23] Albuminuria was measured using an immuno-nephelometric method on a morning spot urine sample and expressed as the albumin-to-creatinine ratio (ACR) on Beckman-Coulter IMMAGE (Beckman-Coulter Instruments, Fullerton, CA; USA). The presence of CKD was defined as an $eGFR_{MDRD} < 60$ ml/min/1.73 m^2, macro-albuminuria (defined as an urinary ACR >30 mg/mmol), or both.

Metabolic syndrome was diagnosed by a modified Adult Treatment Panel (ATP)-III definition, as waist circumference was available only in few patients ($n = 89$). Accordingly with this modified ATP-III definition,[24,25] a patient with type 1 diabetes was classified as having the metabolic syndrome if he/she had at least two of the following four components: (i) BMI >28 kg/m^2 in men or >27 kg/m^2 in women; (ii) triglycerides ≥1.7 mmol/l; (iii) HDL-cholesterol <1.0 mmol/l in men and <1.29 mmol/l in women or receiving lipid-lowering drugs; and (iv) blood pressure ≥ 130/85 mm Hg or receiving anti-hypertensive drugs.

A single ophthalmologist diagnosed diabetic retinopathy using fundoscopy after pupillary dilation according to a clinical disease severity scale (no retinopathy, non-proliferative, proliferative or laser-treated retinopathy). The presence of proliferative retinopathy was confirmed by fundus fluorescein angiography. The presence of atherosclerotic plaques (i.e., stenosis ≥40%) at the level of either the internal or common carotid arteries was also detected by echo-Doppler scanning in all patients.

2.3. Assessment of diabetic peripheral neuropathy

To assess diabetic peripheral neuropathy, we applied a validated Michigan Neuropathy Screening Instrument method (MNSI), as described elsewhere.[22,26] Since this method was designed only for screening purposes, the obtained results can be referred to 'possible' distal symmetric polyneuropathy. A trained nurse administered the MNSI questionnaire to all our patients. This questionnaire consists of 15 "yes or no" questions, including one relevant to general asthenia and one relevant to peripheral vascular disease, as described by the Michigan Clinic.[27] After administration of the MNSI questionnaire, we also performed in all patients: (*i*) *a* foot inspection looking for the presence of deformities (e.g., hammer toes, overlapping toes, hallux valgus, joint subluxation, prominent metatarsal heads), dry skin, callus, infection or ulceration; (*ii*) a non-invasive, quantitative assessment of vibration sensation threshold (VPT) at the dorsum of the great toe using a biothesiometer Vibrotest instead that a 128-Hz tuning fork; and (*iii*) a grading of ankle reflexes (normal, reduced, or absent). The results of physical examination were as follows: 1 point in presence of alterations of the foot at inspection, 1 point to a pathologic ankle reflex (0.5 point when evocable with the Jendrassik maneuver), and 1 point to a VPT higher than 25 Volt. Evaluating both feet, a maximum score of 8 points for each patient was possible.

Table 1
Clinical and biochemical characteristics of adult patients with type 1 diabetes stratified by NAFLD status.

Variables	Without NAFLD ($n = 136$)	With NAFLD ($n = 150$)	p Value
Sex (men, %)	36.0	48.0	0.027
Age (years)	38.8 ± 13.1	47.5 ± 14.2	<0.01
Diabetes duration (years)	13 (8–20)	24 (12–33)	<0.01
BMI (kg/m^2)	22.7 ± 3.4	26.1 ± 4.7	<0.01
Current smokers (%)	22.0	24.0	0.402
Systolic blood pressure (mm Hg)	124.5 ± 16.4	132.1 ± 17.4	<0.01
Diastolic blood pressure (mm Hg)	76.1 ± 7.9	79.7 ± 9.4	<0.05
Fasting glucose (mmol/l)	10.3 ± 4.2	10.8 ± 4.0	0.332
HbA1c (%)	7.8 ± 1.0	8.2 ± 1.2	0.011
Total cholesterol (mmol/l)	4.5 ± 0.9	4.9 ± 1.1	<0.01
LDL cholesterol (mmol/l)	2.5 ± 0.6	2.8 ± 0.9	<0.05
HDL cholesterol (mmol/l)	1.5 ± 0.4	1.3 ± 0.4	0.010
Triglycerides (mmol/l)	0.92 (0.7–1.2)	1.21 (0.8–1.9)	<0.01
$eGFR_{MDRD}$ (ml/min/1.73 m^2)	94 ± 25	83 ± 23	<0.01
Urinary ACR (mg/mmol)	1 (0.7–2.3)	4 (1.1–10)	<0.01
AST (U/l)	17 (13–25)	18 (13–24)	0.091
ALT (U/l)	18 (14–24)	21 (14–29)	<0.05
GGT (U/l)	14 (10–21)	21 (13–39)	<0.01
Hypertension (%)	30.8	60.7	<0.01
Metabolic syndrome (%)	21.3	52.7	<0.01
CKD (%)	4.6	22.7	<0.01
Diabetic retinopathy, any degree (%)	54.8	73.9	<0.01
Carotid artery stenosis ≥40% (%)	1.0	9.3	<0.01
Daily total insulin units (U)	36 (25–48)	45 (32–61)	<0.01
Anti-hypertensive therapy (%)	18.3	51.3	<0.01
Lipid-lowering therapy (%)	17.2	53.4	<0.01
Distal symmetric polyneuropathy (%)	17.1	51.0	<0.001

Sample size, $n = 286$. Data are expressed as means ± SD, medians and interquartile ranges (IQR) or percentages. Abbreviations: ACR, urinary albumin-to-creatinine ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CKD, chronic kidney disease; $eGFR_{MDRD}$, estimated glomerular filtration rate (by the MDRD study equation); GGT, gamma-glutamyltransferase, HbA1c, hemoglobin A1c.
Notes: CKD was defined as $eGFR_{MDRD}$ < 60 ml/min/1.73 m^2 and/or macro-albuminuria; metabolic syndrome was defined by a modified ATP III definition; hypertension was defined as blood pressure ≥ 130/85 mm Hg or drug treatment.

Presence of a score higher than 2 was considered to be pathological. A further evaluation of sensitivity was performed by Semmes–Weinstein monofilament (a plastic handle connected to a nylon monofilament, which measures clinically significant large-fiber neuropathy by buckling at 10 g of force). Based on a 9-point score, the monofilament test was considered pathological when the patient was not able to correctly detect more than 5 points.[28] In the literature, the number of points to be pressed (from one to ten) varies widely. In this study, data on cardiovascular autonomic neuropathy were not available.

2.4. Liver ultrasonography

Liver ultrasonography was performed by experienced radiologists, who were blinded to the patients' clinical details. Hepatic steatosis was diagnosed on the basis of characteristic ultrasonographic characteristics, such as diffuse hyperechogenicity of the liver relative to the kidneys, ultrasonography beam attenuation, and poor visualization of the intrahepatic vessel borders and diaphragm.[29] It is known that ultrasonography has a good sensitivity and specificity for detecting moderate-to-severe hepatic steatosis, while its sensitivity is reduced when the hepatic fat infiltration detected by liver biopsy is less than 25%–30%.[30] In this study, a semi-quantitative ultrasonographic scoring of the degree of hepatic steatosis was not available.

2.5. Statistical analysis

Data are expressed as means ± SD, medians and interquartile ranges (IQR) or proportions. Differences in clinical and biochemical characteristics of participants were assessed using the unpaired Student's t test for normally distributed variables and the Mann–Whitney U test for non-normally distributed variables (i.e., diabetes duration, serum liver enzymes, triglycerides, albuminuria and daily insulin dose). The chi-squared test was used to test for between-group differences among the categorical variables (Table 1). Logistic regression analysis was used to examine the association between NAFLD and risk of prevalent distal symmetric polyneuropathy after adjustment for diabetes-related variables and other potential confounding factors. As reported in Table 2, five forced-entry logistic regression models were performed as follows: the first model was unadjusted; the second model was adjusted for age and sex (adjusted model 1); and the third model was adjusted for age, sex, diabetes duration, HbA1c, smoking history and presence of the metabolic syndrome (adjusted model 2). The fourth model was additionally adjusted for CKD, diabetic retinopathy and presence of carotid artery stenoses ≥40% (adjusted model 3). Finally, we also performed a fifth model (adjusted model 4) adjusted for age, sex, duration of diabetes, HbA1c, smoking, BMI, hypertension, atherogenic dyslipidemia, CKD, diabetic retinopathy and presence of carotid artery stenoses ≥ 40%. Covariates included in these multivariate logistic regression models were chosen as potential confounding factors based on their significance in univariate analyses or based on their biological plausibility. A p-value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS software, version 21.0 (IBM Corp, Armonk, NY).

Table 2
Logistic regression analyses—association between NAFLD and the risk of prevalent distal symmetric polyneuropathy in adult patients with type 1 diabetes.

Logistic regression models	Odds ratio	95% CI	p Value
NAFLD (yes vs. no)			
Unadjusted model	5.32	3.1–9.3	<0.001
Adjusted model 1	4.09	2.3–7.4	<0.001
Adjusted model 2	2.52	1.3–4.9	<0.01
Adjusted model 3	2.23	1.1–4.8	<0.05
Adjusted model 4	2.47	1.1–5.5	<0.05
Other independent predictors in adjusted model 3			
Diabetes duration (years)	1.09	1.05–1.2	<0.001
HbA1c (%)	1.52	1.1–2.1	<0.01
Diabetic retinopathy (yes vs. no)	1.79	1.1–2.9	<0.05

Sample size, $n = 286$. Data are presented as odds ratios (±95% confidence intervals, CI) by logistic regression analyses. Distal symmetric polyneuropathy was the dependent variable in all regression models.
Other covariates included in these multivariate logistic regression models, along with NAFLD, were as follows: *model 1*: adjusted for age and sex; *model 2*: adjustment for age, sex, duration of diabetes, HbA1c, smoking history and presence of the metabolic syndrome (by a modified ATP III definition); *model 3*: adjustment for the same variables included in model 2 *plus* CKD (i.e., $eGFR_{MDRD}$ < 60 ml/min/1.73 m^2 and/or macro-albuminuria), diabetic retinopathy and presence of carotid artery stenoses ≥ 40%; *model 4*: adjustment for age, sex, duration of diabetes, HbA1c, smoking, BMI, hypertension (i.e., blood pressure ≥ 130/85 mm Hg or drug treatment), atherogenic dyslipidemia, CKD, diabetic retinopathy and presence of carotid artery stenoses ≥ 40%.

3. Results

Overall, 150 (52.4%) patients had NAFLD (i.e., hepatic steatosis on ultrasonography among patients with no history of excessive alcohol consumption or other known causes of chronic liver diseases), while the remaining 136 (47.6%) patients did not. Moreover, the prevalence of distal symmetric polyneuropathy in the entire sample was 35.3% ($n = 101$). In our electronic database, the actual scores for MNSI questionnaire, VPT and MNSI examination were available only for a subgroup of 123 patients (43% of total) because only some operators had also reported the actual scores, whereas others had reported exclusively the semi-quantitative information about the severity of distal symmetric polyneuropathy (i.e., mild, moderate, or severe) without simultaneously reporting the actual scores for each MNSI questionnaire, VPT or MNSI examination. Among patients with distal

symmetric polyneuropathy, the mean scores for MNSI questionnaire, VPT and MNSI examination were 5.7 ± 2.9, 33.2 ± 7.4 Volt and 4.1 ± 1.3, respectively. Monofilament testing was pathological in 41% of these patients. Overall, approximately 50% of our patients with distal symmetric polyneuropathy had a moderate-to-severe neuropathy. By study design, no patients had foot ulcers and prior history of amputations.

Table 1 summarizes the main clinical and biochemical characteristics of adult patients with type 1 diabetes grouped by presence or absence of NAFLD. Compared to those without NAFLD, patients with NAFLD were older, more likely to be male and obese, had longer duration of diabetes and higher values of systolic/diastolic blood pressure, HbA1c, serum lipids, albuminuria and liver enzymes (although many patients with NAFLD had fairly normal serum liver enzyme levels). Moreover, they were also more likely to be treated with lipid-lowering and anti-hypertensive drugs, and had lower $eGFR_{MDRD}$ and greater prevalence of the ATP-III defined metabolic syndrome, CKD, diabetic retinopathy and carotid artery stenoses ≥40%. Notably, patients with NAFLD had a remarkably higher prevalence of distal symmetric polyneuropathy compared with their counterparts without NAFLD (51.0% vs. 17.1%, $p < 0.001$).

Fig. 1 shows the prevalence of distal symmetric polyneuropathy in patients stratified by both NAFLD status and HbA1c tertiles (i.e., 1st tertile: ≤7.5%, 2nd tertile: 7.6%–8.1%, and 3rd tertile: >8.1%). Patients with NAFLD and in the 3rd tertile of HbA1c had the highest prevalence of distal symmetric polyneuropathy, whereas those with NAFLD in the 1st tertile of HbA1c had the lowest prevalence of distal symmetric polyneuropathy. Notably and most importantly, in each HbA1c tertile, patients with NAFLD had always a significantly higher prevalence of distal symmetric polyneuropathy than those without NAFLD.

We also compared the baseline clinical and biochemical characteristics of patients stratified by presence or absence of distal symmetric polyneuropathy. Patients with distal symmetric polyneuropathy were older (50.7 ± 14 vs. 38.6 ± 12 years, $p < 0.01$), more likely to be obese (BMI 25.6 ± 4.8 vs. 23.8 ± 4.2 kg/m^2, $p < 0.01$) and had a longer duration of diabetes (median and IQR: 30 [20–37] vs. 12 [8–20] years, $p < 0.01$) compared to those without distal symmetric polyneuropathy. Moreover, they were also more likely to be treated with lipid-lowering and anti-hypertensive drugs, and had significantly lower $eGFR_{MDRD}$, higher values of albuminuria, serum ALT and GGT, and greater prevalence of metabolic syndrome, CKD, diabetic retinopathy or carotid artery stenoses ≥40% than those without symmetric polyneuropathy (data not shown). Notably, NAFLD prevalence was remarkably greater in patients with distal symmetric polyneuropathy than in those without (76.5% vs. 38.0%, $p < 0.01$).

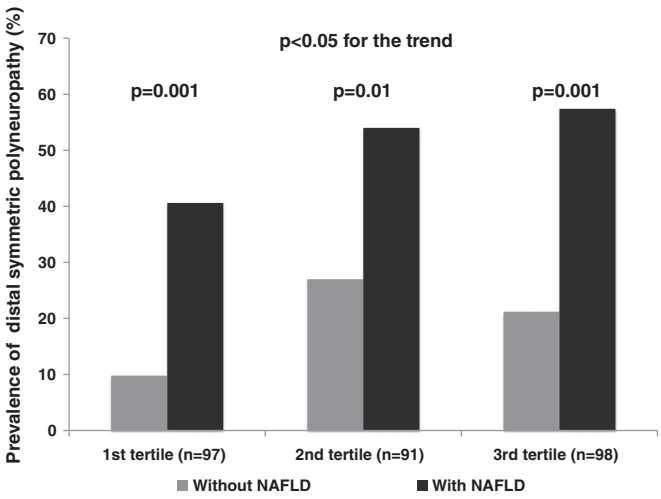

Fig. 1. Prevalence of distal symmetric polyneuropathy in adult patients with type 1 diabetes stratified simultaneously by both NAFLD status and tertiles of hemoglobin A1c (i.e., 1st tertile: ≤7.5%, 2nd tertile: 7.6%–8.1%, and 3rd tertile: >8.1%). *p*-Values = 0.001–0.01 for between-group differences and $p < 0.05$ for the overall trend by the χ^2 test.

Table 2 shows the effect of adjustment for traditional CVD risk factors and diabetes-related variables on the association between NAFLD and the risk of distal symmetric polyneuropathy. In univariable regression analysis, NAFLD was associated with an approximately 5-fold increased risk of prevalent distal symmetric polyneuropathy (OR 5.32, 95% CI 3.1–9.3, $p < 0.001$). This association remained significant after adjustment for age and sex (model 1). Furthermore, the strength of this association was only partially attenuated after adjustment for age, sex, diabetes duration, HbA1c, smoking and presence of the metabolic syndrome (model 2). When we additionally adjusted our results for the presence of CKD, diabetic retinopathy and carotid artery stenoses ≥40% the strong association between NAFLD and distal symmetric polyneuropathy remained unchanged (model 3). As also shown in Table 2, in regression model 3, other variables (along with NAFLD) that were independently associated with distal symmetric polyneuropathy were longer duration of diabetes, higher HbA1c and presence of diabetic retinopathy. To examine whether the inclusion of hypertension or anti-hypertensive treatment or blood pressure values in the cluster of the metabolic syndrome might have smoothed the impact of hypertension per se on distal symmetric polyneuropathy, we have also performed a regression model 4 where we adjusted for the individual components of the metabolic syndrome (instead of the metabolic syndrome as a clinical category) and the same set of covariates of model 3. Also in this case, the strong association between NAFLD and distal symmetric polyneuropathy remained essentially unchanged. Hypertension was not independently associated with distal symmetric polyneuropathy.

4. Discussion

Distal symmetric polyneuropathy, or diabetic peripheral neuropathy, is very common and often the most difficult microvascular complication to diagnose and manage because it is frequently asymptomatic and mostly untreatable, except for symptomatic measures. Distal symmetric polyneuropathy is not only linked with the development of foot complications and subsequent lower limb amputations, but also strongly associated with an increased risk of CVD complications in patients with type 1 or type 2 diabetes.[13–15] For instance, the EURODIAB IDDM Complications Study, including 2787 adult patients with type 1 diabetes, has clearly documented that distal symmetric polyneuropathy is a powerful risk factor for future mortality, exceeding even the effect of traditional CVD risk factors.[14]

Over the last decade, it has become increasingly evident that NAFLD is associated not only with increased liver-related mortality and morbidity, but also with an increased prevalence and incidence of CVD and CKD both in patients with and without diabetes.[1–9]

To our knowledge, this is the largest cross-sectional study aimed at examining the association between ultrasound-diagnosed NAFLD and distal symmetric polyneuropathy in type 1 diabetic adults of European extraction.

The main findings of our study are as follows: (1) type 1 diabetic adults with NAFLD on ultrasonography had a substantially higher prevalence of distal symmetric polyneuropathy than those without NAFLD; (2) when we stratified patients by NAFLD status and HbA1c tertiles, patients with NAFLD in the highest tertile of HbA1c (i.e., >8.1%) had the greatest prevalence of distal symmetric polyneuropathy, whereas patients with NAFLD in the 1st tertile of HbA1c had the lowest prevalence of distal symmetric polyneuropathy; however, it is important to remember that HbA1c level reflects only the glycemic control of a relatively short period (approximately 3 months) and it is not a mean value of longer observation periods; (3) logistic regression analysis showed that NAFLD was associated with an approximately 5-fold increased risk of prevalent distal

symmetric polyneuropathy; and (4) this association remained statistically significant even after adjustment for diabetes-related variables (i.e., diabetes duration, HbA1c and diabetic retinopathy) and established cardio-metabolic risk factors (including also the presence of metabolic syndrome features).

Previous epidemiological studies have reported a high prevalence of the metabolic syndrome (ranging from 20% to 50%) in type 1 diabetic adults[31,32] that might confer a predisposition to develop NAFLD in this patient population. Notably, our findings confirm previous observations demonstrating that NAFLD is a highly prevalent pathologic condition in type 1 diabetic adults that affects up to 50% of these patients.[33–35] Moreover, our findings also extend previously published studies that have shown that ultrasound-diagnosed NAFLD is associated with an increased prevalence of asymptomatic/symptomatic CVD[33] as well as an increased risk of CKD and diabetic retinopathy in patients with type 1 diabetes, independently of several cardio-renal risk factors.[34–36] Collectively, these findings call for a more active and systematic search for NAFLD in type 1 diabetic adults.

At present, published studies that examined the existence of an association between NAFLD and distal symmetric polyneuropathy are very few, have provided conflicting results and have been conducted mainly in Asian populations with type 2 diabetes.[19–21] In fact, in a retrospective study involving 927 South Korean patients with type 2 diabetes, Kim et al.[19] did not find any significant difference in the prevalence of diabetic peripheral neuropathy among patients with and without NAFLD. Lv et al.[20] showed that NAFLD on ultrasonography was associated with lower prevalence rates of both distal symmetric polyneuropathy and diabetic nephropathy in a sample of 1217 Chinese inpatients with type 2 diabetes. More recently, Williams et al.[21] studied two small cohorts of Australian diabetic people from a tertiary diabetes center (cohort 1, $n = 456$ with type 1 or 2 diabetes, and cohort 2, $n = 106$ with type 2 diabetes). All underwent a detailed assessment, including VPT measurement. NAFLD fibrosis score, a non-invasive marker of advanced fibrosis, was calculated for all with available data. Cohort 2 also had liver ultrasound examination and transient elastography. These investigators reported that higher VPT was significantly associated with higher markers of liver fibrosis due to NAFLD in both cohorts. Moreover, in this study, higher NAFLD fibrosis score was also associated with a greater risk of progression of VPT over a median period of 2.2 years.[21]

The most obvious explanation for our findings is that the strong association between NAFLD and distal symmetric polyneuropathy arises from the coexistence of established risk factors for diabetic neuropathy, such as longer duration of diabetes, poor glycemic control, smoking history, presence of metabolic syndrome, diabetic retinopathy or CKD. However, it is important to underline that, in our study, the association between NAFLD and the risk of prevalent distal symmetric polyneuropathy remained statistically significant even after adjusting for all these coexisting clinical risk factors. This finding strongly suggests that additional NAFLD-related mechanisms, beyond of the shared cardio-metabolic risk factors, might be, at least in part, responsible for the association between NAFLD and distal symmetric polyneuropathy. To date, a clear understanding of the pathophysiological pathways linking NAFLD to the development of distal symmetric polyneuropathy remains elusive. However, accumulating evidence suggests that NAFLD, especially in its necro-inflammatory form (NASH), exacerbates hepatic and peripheral insulin resistance, confers a predisposition to atherogenic dyslipidemia, and causes the release of several proinflammatory, procoagulant, prooxidant and profibrogenic mediators[3,5–7,37,38] that may play important roles in the pathophysiology of distal symmetric polyneuropathy. For example, some studies suggested that atherogenic dyslipidemia may directly induce nerve damage through lipotoxicity of free fatty acids and, indirectly, by free fatty acids stimulating a systemic inflammatory cytokine cascade and increasing insulin resistance.[38,39] Furthermore, several experimental studies have clearly demonstrated that proinflammatory and prooxidant mediators play a central role in the pathophysiology of distal symmetric polyneuropathy.[11,39–42] However, further studies are required to better elucidate the underlying mechanisms by which NAFLD may contribute to the development and progression of distal symmetric polyneuropathy.

Our study has some important limitations that should be mentioned. Firstly, the retrospective, cross-sectional design of the study limits our ability to determinate the causality and temporality of these observations. Secondly, we cannot exclude a possible selection bias of excluding patients who had missing liver ultrasound examinations (although no significant differences were found in demographic/laboratory variables and diabetic peripheral polyneuropathy between patients with and without liver ultrasound examination). In addition, another possible selection bias of this study is that we included patients with type 1 diabetes, who attended a foot-screening program (i.e., individuals at high risk for neuropathy; indeed, a large part of our participants had a moderate-to-severe symmetric polyneuropathy). Thus, our results cannot be necessarily generalizable to other diabetic populations (e.g., individuals with mild symmetric polyneuropathy). Thirdly, the diagnosis of distal symmetric polyneuropathy was not confirmed through measurement of nerve-conduction velocities or skin biopsy, which represent the 'gold standard' methods for diagnosing diabetic peripheral polyneuropathy. Indeed, these invasive neurologic tests are restricted only to selected patients and are not suitable for routinely use in clinical practice, as they are time-consuming, patient-unfriendly, and costly, and need to be performed by an expert operator. Conversely, we used the MNSI clinical score. Although the MNSI clinical score dates back to 1994, it is still considered valid and it has been recently used to measure peripheral neuropathy in high-risk non-diabetic, pre-diabetic and newly diagnosed diabetic patients.[26] Furthermore, we used a biothesiometer instead of a 128-Hz tuning fork, as it is a simple, reproducible and more accurate test than the 128-Hz tuning fork.[43] Importantly, in our patients, the assessment of diabetic peripheral neuropathy by biothesiometer was based on VPT values that have been previously validated in diabetic and non-diabetic Italian individuals.[44] Moreover, since our patients were participating in a foot-screening program, we used a pathological cut-off of 25 Volt, which is a VPT value associated with an increased risk of foot ulcers.[45] Fourthly, although we adjusted our results for a number of potential confounding factors, we cannot definitely exclude that residual and unmeasured factors (e.g., cardiovascular autonomic neuropathy that is also associated with NAFLD[46]) might partly explain the observed association between NAFLD and distal symmetric polyneuropathy. Finally, the diagnosis of NAFLD was based on ultrasonography, which has a relatively low sensitivity in the presence of smaller amounts of hepatic steatosis (i.e., <20%–30%), and was not confirmed through liver biopsy (the 'gold standard'). However, we believe that it would have been unethical to perform liver biopsies in our diabetic patients with normal or only moderately elevated serum liver enzymes. In a recent meta-analysis, Hernaez et al.[30] showed that ultrasonography enables a reliable and accurate detection of mild-to-moderate hepatic steatosis compared to histology. In fact, these investigators reported that the overall sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio of ultrasonography for detecting moderate-to-severe fatty liver, compared to histology, were 84.8%, 93.6%, 13.3 and 0.16, respectively.[30] Thus, although some non-differential misclassification of NAFLD based on ultrasonography is likely, this limitation would attenuate the magnitude of our effect measures toward null; therefore, our results are most likely a conservative estimate of the association between NAFLD and distal symmetric polyneuropathy.

Despite these limitations, our study has several important strengths, such as the relatively large sample size, the performance of neurologic examinations/tests by the same examiners (so reducing the inter-observer variability), the ability to adjust for multiple

established risk factors, and the exclusion of patients with important comorbidities, such as cirrhosis, end-stage renal disease or cancer. Indeed, we believe that including patients with these complications might have confounded our interpretation of data.

In conclusion, this retrospective, cross-sectional study is the first to show that NAFLD as diagnosed by ultrasonography was significantly associated with a fivefold increased risk of prevalent distal symmetric polyneuropathy in adult patients with type 1 diabetes. Notably, this association was independent of multiple established cardio-metabolic risk factors (including also glycemic control and metabolic syndrome features). Further research is needed to determine whether NAFLD increases the risk of developing distal symmetric polyneuropathy, and to elucidate whether improvement in NAFLD (or future treatments for NAFLD) will help to delay or prevent the development and progression of distal symmetric polyneuropathy in type 1 diabetic adults.

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