ORIGINAL ARTICLE

Evaluation of sudomotor function in adult patients with long-lasting type 1 diabetes

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KEY WORDS

diabetes, microvascular complications, neuropathy, small nerve fibers, sudomotor function

ABSTRACT

INTRODUCTION The function of the sweat glands appears to be impaired in patients with diabetic complications.

OBJECTIVES The aim of the study was to evaluate sudomotor function in adult patients with type 1 diabetes (DM1) and healthy controls and its relationship with metabolic control and diabetic complications.

PATIENTS AND METHODS The study group included 404 patients with DM1 (194 women), aged 41 years (interquartile range [IQR], 32–51 years) and with disease duration of 23 years (IQR, 18–31 years). The control group included 84 healthy volunteers. Electrochemical skin conductance (ESC) in the feet and hands was measured in both groups.

RESULTS Patients with DM1 had lower ESC than controls (feet: 80 μS [IQR, 65–85 μS] vs 83 μS [IQR, 78.5–87 μS], $P < 0.001$; hands: 63 μS (IQR, 51–75 μS) vs 69 μS (IQR, 61.5–78.5 μS), $P < 0.001$). In the study group, there was a negative correlation between ESC and patients' age, duration of diabetes, waist-to-hip ratio, skin autofluorescence, vibration perception threshold, as well as hemoglobin A_{1c} and triglyceride levels, and a positive correlation with estimated glomerular filtration rate. Microvascular complications were diagnosed in 73.3% of the patients. Patients with retinopathy, diabetic kidney disease, peripheral neuropathy, and cardiac autonomic neuropathy had lower ESC in the feet and hands compared with those without complications. In multivariate logistic regression models, ESC was associated with the presence of any microvascular complications independently of potential confounders.

CONCLUSIONS Diabetic microangiopathy, and in particular neuropathy, is related with reduced sudomotor function in DM1. A longer duration of diabetes, worse metabolic control, and reduced renal function are associated with greater sudomotor dysfunction.

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INTRODUCTION Microvascular complications are an important concern in the course and management of diabetes. Despite the availability of screening tests, they are often diagnosed too late, reduce the quality of life, lead to disability, and increase mortality and morbidity in diabetic patients. Therefore, new accurate and noninvasive methods are needed for the early diagnosis of these complications.

Microangiopathy is associated with dysfunction and structural changes of small vessels. A neurovascular concept of diabetic complications has been postulated.[1] Vessel wall tension and blood flow are regulated by the autonomic nervous system. It is suggested that impaired blood flow in nerve nutrient vessels leads to degeneration of peripheral nerves.[2,3] The perfusion deficit causes endoneurial hypoxia sufficient to compromise nerve function and initiates neurodegeneration.[4,5] Therefore, impaired microvascular circulation observed in diabetes is reflected in impaired function of the peripheral nervous system.[6] On the other hand, neurodegeneration in the form of the neuronal apoptosis and reactive gliosis has recently been postulated as early changes in diabetic retinopathy. Elimination of neurons is preceded by functional abnormalities within the retina.[7] Therefore, the dysfunction of the neurons and vessels in diabetes cannot be separated.

The sweat glands are innervated by thin, unmyelinated C-fibers. Degeneration of small fibers

TABLE 1 Clinical characteristics of the study and control groups

Parameter	Healthy controls (n = 84)	Diabetic patients (n = 404)	*P* value[a]
age, y	40 (32–51)	41 (32–51)	0.68
sex, women/men	42 (50)/42 (50)	194 (48)/210 (52)	0.74
duration of diabetes, y	–	23 (18–30.5)	–
smoking	15 (17.9)	121 (30.0)	0.025
history of hypertension	12 (14.3)	185 (45.8)	<0.001
BMI, kg/m^2	24 (22–27)	25 (23–28)	0.12
WHR	0.87 (0.8–0.93)	0.86 (0.80–0.93)	0.99
HbA_{1c}, %	–	8.0 (7.2–8.9)	–
hs-CRP, mg/l	0.7 (0.5–1.4)	1.3 (0.6–2.5)	0.017
TG, mmol/l	1 (0.6–1.4)	1.1 (0.8–1.4)	0.07
LDL cholesterol, mmol/l	3 (2.3–3.8)	2.8 (2.2–3.6)	0.34
HDL cholesterol, mmol/l	1.7 (1.3–1.9)	1.7 (1.4–2.0)	0.33
creatinine, mg/dl	0.86 (0.73–0.94)	0.88 (0.78–1.01)	0.13
eGFR (MDRD), ml/min/1.73 m^2	88 (82–95)	90 (77–102)	0.82
skin AF, AU	2 (1.8–2.4)	2.3 (2–2.7)	<0.001
VPT, V	–	18.8 (13.9–29.8)	–
Michigan Neuropathy Scale, n	–	3 (1–7)	–
ESC, feet, µS	83 (78.5–87)	80 (65–85)	<0.001
ESC, hands, µS	69 (61.5–78.5)	63 (51–75)	<0.001
risk of cardiac autonomic neuropathy, %	7.5 (0–18)	21 (9–33)	<0.001

Data are presented as median (interquartile range) or number (percentage) of patients.

a diabetic patients vs healthy controls; the Mann–Whitney test for continuous variables and the χ^2 test for categorical variables were used; a *P* value of less than 0.05 was considered statistically significant.

Abbreviations: AF, autofluorescence, BMI, body mass index; eGFR, estimated glomerular filtration rate, ESC, electrochemical skin conductance; HbA_{1c}, hemoglobin A_{1c}; HDL, high-density lipoprotein; hs-CRP, high-sensitive C-reactive protein; LDL, low-density lipoprotein; MDRD, Modification of Diet in Renal Disease; TG, triglyceride; VPT, vibration perception threshold; WHR, waist-to-hip ratio

reduces the innervation of the sweat glands and impairs their function, which is the clinical manifestation of small fiber neuropathy.[8,9] Sudomotor function appears to be impaired in patients with diabetic complications. The 2010 Guidelines of the European Federation of Neurological Societies and the Peripheral Nerve Society acknowledged intraepidermal nerve fiber density (IENFD), assessed in skin biopsy, as the most reliable and effective technique for confirming the clinical diagnosis of small fiber neuropathy.[10] However, skin biopsy is an invasive procedure that is not widely used for assessing diabetic neuropathy. There have been attempts to assess early nerve damage by confocal corneal microscopy; however, the method is costly and the availability of trained personnel is greatly limited.[11] The evaluation of sudomotor function might not only allow a simple assessment of small fiber neuropathy but possibly also of diabetic microangiopathy in general. SUDOSCAN+ is a device used for noninvasive assessment of neuropathy on the basis of the function of the sweat glands, but it has never been examined in a large homogenous group of patients with type 1 diabetes (DM1).

Therefore, the aim of this study was to evaluate sudomotor function using the SUDOSCAN+ device in adult patients with DM1 and healthy controls, as well as to assess the relationship of sudomotor function with metabolic control and diabetic complications.

PATIENTS AND METHODS

Patients Our study involved 404 patients (194 women) with DM1, treated at the Department of Internal Medicine and Diabetology of the Poznan University of Medical Sciences (Poznań, Poland) between the years 2013 and 2015. The minimum duration of diabetes was 5 years. The exclusion criteria were as follows: pregnancy, mental and neurological disorders, alcohol abuse, active foot ulceration or limb amputation in the past, and implanted electronic devices. The median age of patients was 41 years (interquartile range [IQR], 32–51 years). The median disease duration was 23 years (IQR, 18–31 years). The clinical characteristic of the study population are presented in TABLE 1. All patients gave written informed consent to participate in the study, which was approved by the local Bioethics Committee of the Poznan University of Medical Sciences. Participants were divided into subgroups, depending on the presence or absence of microangiopathy. The healthy control group consisted of 84 age- and sex-matched volunteers (hospital employees and their families), including 42 women. The median age of the control group was 40 years (IQR, 32–51 years).

Data collection All patients completed a questionnaire, which contained information on the duration of diabetes, family history, medications, comorbidities, history of smoking, and the Michigan Neuropathy Scale.[12] All patients underwent a physical examination, which included anthropometric measurements.

Laboratory tests Blood samples were collected in a fasting state defined as no caloric intake for at least 8 hours. The glucose concentration in venous plasma, as well as the serum concentrations of total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides (TGs), and creatinine were measured using standard methods. The serum concentration of C-reactive protein was measured using a highly sensitive method. Hemoglobin A_{1c} (HbA_{1c}) was determined by high-performance liquid chromatography. The estimated glomerular filtration rate (eGFR) was calculated according to the Modification of Diet in Renal Disease Study Group formula. Albuminuria was assessed by measuring urinary albumin excretion over a 24-hour period using an immunoturbidimetric test.

Evaluation of the skin content of advanced glycation end products of proteins by autofluorescence measurement To evaluate long-term glycemic control, the degree of accumulation of advanced glycation

end products (AGEs) of proteins in the skin was assessed using the fluorescent properties of the tissue. The autofluorescence (AF) ratio was measured using the AGE-Reader (DiagnOptics, Groningen, the Netherlands). The device has a source of ultraviolet radiation with a wavelength range of 300 to 420 nm and an optic spectrometer. AF is the quotient of the mean AF intensity of the light emitted by the skin in the wavelength range of 420 to 600 nm and the average light intensity in the wavelength range of 300 to 420 nm emitted by the light source. AF is multiplied by 100 and expressed in arbitrary units. For each patient, AF was measured 3 times in a series. The result is the arithmetic mean of these 3 measurements. Each test lasted 30 seconds and was performed at the ventral side of the forearm, about 5 cm distal to the elbow.[13]

Assessment of microvascular complications Diabetic retinopathy was diagnosed using direct ophthalmoscopy through dilated pupils, followed in all patients by fundus photography. Fundus examinations were performed using an indirect Volk lens. Subsequently, using a 45° digital camera (VISUCAM, Zeiss, Oberkochen, Germany), 2 fundus photographs were taken of each eye: one centered on the fovea and the other centered on the optic disc. The results of both ophthalmoscopy and fundus photographs were evaluated for all patients by the same ophthalmologist with experience in diabetic retinopathy. Patients were divided into 2 groups: a group with any diagnosed stage of retinopathy and the group without retinopathy.

Diabetic kidney disease (DKD) was detected at the stage of albuminuria. Albuminuria was defined as a urinary albumin excretion rate between 30 and 300 mg/24 h in 2 of 3 samples collected over a 3-month period, after the exclusion of secondary causes of proteinuria (urinary tract infection, heart failure, acute febrile illness, hematuria, or excessive physical activity). DKD was defined as the presence of albuminuria or eGFR of less than 60 ml/min/1.73 m^2 (or both).[14]

Evaluation of peripheral neuropathy Our study was based on the Toronto definition of probable peripheral neuropathy.[15] Symptoms were assessed on the basis of information collected during medical history. In a standard physical examination, the ankle reflex and peripheral sensation (temperature using a ThipTherm, touch using a 10-g monofilament, vibration using a 128-Hz tuning fork) were evaluated. Diabetic neuropathy was diagnosed as the presence of 2 or more of the following: the presence of symptoms, lack of the ankle reflex, and impaired sensation of touch, temperature, or vibration.

Vibration perception threshold Vibration perception threshold (VPT) was assessed using a neurothesiometer (Horwell Neurothesiometer NEU 1, Scientific Laboratory Supplies, Nottingham, United Kingdom). The voltage applied to the vibrator is adjusted by a rotary knob. The set range was from 0 to 50 volts with 1-volt increments. The vibration frequency is 50 Herz. The result is the arithmetic mean of the rates measured at 8 points on each foot.

Assessment of autonomic neuropathy The assessment of cardiac autonomic neuropathy (CAN) was performed using the ProsciCard III program (Medi-Syst GmbH, Linden, Germany). Heart rate variability was monitored under the influence of certain standardized stimuli (while in the supine position, during deep breathing, Valsalva maneuver, orthostatic test). During the test, an electrocardiogram was monitored on a computer screen. The program recognized the R wave and calculated consecutive R-R intervals and their variability. Based on the analysis of the spread of R-R intervals, specific parameters were calculated to evaluate CAN, which were then compared to the standard values for age and sex. Cardiac autonomic neuropathy was diagnosed if 2 of the 4 tests were abnormal.

SUDOSCAN+ device SUDOSCAN+ (Impeto Medical, Paris, France) consists of 2 sets of electrodes (one set for the feet, the other for the hands), which are connected to a computer for data analysis. The patient places his or her hands and feet on steel electrodes. The test is noninvasive and painless. It takes about 2 minutes, during which the low-voltage current (<4 V) flows through the electrodes. The device records the electrochemical reaction between the sweat chlorides and the stainless-steel electrodes, which is expressed in conductivity units. ESC is the ratio between the current generated and the constant direct current stimulus applied on the electrodes, which is expressed in microsiemens (μS). A lower ESC value denotes worse sudomotor function. The normal ESC values provided by the manufacturer are as follows: for women, 75 μS (IQR, 57–87 μS) in the hands and 83.5 μS (IQR, 71–90 μS)in the feet; and for men, 76 μS (IQR, 56–88.5 μS) in the hands and 82.5 μS (IQR, 70–90.5 μS) in the feet. Additionally, risk for cardiac autonomic neuropathy (RCAN) based on demographic data (body mass index, age) and ESC was calculated by the SUDOSCAN software.[16-18]

Patients were tested on empty stomach, at a temperature of 21 ±1°C and air humidity of 40% to 50%. The temperature and humidity were measured by a thermohygrometer (Breuer HM 16, Beurer GmbH, Ulm, Germany). Patients were asked to clean their skin with soap and water before the test.

Statistical analysis The Kolmogorov–Smirnov test with Lilliefors correction was used to test for normality. A regression analysis by Passig and Babloc was used to assess the reproducibility of the SUDOSCAN test.

The Mann–Whitney, Kruskal–Wallis, and χ^2 tests, as appropriate, were used for comparative

assessment. The association between conductivity of the skin and the assessed variables was evaluated using the Spearman correlation coefficient. A multiple linear regression analysis was used to identify significant predictors of ESC. A univariate logistic regression analysis was used to determine factors associated with diabetic microvascular complications, and multivariate logistic regression was used to control for possible confounders. Multivariate regression models included the following variables, which were associated with statistically significant differences in comparative assessment: sex, duration of diabetes, age, HbA_{1c}, eGFR, TG, waist-to-hip ratio (WHR), history of hypertension, and ESC in the hands or feet. Receiver operating characteristics (ROC) curve statistics were applied to calculate sensitivity and specificity and to determine the diagnostic accuracy of the tests. The method described by DeLong was used for comparing the areas under the ROC curves (AUC). The results of comparative analyses were presented as medians and IQRs or as number and percentage of patients. All tests were 2-sided, and a *P* value of less than 0.05 was considered statistically significant. Data were analyzed using Statistica version 10 (StatSoft Inc., Tulsa, Oklahoma, United States) and MedCalc Statistical Software version 15.6.1 (MedCalc Software bvba, Ostend, Belgium).

RESULTS To evaluate the reproducibility of the test, the ESC was assessed in a group of 30 patients with DM1 using the SUDOSCAN+ device on 2 consecutive days. The test was performed under the same conditions and at the same time of the day. The regression analysis by Passig and Bablock demonstrated a linear relationship between the results of measurements performed on different days and confirmed the reproducibility of the assays. In relation to both the ESC in the feet and ESC in the hands, the cut-off ratio was not significantly different from 0, while the slope ratio was close to unity. Charts and regression equations with confidence intervals (CIs) of coefficients are shown in **FIGURE 1**.

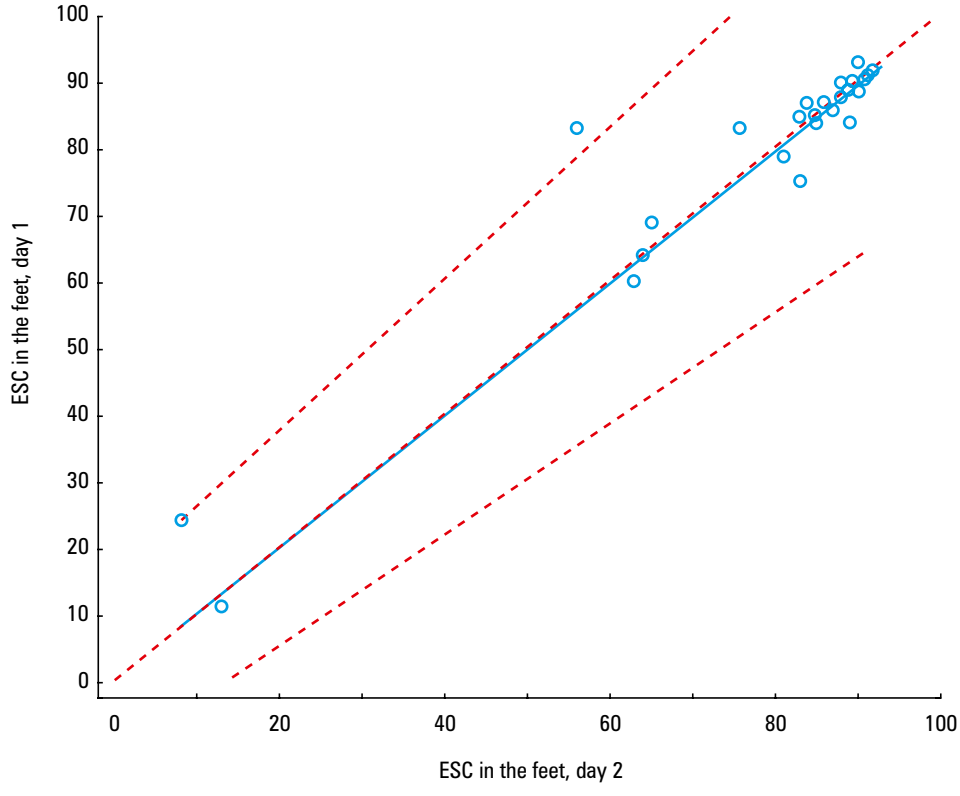
In the study group, with a median duration of diabetes of 23 years, peripheral neuropathy was diagnosed in 179 patients (44.3%), autonomic neuropathy in 79 (19.9%), diabetic retinopathy in 261 (64.8%), DKD in 79 (19.7%), and 296 patients had at least one type of microvascular complication (73.3%). The median value of ESC in the feet was 80 µS (IQR, 65–85 µS), and in the hands—63 µS (IQR, 51–75 µS). The estimated RCAN was 21% (IQR, 9%–33%). Patients with DM1 had significantly lower ESC in the feet and hands than healthy controls (feet, 80 µS [IQR, 65–85 µS] vs 83 µS [IQR, 78.5–87 µS], *P* <0.001; hands, 63 µS [IQR, 51–75 µS] vs 69 µS [IQR, 61.5–78.5 µS], *P* <0.001). Also, the subgroup of patients with DM1 and peripheral neuropathy had significantly lower ESC in the feet and hands compared with that in healthy controls (feet, 69 µS [IQR, 46–81 µS] vs 83 µS [IQR, 78.5–87 µS], *P* <0.001; hands, 56 µS [IQR, 39–69 µS] vs 69 µS [IQR, 61.5–78.5 µS], *P* <0.001). However, there was no statistical difference in ESC between DM1 patients without neuropathy and healthy controls (feet, 83 µS [IQR, 76–87 µS] vs 83 µS [IQR, 78.5–87 µS], *P* = 0.73; hands, 69 µS [IQR, 60–78 µS] vs 69 µS [IQR, 61.5–78.5 µS], *P* = 0.45).

DM1 patients with peripheral neuropathy, compared with those without clinical neuropathy, had lower ESC in the feet (69 µS [IQR, 46–81 µS] vs 83 µS [IQR, 76–87 µS]; *P* <0.001), lower ESC in the hands (56 µS [IQR, 39–69 µS] vs 69 µS [IQR, 60–78 µS], *P* <0.001), and higher estimated RCAN (31% [IQR, 18%–40%] vs 15% [IQR, 3%–24%], *P* <0.001) (**TABLE 2**). Patients with peripheral neuropathy were significantly older, had longer disease duration, higher WHR, higher HbA_{1c} value, higher skin AF ratio, higher serum triglyceride concentration, higher VPT, higher score in the Michigan Neuropathy Scale, and lower eGFR (**TABLE 1**). Moreover, patients with CAN, diabetic retinopathy, DKD, and with at least one type of microvascular complications, compared with patients without any of these complications, demonstrated significantly lower ESC in the feet, ESC in the hands, and higher RCAN. The results are presented in **TABLE 2**.

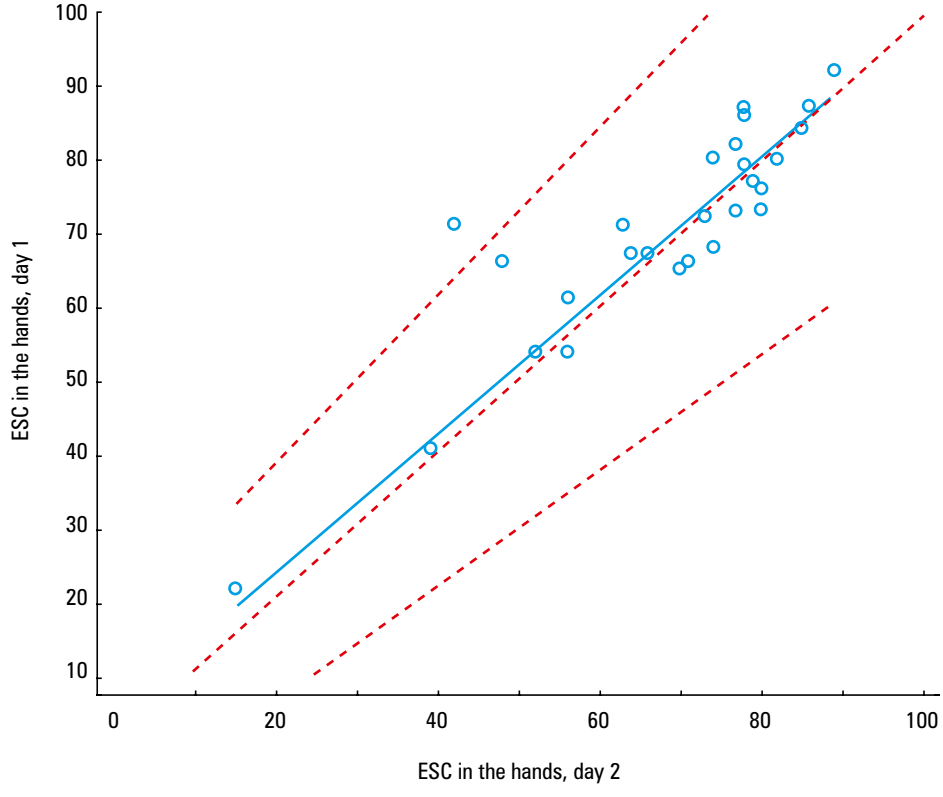
In the study group, we found a negative correlation between ESC in the feet and hands and the patients' age (*Rs* = –0.41, *P* <0.001 and *Rs* = –0.40, *P* <0.001, respectively), duration of diabetes (*Rs* = –0.33, *P* <0.001 and *Rs* = –0.30, *P* <0.001, respectively), WHR (*Rs* = –0.11, *P* <0.03 and *Rs* = –0.13, *P* <0.02, respectively), HbA_{1c} (*Rs* = –0.13, *P* <0.01 and *Rs* = –0.12, *P* <0.02, respectively), skin AF (*Rs* = –0.34, *P* <0.001 and *Rs* = –0.30, *P* <0.001, respectively), VPT (*Rs* = –0.51, *P* <0.001 and *Rs* = –0.40, *P* <0.001, respectively), Michigan Neuropathy Scale (*Rs* = –0.31, *P* <0.001 and *Rs* = –0.31, *P* <0.001, respectively), TG level (*Rs* = –0.18, *P* <0.001 and *Rs* = –0.14, *P* <0.001, respectively), and a positive correlation with eGFR (*Rs* = 0.38, *P* <0.001 and *Rs* = 0.31, *P* <0.001, respectively).

In the multiple linear regression model that included age, duration of diabetes, sex, HbA_{1c} level, skin AF, presence of retinopathy, DKD, peripheral neuropathy, and CAN, ESC in the feet was independently associated with the patient's age (coefficient [B], –0.21; 95% CI, –0.41 to –0.01; *P* = 0.04), HbA_{1c} level (B, –1.87; 95% CI, –3.15 to –0.6; *P* = 0.004), skin AF (B, –2.7; 95% CI, –5.22 to –0.17; *P* = 0.04), peripheral neuropathy (B, 4.14; 95% CI, 2.01–6.26; *P* <0.001], autonomic neuropathy (B, 5.42; 95% CI, 3.01–7.86; *P* <0.001), and sex (B, –1.98; 95% CI, –3.70 to –0.26; *P* <0.02); R^2 = 0.32. ESC in the hands was associated with age (B: –0.32; 95% CI, –0.5 to –0.14; *P* <0.001), HbA_{1c} level (B, –1.59; 95% CI, –2.7 to –0.46; *P* = 0.006), peripheral neuropathy (B, 3.04; 95% CI, 1.15 to –4.93; *P* = 0.002), and autonomic neuropathy (B, 3.08; 95% CI, 0.93–5.22; *P* = 0.005); R^2 = 0.28 (**TABLE 3**).

FIGURE 1
Reproducibility of the electrochemical skin conductance (ESC) evaluation on 2 consecutive days; regression analysis by Passig and Bablock



ESC in the feet: y = 1.0 (0.83–1.14) × + 0.00 (–11.59–15.0)



ESC in the hands: y = 0.94 (0.79–1.14) × + 5.44 (–9.39–16.24)

In the univariate logistic regression model, ESC in the feet and hands was associated with the presence of retinopathy (odds ratio [OR], 0.95; 95% CI, 0.94–0.97; *P* <0.001 and OR, 0.97; 95% CI, 0.95–0.98; *P* <0.001; respectively), DKD (OR, 0.98; 95% CI, 0.97–0.99; *P* <0.001 and OR, 0.98; 95% CI, 0.96–0.99; *P* <0.001; respectively), peripheral neuropathy (OR, 0.94; 95% CI, 0.93–0.96; *P* <0.001 and OR, 0.95; 95% CI, 0.94–0.96; *P* <0.001; respectively), CAN (OR,

TABLE 2 Comparison of the subgroups with and without microvascular complications and neuropathy

Parameter		Diabetic retinopathy vs no diabetic retinopathy	DKD vs no DKD	Peripheral neuropathy vs no peripheral neuropathy	CAN vs no CAN	Microvascular complications vs no microvascular complications
no. of patients, n		261 vs 142	79 vs 323	179 vs 225	79 vs 318	296 vs 108
age, y		44 (35–54) vs 35 (27–43), P <0.001	44 (34–52) vs 40 (32–51), P = 0.03	49 (39–57) vs 36 (29–43), P <0.001	48 (33–56) vs 40 (32–49), P <0.001	44 (34–54) vs 33 (25–40), P <0.001
sex, women/men, n		120/141 vs 73/69, P = 0.3	37/42 vs 156/167, P = 0.82	87/92 vs 107/118, P = 0.91	39/40 vs 152/166, P = 0.8	138/158 vs 56/52, P = 0.35
duration of diabetes, y		27 (20–32) vs 19 (14–23), P <0.001	26 (21–34) vs 22 (17–30), P = 0.002	27 (20–33) vs 21 (16–29), P <0.001	28 (20–35) vs 22 (17–30), P <0.001	26 (20–32) vs 18 (14–22), P <0.001
smokin, n (%)		86 (33) vs 35 (24.7), P = 0.08	25 (31.7) vs 96 (29.7), P = 0.74	53 (29.6) vs 68 (30.2), P = 0.98	28 (35.4) vs 91 (28.6), P = 0.24	95 (32.1) vs 26 (24.1), P = 0.12
history of hypertension, n (%)		151 (57.9) vs 33 (22.2), P <0.001	57 (72.2) vs 126 (31), P <0.001	109 (60.9) vs 76 (33.8), P <0.001	54 (68.4) vs 128 (40.3), P <0.001	164 (55.4) vs 21 (19.4), P <0.001
BMI, kg/m^2		25 (23–29) vs 25 (22–27), P = 0.02	25 (24–29) vs 25 (23–28), P = 0.3	25 (22–28) vs 25 (23–28), P = 0.34	24 (22–27) vs 25 (23–29), P = 0.01	25 (23–28) vs 25 (23–28), P = 0.43
WHR		0.88 (0.82–0.94) vs 0.84 (0.78–0.9), P <0.001	0.9 (0.83–0.96) vs 0.86 (0.8–0.93), P = 0.003	0.89 (0.82–0.94) vs 0.85 (0.79–0.92), P <0.001	0.87 (0.81–0.92) vs 0.86 (0.8–0.94), P = 0.81	0.87 (0.82–0.94) vs 0.83 (0.77–0.9), P <0.001
HbA_{1c}, %		8.1 (7.2–9) vs 7.8 (7.1–8.6), P = 0.013	8 (7–9.2) vs 8 (7.2–8.8), P = 0.92	8.2 (7.4–9.2) vs 7.8 (7.1–8.7), P = 0.015	8.4 (7.3–9.5) vs 7.8 (7.1–8.7), P = 0.019	8.1 (7.2–9) vs 7.8 (7–8.5), P = 0.01
hs-CRP, mg/l		1.4 (0.6–2.6) vs 1.1 (0.5–2.2), P = 0.09	1.7 (0.7–2.6) vs 1.2 (0.6–2.5), P = 0.05	1.2 (0.6–2.5) vs 1.4 (0.6–2.5), P = 0.7	1.5 (0.6–2.4) vs 1.2 (0.6–2.5), P = 0.99	1.4 (0.6–2.5) vs 1.1 (0.5–2.2), P = 0.07
TG, mmol/l		1.1 (0.9–1.5) vs 0.9 (0.8–1.2), P <0.001	1.4 (1.1–1.8) vs 1 (0.8–1.3), P <0.001	1.1 (0.9–1.5) vs 1 (0.8–1.3), P = 0.001	1.2 (0.94–1.6) vs 1 (0.8–1.3), P = 0.002	1.1 (0.9–1.5) vs 0.9 (0.7–1.2), P <0.001
LDL cholesterol, mmol/l		2.9 (2.3–3.6) vs 2.7 (2.2–3.5), P = 0.35	3 (2.3–3.6) vs 2.8 (2.2–3.6). P = 0.54	2.8 (2.4–3.6) vs 2.8 (2.2–3.6), P = 0.5	2.9 (2.2–3.5) vs 2.8 (2.2–3.6), P = 0.91	2.9 (2.3–3.6) vs 2.7 (2.2–3.5), P = 0.18
HDL cholesterol, mmol/l		1.7 (1.4–2) vs 1.7 (1.4–2.1), P = 0.56	1.6 (1.3–2) vs 1.7 (1.4–2.1), P = 0.06	1.7 (1.4–2.1) vs 1.7 (1.4–2), P = 0.89	1.6 (1.3–2.1) vs 1.7 (1.4–2), P = 0.49	1.7 (1.4–2) vs 1.7 (1.4–2.1), P = 0.96
TSH, µIU/ml		1.7 (1.1–2.5) vs 1.5 (1.1–2.3), P = 0.42	1.5 (1.1–2.2) vs 1.6 (1.1–2.4), P = 0.49	1.5 (1.0–2.4) vs 1.7 (1.2–2.4), P = 0.3	1.5 (1.1–2.4) vs 1.7 (1.1–2.4), P = 0.47	1.6 (1.2–2.4) vs 1.6 (1–2.4), P = 0.91
creatinine, mg/dl		0.91 (0.81–1.04) vs 0.84 (0.75–0.93), P <0.001	1.13 (1–1.36) vs 0.85 (0.76–0.95), P <0.001	0.92 (0.8–1.07) vs 0.87 (0.77–0.97), P = 0.002	0.96 (0.84–1.23) vs 0.87 (0.77–0.98), P <0.001	0.9 (0.79–1.04) vs 0.85 (0.77–0.93), P = 0.002
eGFR (MDRD), ml/min/1.73 m^2		87 (72–98) vs 96 (84–108), P <0.001	68 (52–81) vs 94 (82–105), P <0.001	81 (71–94) vs 96 (84–107), P <0.001	77 (58–91) vs 92 (81–104), P <0.001	87 (73–98) vs 97 (87–108), P <0.001
skin AF, AU		2.4 (2.1–2.8) vs 2.1 (1.8–2.4), P <0.001	2.5 (2.2–2.9) vs 2.2 (2–2.6), P <0.001	2.5 (2.2–2.9) vs 2.1 (1.8–2.5), P <0.001	2.6 (2.3–3.1) vs 2.2 (2.0–2.6), P <0.001	2.4 (2.1–2.8) vs 2 (1.8–2.2), P <0.001
VPT, V		23 (15–32) vs 15 (11–20), P <0.001	26 (18–32) vs 17 (13–27), P <0.001	31 (25–38) vs 15 (12–18), P <0.001	27 (23–35) vs 17 (14–26), P <0.001	24 (16–33) vs 14 (10–17), P <0.001
Michigan Neuropathy Scale, n		5 (2–7) vs 2 (1–4), P <0.001	6 (2–7) vs 3 (1–6), P = 0.009	7 (4–8) vs 2 (1–4), P <0.001	6 (2–8) vs 3 (1–6), P = 0.005	5 (2–7) vs 1 (1–3), P <0.001
ESC, µS	feet	75 (56–83) vs 83 (78–87), P <0.001	69 (46–81) vs 80 (68–86), P <0.001	69 (46–81) vs 83 (76–87), P <0.001	64 (35–76) vs 81 (71–86), P <0.001	75 (59–83) vs 84 (81–87), P <0.001
	hands	60 (48–72) vs 70 (60–78), P <0.001	57 (42–69) vs 66 (53–76), P <0.001	56 (39–69) vs 69 (60–78), P <0.001	54 (35–62) vs 68 (54–76), P <0.001	60 (48–720 vs 72 (64–80), P <0.001
RCAN		26 (14–36) vs 13 (2–22), P <0.001	28 (15–37) vs 19 (7–32), P <0.001	31 (18–40) vs 15 (3–24), P <0.001	30 (18–38) vs 18 (7–31), P <0.001	26 (13–36) vs 10 (1–20), P <0.001

Data are presented as median (interquartile range) unless otherwise stated. The groups were compared using the Mann–Whitney test for continuous variables and the χ^2 test for categorical variables. Abbreviations: CAN, cardiac autonomic neuropathy; DKD, diabetic kidney disease; RCAN, risk for cardiac autonomic neuropathy; TSH, thyroid-stimulating hormone; others, see **TABLE 1**

TABLE 3 Associations between electrochemical skin conductance and selected variables in a multiple linear regression model

Parameter	ESC in the feet B, 95% CI	*P* value	ESC in the hands B, 95% CI	*P* value
age	–0.21 (–0.41 to –0.01)	0.04	–0.32 (–0.50 to –0.14)	<0.001
duration of diabetes	–0.04 (–0.28–0.20)	0.74	–0.13 (–0.34–0.09)	0.24
HbA_{1c}	–1.87 (–3.15 to –0.60)	<0.001	–1.59 (–2.73 to –0.46)	0.01
skin AF	–2.70 (–5.22 to –0.17)	0.04	–1.45 (–3.70–0.80)	0.20
sex (men)	–1.98 (–3.70 to –0.26)	0.02	–1.49 (–3.02–0.04)	0.06
retinopathy	1.91 (–0.15–3.97)	0.07	0.62 (–1.21–2.45)	0.51
DKD	1.55 (–0.85–3.95)	0.21	0.56 (–1.58–2.69)	0.61
peripheral neuropathy	4.14 (2.01–6.26)	<0.001	3.04 (1.15–4.93)	<0.001
CAN	5.42 (3.01–7.83)	<0.001	3.08 (0.93–5.22)	<0.001

A *P* value of less than 0.05 was considered significant.

Abbreviations: B, coefficient; CI, confidence interval; others, see TABLES 1 and 2

0.96; 95% CI, 0.95–0.97; *P* <0.001 and OR, 0.96; 95% CI, 0.95–0.97; *P* <0.001; respectively). In addition, the multivariate analysis demonstrated that ESC in the feet and ESC in the hands were independent predictors of retinopathy, DKD, peripheral neuropathy, and CAN (TABLE 4).

The discriminative value of ESC in the feet to identify patients with peripheral neuropathy in the clinical examination was slightly better than that of ESC in the hands: AUC, 0.77 (95% CI, 0.72–0.81) vs AUC, 0.72 (95% CI, 0.68–0.77), *P* = 0.041. The value of the RCAN score to discriminate these groups was similar to both mean ESC values: AUC, 0.76 (95% CI, 0.71–0.80), *P* = 0.63 for RCAN vs ESC in the feet and *P* = 0.25 for RCAN vs ESC in the hands. When choosing a cut-off point of 79 µS or less for ESC in the feet (optimal Youden index), the sensitivity was 72%, specificity was 68%, and the Youden index was 0.4. The AUC was 0.77.

Similarly, the discriminative value of ESC in the feet to identify patients with autonomic neuropathy did not differ significantly from the value of ESC in the hands: AUC, 0.77 (95% CI, 0.72–0.81) vs AUC, 0.73 (95% CI, 0.68–0.77), *P* = 0.11. The value of the RCAN score to discriminate these groups was lower than each of the mean ESC values: AUC, 0.66 (95% CI, 0.61–0.71); *P* = 0.05 compared with the AUC for ESC in the hands and *P* <0.001 for comparison with the AUC of ESC in the feet. When choosing a cut-off point of 79 µS or less for the ESC in the feet (optimal Youden index), the sensitivity was 78%, specificity was 67%, and the Youden index was 0.4.

The discriminative value of ESC in the feet and in the hands to identify patients with retinopathy was as follows: AUC, 0.69 (95% CI, 0.65–0.74), *P* <0.001 and AUC, 0.66 (95% CI, 0.62–0.71), *P* <0.001, respectively. When choosing a cut-off point of 78 µS or less for ESC in the feet (optimal Youden index), the sensitivity was 58%, specificity was 73%, and the Youden index was 0.3.

The discriminative value of ESC in the feet and in the hands to identify patients with DKD was as follows: AUC, 0.67 (95% CI, 0.62–0.71), *P* <0.001 and AUC, 0.64 (95% CI, 0.59–0.69), *P* <0.001, respectively. When choosing a cut-off point of 72 µS or less for ESC in the feet (optimal Youden index), the sensitivity was 58%, specificity was 70%, and the Youden index was 0.3.

DISCUSSION This study involved a large homogeneous group of DM1 patients from Poland. It demonstrated a significant association between reduced ESC, measured using the SUDOSCAN+ device, and the occurrence of microvascular complications in patients with DM1, especially those with peripheral and autonomic neuropathy.

In diabetic patients, Gin et al[19] observed an association between ESC and VPT measured using a neurothesiometer. Yajnik et al[17] obtained similar results in patients with type 2 diabetes (DM2). Our study also revealed a correlation between VPT and ESC in DM1 patients, which is understandable because small-fiber damage precedes the degeneration of thick myelin fibers, responsible for the sensation of vibration. Yajnik et al[17] also reported lower ESC in the elderly, with longer duration of diabetes and higher HbA_{1c} levels. In addition, they documented a reduced ESC in patients with autonomic neuropathy diagnosed using standard methods.[18] Casellini et al[9] observed a reduction of ESC in patients with DM1 and DM2, diagnosed with peripheral and autonomic neuropathy. Similar results were obtained by Smith et al[16] in a small group of patients with either DM2 or impaired glucose tolerance. To our knowledge, we are the first to report the association between decreased ESC and the presence of peripheral neuropathy in a homogeneous group of patients with DM1. We have also identifed the correlation between ESC and renal function. A similar association between impaired skin conductance and DKD was reported by Freedman et al,[20] in an ethnically diverse group of patients with DM2. Similarly, Calvet et al,[8] in a group of 52 patients with DM1 and 115 patients with DM2, reported lower values of ESC in

TABLE 4 Variables related to the presence of microvascular complications in multivariate logistic regression

Parameter	Diabetic retinopathy		DKD		Peripheral neuropathy		CAN		Microvascular complications	
	P value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)
age, y	NS	0.99 (0.96–1.02)	0.01	0.95 (0.91–0.99)	<0.001	1.08 (1.05–1.11)	NS	1 (0.97–1.03)	NS	1.02 (0.99–1.06)
sex, women/men, n	NS	1.22 (0.67–2.22)	0.007	3.43 (1.4–8.42)	NS	1.4 (0.76–2.59)	0.01	2.74 (1.27–5.93)	NS	1.39 (0.72–2.69
duration of diabetes, y	<0.001	1.07 (1.03–1.12)	NS	1.01 (0.97–1.06)	NS	0.98 (0.9501.02)	NS	1.02 (0.98–1.06)	0.002	1.07 (1.03–1.12)
history of hypertension	0.002	2.42 (1.38–4.23)	NS	1.53 (0.73–3.21)	NS	1.2 (0.69–2.1)	NS	1.94 (0.98–3.85)	0.01	2.25 (1.18–4.3)
WHR	NS	9.08 (0.26–323.6)	NS	9.3 (0.1–895.7)	NS	1.04 (0.05–24.0)	0.02	0.01 (0.01–0.38)	NS	3.06 (0.05–175.5)
HbA_{1c}, %	NS	1.11(0.93–1.32)	NS	0.96 (0.74–1.25)	0.001	1.37 (1.13–1.66)	0.02	1.29 (1.03–1.6)	0.003	1.35 (1.1–1.65)
TG, mmol/l	NS	1.09 (0.75–1.61)	NS	1.33 (0.83–2.15)	NS	1.06 (0.73–1.54)	NS	1.11 (0.66–1.87)	NS	1.07 (0.7–1.63)
eGFR (MDRD), ml/min/1.73 m^2	NS	0.99 (0.98–1)	<0.001	0.9 (0.88–0.93)	NS	0.99 (0.98–1.0)	<0.001	0.97 (0.96–0.99)	NS	1.0 (0.98–1.01)
ESC in the feet	<0.001	0.97 (0.95–0.99)	NS	0.99 (0.97–1.0)	<0.001	0.96 (0.94–0.97)	<0.001	0.97 (0.95–0.98)	<0.001	0.95 (0.92–0.98)

A *P* value of less than 0.05 was considered significant.

Abbreviations: NS, nonsignificant; OR, odds ratio; others, see TABLES 1, 2, and 3

patients with decreased eGFR, as compared with patients with non-DKD. The presence of any diabetic microangiopathy complications was also associated with lower ESC in the study by Eranki et al.[21] However, our study revealed the strongest association between ESC and peripheral autonomic neuropathy from any of diabetic complications. Our results also revealed the correlation between ESC and serum TG levels, which supports the concept that dyslipidemia is instrumental in the progression of diabetic neuropathy. The association between TG levels and neuropathy was previously evaluated by Wiggin et al.[22] Similar conclusions were drawn by Keenen et al[23] who assessed risk factors for neuropathy in patients with a history of DM1 exceeding 50 years.

We did not show any difference in sudomotor function between healthy controls and patients with DM1 without peripheral neuropathy, in contrast to patients with neuropathy, in whom sudomotor function was reduced. The same result was obtained by Selvarajah et al.[24] Based on this observation, we believe that the evaluation of sudomotor function may be considered a useful method to identify patients with neuropathy. The fact that patients with good metabolic control and healthy subjects had similar, unaffected sudomotor function further supports the significance of metabolic control in the prevention of chronic complications of diabetes. In the present study, we also found a correlation between ESC and an increased accumulation of protein AGEs in the skin of patients with DM1, which to our knowledge had not been reported before. Skin AF has been demonstrated as a reliable marker of past glycemic control of diabetes. Our earlier results showed a relationship between skin AF and the presence of peripheral neuropathy and its correlation with IENFD.[13,25] Interestingly, we found a much stronger correlation between ESC and skin AF than with HbA_{1c} levels. From all the mechanisms involved in the pathogenesis of diabetic neuropathy, one of the most important element is the increased formation of AGEs, just as is the alternating activity of glucose through the polyol pathway, increased expression of the nuclear factor NF-kB, and the activation of the mitogen-activated protein kinase signaling pathway during hyperglycemia. AGEs form a network of crosslinks, which interfere with the function of most cells and tissues of the body by combining with one another and with the long-living proteins. AGEs, via specific receptors, activate endothelial cells, monocytes, macrophages, and mesangial cells. Stimulation of these cells leads to the release of proinflammatory cytokines (interleukins 1 and 6, tumor necrosis factor), production of toxic oxygen species, and the activation of transcription factors. The accompanying oxidative stress and inflammation cause damage to cells, in particular the structure of the vascular wall.[26] It has been also suggested that peripheral nerve degeneration occurs due to impaired flow of nutrient in the vessels (microangiopathy).

In addition, the microvascular wall tension, and thus blood flow, is regulated by the peripheral autonomic nervous system. Regardless of the main cause, the impairment of flow in small vessels induces local hypoxia inside the neuron, which initiates the process of neurodegeneration.[27] A simultaneous analysis of AF and ESC could help estimate the risk of complications, particularly diabetic neuropathy.

In the present study, we also found that diagnostic accuracy of the electrochemical skin conduction measurement for peripheral and autonomic neuropathy is higher for ESC in the feet than for that in the hands. This result is consistent with a previous study. The observed differences between the various reports in the field of diagnostic value of the ESC measurement may result from the methods used to assess neuropathy and different groups of patients.[8,24,28] Similarly to Gin et al[19] and Mayaudon et al,[28] our study showed good reproducibility of the assay.

Our study has several limitations. The case-control design is less powerful than the prospective design of the study. Sensitivity, specificity, and discriminative value of ESC to identify peripheral and autonomic neuropathy are slightly lower than those reported in some previous studies, which may limit the power of our analyses. The study did not include nerve conduction measurement or skin biopsy analyses that might have increased the sensitivity of diagnosing neuropathy. Also investigation using the SUDOSCAN+ device has specific limitations. The test cannot be performed in patients with an active foot ulcer, amputations, or implanted electrical devices.

In conclusion, diabetic microangiopathy, and in particular neuropathy, is related to reduced sudomotor function in DM1. A longer duration of diabetes, worse metabolic control, and reduced renal function are associated with greater sudomotor dysfunction.

Contribution statement BW-W conceived the idea of the study. AG and AA contributed to the design of the research. All authors were involved in data collection. AG and SP analyzed the data. AA, DZ-Z, and BW-W coordinated funding for the project. All authors edited, reviewed, and approved the final version of the manuscript.

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