STUDY PROTOCOL

Study information

Title	Feasibility and effectiveness of electrochemical dermal conductance measurement for the screening of diabetic neuropathy in Primary Care. DECODING Study (Dermal Electrochemical Conductance in Diabetic Neuropathy). Rationale and design.
Ethical committee protocol number	P14/147
Protocol version identifier	1.0
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ClinicalTrials.gov register number	Study not yet registered
Intervention	Dermal Electrochemical Conductance
Research question and objectives	To evaluate the feasibility, effectiveness and performance of a new technique which measures DEC (sudomotor reflex) in the screening of diabetic neuropathy in Primary Care.
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Abbreviations

CHOD-PAP: Cholesterol oxidase phenol-aminophenazone

CI95%: Confidence interval 95%

CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration

DEC: Dermal Electrochemical Conductance

DECODING: Dermal Electrochemical Conductance in Diabetic Neuropathy

DM: Diabetes mellitus

DSPN: distal symmetric polyneuropathy

EMG: electromiography

HLD: High density lipoprotein

HPLC: High Resolution Liquid Chromatography

ICS: *Institut Català de la Salut* – Catalan Institute of Health

IDIAP : *Institut d'Investigació en Atenció Primària* – Primary Care Research Institute

IFG: Impaired fasting glucose

IGT: Impaired glucose tolerance

LDL: Low density lipoprotein

MDRD: Modification of Diet in Renal Disease

MFT: Monofilament testing

MNI : Michigan Neuropathy Instrument

NDS: Neuropathy Disability Score

NSS : Neuropathy Symptoms Score

OGTT: Oral glucose tolerance test

PN: polyneuropathy

QOL-DN: Quality of Life Questionnaire-Diabetic Neuropathy

ROC: Receptor Operative Characteristics

UENS: Utah Early Neuropathy Scale

VO_2 max: Maximum Oxygen Volume

WHO: World Health Organization

ABSTRACT:

Diabetes mellitus is the leading cause of polyneuropathy in the Western world. Diabetic neuropathy is a frequent complication of diabetes and may have great clinical transcendence due to pain and possible ulceration of the lower extremities. It is also a relevant cause of morbidity and mortality in patients with diabetes. Although the cause of polyneuropathy in patients with diabetes is only partially known, it has been associated with chronic hyperglycaemia suggesting the possible aetiopathogenic implication of advanced glycosylation end-products. The strategy of choice in the medical management of diabetic neuropathy is early detection since glycaemic control and the use of certain drugs may prevent or slow the development of this disease. Diabetic neuropathy most often presents with a dysfunction of unmyelinated C-fibers, manifested as an alteration of the sweat reflex of the eccrine glands. This dysfunction can now be demonstrated using a newly developed technology which measures dermal electrochemical conductivity. This noninvasive test is easy and cost-effective. The aim of the present study is to evaluate the feasibility and effectiveness of dermal electrochemical conductance measurement (quantitative expression of the sudomotor reflex) as a screening test for the diagnosis of diabetic neuropathy in patients in primary care.

INTRODUCTION

The prevalence of diabetes mellitus (DM) is very high in Spain, being nearly 14% according to oral glucose tolerance test (OGTT) results (1). The management of DM requires a significant consumption of healthcare resources, mainly in relation to the care of vascular complications. Among the late microvascular events which may develop in patients with DM, polyneuropathy (PN) is the most common and disabling, and is the leading cause of morbidity and mortality in these patients (2). Indeed, in Spain, the leading cause of neuropathy is DM, with its prevalence increasing with the presence of DM and other risk factors such as obesity (3).

PN is defined as the presence of symptoms and/or signs of peripheral nerve dysfunction in people with DM after the exclusion of other possible causes (4). The Toronto Panel Consensus on PN defined this disorder as "symmetrical, depending on large fibers, sensory-motor attributable to metabolic and microvessel disorders, as a result of chronic hyperglycemia and other risk factors" (2). In patients with PN, thin fibers (autonomic system - sweating) and thermal and tactile sensitivity are first affected, followed by the involvement of large fibers, presenting an altered vibrating sensation which eventually alters electromyography (EMG) patterns. Therefore, dysfunction of sweat reflex in small distal fibers is one of the earliest changes to be detected in these patients (5).

The most common clinical presentation of PN is distal symmetric polyneuropathy (DSPN), being predominantly sensory in 80% of cases (3). Pain is the most important symptom, being described as burning or flashing, lancinating, deep, and with frequent exacerbations during rest (4). Pain often affects the quality of life of these patients, and

it is a frequent cause of depression and/or anxiety (6). Moreover, some patients may develop hypoesthesia, which may lead to severe foot lesions (7).

The prevalence of DSPN varies greatly according to the population, definition and detection method. The Rochester study, including more than 64,000 patients, reported the prevalence of PN to be between 66% and 59% for type1 DM and type 2 DM, respectively (8). The 3rd report of the Technical Study Group of Diabetes of the World Health Organization (WHO) described a prevalence of 40% (8), and 50% in patients with more than 25 years of DM evolution. Pirart *et al*. (9) reported a prevalence ranging from 25 to 48% (7,10-17), while in Spain, Cabezas-Cerrato *et al.* published a figure of 24.1% (11). DSPN-related factors are: age, DM duration, metabolic control, male gender, acute myocardial infarction, hyperlipidaemia (especially hypertriglyceridaemia), smoking, and general cardiovascular risk factors (2,15,16,18). Puig *et al.* (15) also included urinary albumin excretion as a risk factor of presenting DSPN.

The diagnosis of DSPN is commonly made based on signs and symptoms and usually includes the use of several questionnaires such as the Neuropathy Disability Score (NDS), the Neuropathy Symptoms Score (NSS) and the Michigan Neuropathy Instrument (MNI). These questionnaires are easy to perform and are reproducible, sensitive and adequate for use in a screening program (16). Additionally, It was included a short scale (UENS - Utah Early Neuropathy Scale) to screen early neuropathy (17). This sensitive, fast and practical test, has 5 items and their score ranges from 0 to 42 points.

There are many confirmatory tests, including measurements of nerve conduction velocity (EMG) and biothesiometry or skin biopsy. However, those most commonly used are the measurement of altered sensations using a vibrating tuning fork with 128 Hz and/or pressure with Semmes-Weinstein 5:07 monofilament (18). Monofilament testing (MFT) is widely accepted and recommended by all scientific societies because of its validity, predictive risk, efficiency and simplicity. Feng *et al.* (19) reported that MFT has a sensitivity of 57-93%, a specificity of 75-100%, a positive predictive value of 36-94% and a negative predictive value of 84-100% compared to the measurement of nerve velocity by EMG. Although electrophysiological measures are more objective and reproducible, they are limited in that they only detect dysfunction based on the presence of thicker and faster (myelinated) fibers and show their involvement later. Consequently, EMG is a specific, albeit very insensitive, test.

Recently developed non invasive techniques are more reproducible and reliable for the detection of early dysfunction of small fibers. One of these new techniques involves the measurement of **dermal electrochemical conductance** (**DEC**) or sudomotor dysfunction index and has been evaluated by well-designed studies (Calvet, Dupin, Winiecki, Black, 2013; Casellini 2013; Devigili 2008; Peltier 2009) which support its use as a screening test (20).

Ramachandran *et al* (21) studied the use of DEC to detect diabetes and other disorders of glucose metabolism. In a study on the use of DEC Casellini *et al* (5) applied a PN test which showed a low sensitivity of 78% and a specificity of 92% in diabetic patients without neuropathy compared to other subjects with neuropathy and a control group. In this latter study, correlation with clinical parameters showed adequate reproducibility of

the results, particularly in regard to the measurements of the feet (5). Several other studies (22) also obtained significantly lower DEC values on comparing diabetic patients and controls. In a study of patients following a 12-month program of intense physical activity, Raisanen *et al* (23) observed a greater improvement in DEC compared to weight, waist circumference or maximum oxygen volume (VO_2 max).

Therefore, taking into account the large number of methods used and the learning curve required to correctly implement these techniques as well as the absence of consensus as to which method is the most adequate to diagnose DSPN, the aim of this study is to validate the usefulness of DEC measurement in the early diagnosis of DSPN compared with traditional techniques in the Primary Care setting.

HYPOTHESIS AND OBJECTIVES

The hypothesis of our study is that the measurement of DEC is feasible, sensitive and specific and more or equally effective to other techniques commonly used in the initial screening of diabetic neuropathy in Primary Care.

Main objective

To evaluate the feasibility, effectiveness and performance of a new technique which measures DEC (sudomotor reflex) in the screening of diabetic neuropathy in Primary Care.

Specific objectives

1. Determine the performance of DEC (quantitative assessment of sudomotor reflex) as a tool for the screening of diabetic neuropathy in Primary Care compared with the Semmes-Weinstein 5:07 MFT when an EMG is used to confirm the presence of diabetic peripheral neuropathy in patients with prediabetes and type 2 diabetes.

2. Determine the performance of DEC (quantitative assessment of sudomotor reflex) as a tool for screening diabetic neuropathy in Primary Care compared with the Semmes-Weinstein 5:07 MFT when the UENS (Utah Early Neuropathy Score) is used to confirm the presence of diabetic peripheral neuropathy in patients with prediabetes and type 2 diabetes.

3. Estimate the cost and cost-effectiveness of the use of DEC in the screening of early diabetic neuropathy in Primary Care.

METHODS

Design

We will perform a blind, prospective study comparing DEC (sudomotor reflex) (Sudoscan®, Impeto Medical, France), EMG, the Semmes-Weinstein 5:07 MFT (10 g), the sensitivity of a vibrating tuning fork 128 Hz, the NDS score and the UENS score in a consecutive series of patients treated in Primary Care.

Sites

The Primary Care teams of Terrassa-Sud and other partners belonging to the Mutua Terrassa reference hospital and those of the University Hospital Sant Joan de Reus reference hospital.

Study subjects

We will consecutively include patients with type 2 DM over 40 years of age, with or without symptoms of neuropathy, attended in Primary Care. We will also include the following two groups of patients matched by age and gender: one including patients with prediabetes (intermediate alterations of glucose metabolism defined as impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) determined by OGTT after 2-hour 75 g oral glucose administration and another including patients without glucose alterations (normal glucose tolerance) (control group).

Three main diagnostic categories (normal, pre-diabetes and diabetes) were defined using the WHO criteria based on 2-h postload glucose [<7.8 (140 mg/dL), 7.8–11.0 mmol/L (140-200 mg/dL) and / or fasting plasma glucose (6.1–6.9 mmol/L; 110-126 mg/dL) and >11.1 mmol/L (>200 mg/dL), respectively.

The exclusion criteria are: Type 1 DM, upper or lower limb amputation (except phalanges), diagnosis of neuropathy not related to diabetes, neuropathy by entrapment, use of psychoactive substances, chronic alcoholism, malnutrition; treatment with beta-blockers, presence of terminal disease or life expectancy less than 3 years. Pregnancy will be ruled out in women (negative pregnancy test) and a possible history of gestational diabetes will also be taken into account.

The study period is from January 1, 2017 to 30 November 2019.

Sample size

To estimate the validity and performance of a screening test that showed a sensitivity of 82%, a precision of 9% and a confidence interval of 95% (CI95%), considering a loss percentage of 20%, this study will include a total of 160 participants. The proportion of diabetes / prediabetes / normal glucose tolerance will be 2:1:1. The contribution by centres will be: 66% from the Mutua de Terrassa (106 cases) and 34% from Reus (54 cases).

Variables and dynamic data collection:

After verifying the inclusion criteria and receiving written informed consent to participate, during the **first visit** to the Primary Care centres the medical history of the patient will be obtained and a physical examination will be performed using the MFT and the NDS and UENS questionnaires will be given to screen for PN. The patient will also undergo DEC quantification using the Sudoscan® device.

The following variables were collected:

- Age, sex and country of origin.

- Family history of type1 or type2 DM: (yes / no).

- In diabetic population, length of diabetes evolution (in years) since diagnosis.

- Toxic habits: smoking (active smoker / ex smoker / non-smoker); drinking (teetotal, occasional drinker, habitual drinker).

- Other complications of diabetes and year of diagnosis (retinopathy, ischemic heart disease, peripheral vascular disease, cerebral vascular disease and diabetic nephropathy) will be collected from the patient medical records.

- Diabetes treatment.

- Other pharmacological treatments related to: hypotension, hypolipidemia, arterial thrombosis, among others.

On physical examination the following variables will be collected:

- Anthropometric data: Height (cm), weight (kg) (using validated clinical scale with stadiometer in light clothing), waist circumference (cm) (using a flexible measuring tape), measuring the point between the navel and upper iliac crest.

- Systolic/diastolic blood pressure; presence of pulses in pedia/posterior tibial arteries. Blood pressure (mmHg) measurements will be made three times while seated after 30 minutes of rest using a validated automatic device, with each measure being made at least one minute apart. The data recorded will be the mean of the second and third readings.

Other data to be collected include:

- Blood/urine tests: OGTT (Except in known DM); glycosylated hemoglobin A1c (High Resolution Liquid Chromatography (HPLC)), glomerular filtration rate [Modification of Diet in Renal Disease (MDRD) / Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)]; lipid profile (total cholesterol, triglycerides and LDL and HDL fractions using the cholesterol oxidase-phenol + aminophenazone (CHOD-PAP) method. These latter parameters should have been determined no more than 6 months previously. If not the determinations should be made in the reference laboratory.

- A score of 8 out of 8 with the Semmes-Weinstein 5:07 MFT(10 g) will be considered as sensitive.

- A NDS score greater than or equal to 6 points will be considered as the presence of PN.

- A UENS score greater than or equal to 10 points will be considered as the presence of PN.

- The determination of vibration sensitivity will be performed using a 128 Hz Rydel-Seiffer tuning fork. The test will be considered positive for PN when vibration of the tuning fork is not perceived when applied to the thumb or external maleolus.

In the **second visit**, done at the reference hospital, a neurologist blinded to previous test results, will perform neurographyc test including sensory conduction study of the median, ulnar and sural nerves, and motor conduction study of the deep peroneal nerve.

The studied variables will be amplitude of compound muscle action potential and distal latency of the motor nerves, and amplitude and distal latency of sensory nerves. DEC determination and the other neuropathy screening and electrophysiological tests will take no longer than one month.

The **third visit** will be carried out in the Primary Care centre where the results of the previous visit will be recorded and the patient will be informed of the results and the diagnosis.

The cost effectiveness of the different diagnostic methods studied will be evaluated and the incremental cost method to detect new cases compared to traditional methods will be calculated. In addition, a computer simulated model will determine the long term as well as the direct and indirect costs of the different methods.

STATISTICAL ANALYSIS

The Chi-square test will be used to analyse qualitative variables and the Student t-test will be performed for quantitative variables. Logistic regression will be used to identify predictors of diabetic neuropathy. Dependent variables (Response) will include the presence of diabetic neuropathy diagnosed by EMG or the NDS / UENS questionnaires. We will compare the performance of DEC with that of the MFT as screening tests of PN.

To determine the validity and reliability of DEC we will calculate the sensitivity, specificity, the positive and negative predictive values, and the positive and negative likelihood ratios. A ROC curve will be used and the area under the curve will be calculated. A p value <.05 will be considered as statistically significant. The analysis will be performed with the statistical packages STATA/SE 12.0 and R for Windows.

LIMITATIONS OF THE STUDY

The main limitation of the present study is accurate diagnosis of diabetic neuropathy since some studies have shown that some cases of diabetic neuropathy present no alterations in the EMG. Indeed, several considerations should be taken into account. Firstly, the EMG test is more specific, albeit not very sensitive, showing positive results in advanced stages of PN. The fingerboard and the NDS questionnaire are commonly used for the diagnosis of diabetic neuropathy, probably because the NDS is carried out prior to EMG and is actually often used to avoid the need for EMG. Therefore, both the EMG and the NDS score, which mainly assess the dysfunction of myelinated fibers provide a good profile for diagnostic confirmation. On the other hand, both the DEC and the MFT are able to diagnose and stage diabetic neuropathy earlier than the previous two tests by the detection of unmyelinated fiber dysfunction. But, another limitation is that MFT is a good test for prediction of a foot ulcer but is certainly insensitive for the detection of early neuropathy. For this reason, we think about the UENS, because of its sensitivity to early sensory loss and ability to record modest anatomic change in sensory function, considering patients with milder neuropathy. Therefore for purposes of simplification and taking into account the possible limitations, we will compare the effectiveness of the measurement of DEC and the use of MFT as diagnostic tools in Primary Care according to whether the true diagnosis is achieved by a) the EMG or b) the score of the NDS or UENS questionnaires.

Obviously there are another tests to detect early neuropathy, i.e. the Norfolk QOL-DN scale; but this questionnaire is not practical in the clinical scenario. Certainly, this test is a good tool to detect unaware neuropathy in patients with diabetes (24). This study used the Norfolk QOL-DN scale on 25000 patients with diabetes and found 6600 patients who were not aware of their neuropathy nor did their physicians know.

Another limitation of this study is the possibility of introducing a selection bias, and thus, we will consecutively include patients with type 2 DM, excluding patients with type 1 DM. We will also include a group of patients without DM or other glucose homeostasis disorders matched by age and gender to a 2:1:1 ratio (80 with DM, 40 with prediabetes and 40 with normal glucose tolerance).

QUALITY ASPECTS

This project will promote a strategy to determine whether the implementation of a new technique simplifies the detection of a major complication of diabetes, such as neuropathy.

Primary Care is clearly the most appropriate setting for the screening of this complication. Indeed, the earlier the diagnosis the better the prognosis.
This strategic action on health is directly linked to:

(A) New medical technologies to promote personalized medicine based on the profiles of the individuals and not the disease.
(B) Translational and clinical research, evidence-based scientific and technological knowledge.
(C) Validation of a promising new technology for early detection of diabetic neuropathy in Primary Care.
(D) Application of evidence in clinical practice. This is a validation study of diagnostic technology implemented in real life conditions which would provide additional knowledge for everyday clinical practice.

It is of note that this study will also determine the cost-effectiveness of this new tool which is essential for the validation of new technologies aimed at different healthcare areas such as Primary Care.

Additionally, this project will establish synergies at different levels such as Primary

Care and Neurology, and the results can be shared with a number of centers, primary care areas and different scientific societies involved in the comprehensive care of patients with diabetes.

The results of previous studies support the feasibility of DEC in Primary Care, although further studies should be aimed at complementing and extending our hypothesis to different types of candidates and determining the cost-effectiveness of the systematic use of DEC.

Technical and collaborative aspects

The number of centres involved is limited, including Primary Care centres of the Catalan Institute of Health (ICS) of Reus (Tarragona) and the Mútua Terrassa (Barcelona).

In addition, IDIAP Jordi Gol and the Pere Virgili Health Research Institute - Hospital de Sant Joan (Reus), and IDIAP Jordi Gol and Mútua Terrassa have signed a cooperation agreement regarding research in the field of primary care.

Author's contributions

All authors substantially contributed to designing the study protocol or to data analysis and interpretation, and to drafting or revising the article. Specifically, JJC, TM and BC formulated the research question, designed the study (by cooperating with FB, RS, JV and GFO), and wrote the research proposal as well as the first draft of the manuscript.

JJC, TM and BC discussed the proposal and his approach to the reality of primary care.

BC, TM, FB, CLM, MGB, IB, NO and GFO critically revising the proposal for

scientifical content.

All authors contributed to the revision of the manuscript, read and approved the final

version.

Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

The research ethics committee board at the Jordi Gol Research Institute (Barcelona)

(www.idiapjordigol.org) approved the protocol (January 2015, reference number

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