

# Diabetic Peripheral Neuropathy as a Predictor for Asymptomatic Myocardial Ischemia in Type 2 Diabetes Mellitus

## Supplementary Material

### Methods

#### 1. *Patients*

Eighty-two adult subjects, with diagnosed Type 2 DM (T2DM), were recruited from the Diabetes Mellitus Outpatient Clinic of the 3rd Department of Internal Medicine, situated at Papageorgiou General Hospital, in Thessaloniki, Greece. Recruitment took place from April 2012, until May 2015. The Ethics Committee of the Papageorgiou General Hospital approved the study's protocol and patients gave their informed consent prior to participation.

The inclusion criteria were:

- type T2DM, according to ADA and IDF criteria[1]
- age>18years

The exclusion criteria were:

- history of myocardial infraction, stroke, coronary revascularization or cardiac bypass
- active liver disease
- any chronic renal disease
- any autoimmune disease
- HIV infection
- malignancy

- primary neurologic disorders (previous spinal injury, a history of lumbar or cervical discopathy, carpal tunnel syndrome, alcoholism, inherited neuropathy)
- vitamin B9 or B12 deficiency
- concomitant use of glucocorticoid, isoniazid or metronidazole.

## **2. Procedures**

### **a. Clinical examination**

The same experienced physician performed a thorough physical examination and recorded a detailed medical and medication history for each participant. Overweight and obesity were defined, according to the World Health Organization Criteria[2]. Waist circumference was measured with a common measuring tape in a horizontal plane, midway between the inferior margin of the ribs and the superior border of the iliac crest and central obesity was diagnosed according to the IDF criteria for people of European Origin[3]. Blood pressure was measured twice with a digital sphygmomanometer (DINAMAP, Johnson & Johnson, Medical INC, Arlington, TX, USA). Due to the diagnosis of T2DM, hypertension was defined as arterial systolic blood pressure (BP)  $BP > 130$  mmHg or diastolic  $BP > 85$  mmHg or as treatment of previously diagnosed hypertension, according to the IDF[3]. Autonomic function was assessed according to two cardiovascular reflex responses after the patients had rested in a supine position for at least five minutes — a change in heart rate and a change in systolic blood pressure on standing. The diagnosis of orthostatic hypotension involved measurement of the BP in both a supine and in a standing position. Difference in the systolic BP between the two positions greater than 20 mmHg, or more than 10 mmHg in the diastolic BP difference was considered indicative of orthostatic hypotension[4]. The RR ratio was calculated by one observer. The RR ratio is the ratio of the longest electrocardiographic RR interval between the 28th and 32nd beats after standing to the shortest interval between the 13th and 17th beats. Abnormal autonomic function was defined as a loss of heart rate variability with an RR ratio of

less than 1.04, postural hypotension with a fall in systolic blood pressure of 20 mm Hg or more, or both[5]. DPN was evaluated with the neuropathy disability score (NDS) by testing the sensations of pain, touch, cold, and vibration in both legs of each patient and assigning a score according to the level of impaired sensation. For this study, an NDS score  $\geq 5$  (maximum 28) was indicative of DPN[6].

Fasting blood samples were obtained from all subjects after a 10-12h overnight fast for the assay of lipid levels [total cholesterol (TC), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and triglycerides (TG)], transaminases (SGOT and SGPT) and Creatinine (Cr) concentrations.

Morning urine samples were collected from each participant for the determination of albumin concentrations. Nephropathy was diagnosed through the calculation of glomerular filtration rate ( $GFR < 60 \text{ mL/min/1.73m}^2$ ) and the presence of albuminuria (defined as urinary excretion of  $> 30 \text{ mg albumin/day}$ )[7]. The Creatinine Clearance Estimate by Cockcroft and Gault was used for the calculation of the GFR.

### ***b. Cardiac imaging***

Myocardial ischemia was diagnosed via electrocardiographically gated Technetium-99m (Tc-99m) Sestamibi Single-photon Emission Computed Tomographic (SPECT) images, a method adding added value to non-gated SPECT perfusion images[8]. The Summed Stress Score (SSS) which represents the extent and severity of a perfusion abnormalities was obtained and considered abnormal when  $\geq 4$ [9]. Additionally the percentage of ischemia was estimated for each patient, as described in the European Association of Nuclear Medicine guidelines[10].

Patients were instructed to fast for a minimum of 4h and withhold beta-blockers, calcium channel blockers, nitrates, methyl-xanthene containing drugs and caffeinated foods and

beverages for at least 24-48h prior to the examination[10]. The camera used was a Philips dual head ADAC  $\gamma$ -camera with the AutoQUANT Plus release 6.5 software.

**Table S1.** Multiple linear regression model predicting the Summed Stress Score (SSS), excluding patients with abnormal RR ratio (sensitivity analysis 1) or patients with ulcers (sensitivity analysis 2)

	Sensitivity analysis 1 (adjusted R <sup>2</sup> =0.22)		Sensitivity analysis 2 (adjusted R <sup>2</sup> =0.25)	
	Standardized coefficients (β)	P-value	Standardized coefficients (β)	P-value
<b>NDS</b>	<b>0.32</b>	<b>0.003</b>	<b>0.24</b>	<b>0.04</b>
Waist	0.22	0.03	0.22	0.04
Age	0.09	0.36	0.21	0.07
Sex (Male)	-0.24	0.02	-0.28	0.01

**Table S2.** Multiple linear regression model predicting the Summed Stress Score (SSS), including the RR ratio instead of NDS into the model

	Univariate analyses		Multivariate analysis (adjusted R <sup>2</sup> =0.18)	
	Spearman's correlation coefficient ( $\rho$ )	P-value	Standardized coefficients ( $\beta$ )	P-value
<b>RR ratio</b>	<b>-0.30</b>	<b>0.005</b>	<b>-0.24</b>	<b>0.02</b>
Waist	0.19	0.089	0.27	0.01
Age	0.31	0.004	0.13	0.21
Sex (Male)	-0.42	<0.001	-0.27	0.009

## References

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