Diabetes Research and Clinical Practice 151 (2019) 152–162

Contents available at ScienceDirect

Diabetes Research and Clinical Practice

journal homepage: www.elsevier.com/locate/diabres

Cardiac syncope recurrence in type 2 diabetes mellitus patients vs. normoglycemics patients: The CARVAS study

Celestino Sardu [a,*], *Pasquale Paolisso* [a], *Matteo Santamaria* [b], *Cosimo Sacra* [b], *Gorizio Pieretti* [c], *Maria Rosaria Rizzo* [a], *Michelangela Barbieri* [a], *Lucia Scisciola* [a], *Gianfranco Nicoletti* [c], *Giuseppe Paolisso* [a], *Raffaele Marfella* [a]

[a] *Department of Advanced Medical and Surgical Sciences, University of Campania "Luigi Vanvitelli", Naples, Italy*
[b] *Cardiovascular and Arrhythmias Department, Juan Paul II Research and Care Foundation, Campobasso, Italy*
[c] *Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy*

ARTICLE INFO

Article history:
Received 19 February 2019
Received in revised form
1 April 2019
Accepted 12 April 2019
Available online 18 April 2019

Keywords:
Type 2 diabetes mellitus
Vaso vagal syncope
Cardiac autonomic dysfunction

ABSTRACT

Study hypothesis: Cardiac autonomic dysfunction might lead to higher vaso vagal syncope (VVS) recurrence rate in type 2 diabetes mellitus (T2DM) patients vs. non diabetics patients.
Background: VVS recurrence might be due to alterations of autonomic system function, as assessed by heart rate variability (HRV). To date, in this study we investigated the correlation between HRV alterations and VVS recurrence at 12 months of follow up in T2DM vs. non T2DM patients.
Materials and methods: In a prospective multicenter study we studied a propensity score matching (PSM) analysis of 121 T2DM vs. 121 non T2DM patients affected by VVS.
Results: T2DM vs. non T2DM patients had at baseline a higher rate of HRV dysfunction, and this was linked to higher rate of VVS recurrence at 12 months of follow up ($p < 0.05$). Blood pressure alterations and lower LF/HF ratio were linked to higher rate of all cause syncope recurrence, and of vasodepressor, cardio inhibitory, and mixed syncope recurrence ($p < 0.05$). Anti hypertensive drug therapies increased the number of vasodepressor and mixed syncope events ($p < 0.05$); alterations of heart rate increased syncope recurrence and mixed syncope recurrence events ($p < 0.05$). Finally, T2DM was linked to higher rate of VVS recurrence, and specifically of vasodepressor and mixed VVS recurrence ($p < 0.05$).
Conclusions: T2DM patients have alterations of the autonomic nervous system, as result of cardiac autonomic neuropathy. However, T2DM diagnosis and autonomic dysfunction assessed by HRV alterations predicted VVS recurrence.

* Corresponding author at: Piazza Miraglia, 2, 80138 Naples, Italy.
E-mail addresses: drsarducele@gmail.com (C. Sardu), matteo.santamaria@fgps.it (M. Santamaria), cosimo.sacra@fgps.it (C. Sacra), gorizio.pieretti@unicampania.it (G. Pieretti), mariarosaria.rizzo@unicampania.it (M.R. Rizzo), michelangela.barbieri@unicampania.it (M. Barbieri), lucia.scisciola@unicampania.it (L. Scisciola), Gianfranco.nicoletti@unicampania.it (G. Nicoletti), giuseppe.paolisso@unicampania.it (G. Paolisso), raffaele.marfella@unicampania.it (R. Marfella).
https://doi.org/10.1016/j.diabres.2019.04.015

1. Introduction

Vaso vagal syncope (VVS) recurrence is a relevant clinical problem [1]. Indeed, despite the rapid onset, the short duration of the acute crisis, and the spontaneous complete recovery after the event, VVS may be complicated by physical injury and worse prognosis [1–2]. However, VVS has a frequency between 15% and 39%, with annual number of episodes about 18.1–39.7 per 1000 patients [3]. To date, VVS has an incidence of 6.2 per 1000 person-years, that grows up after 70 years of age with rate annual 19.5 per thousand individuals after 80 years [3]. However, VVS recurrence rate is about 35%, causing a physical injury until the 29% [3]. The patients with type 2 diabetes mellitus (T2DM) represent a percentage about the 30% of all the VVS subjects [4]. Different mechanisms might explain the link between VVS recurrence and T2DM, such as the abnormalities of autonomic nervous system [5]. The autonomic nervous system regulates the hemodynamic stability by maintaining a stable blood pressure and the heart rate under normal and abnormal physiologic conditions [5]. Consequently, the dysfunction of this complex regulatory system, and of its interaction with sensor systems as baroreceptors, mechanoreceptors, and chemoreceptors might alter the vascular reactivity, leading to the VVS and its recurrence [5]. Moreover, autonomic system alterations might cause both VVS event and VVS recurrence, as the result of an inappropriate response of the autonomic nervous system, with excessive vagal tone, and sympathetic tone withdrawal [2]. As first, T2DM patients have higher rate of autonomic system dysfunction [6]. Secondly, in T2DM patients the cardiac autonomic deregulation might be persistent, severe and known as cardiac autonomic neuropathy (CAN), [6]. To date, in T2DM with CAN there is a parasympathetic heart denervation, with an early augmentation of sympathetic tone, then leading to impaired heart rate variability (HRV), resting tachycardia, exercise intolerance, abnormal blood pressure regulation, and orthostatic hypotension [7]. However, in T2DM patients all these alterations look to be the compensatory response of the cardiac sympathetic tone increase to subclinical peripheral denervation [7]. Actually, the association between T2DM and autonomic dysfunction, such as between T2DM and VVS event and its recurrence is not well established and under investigated. Therefore, the recent studies cannot come to definitive conclusions about the link between T2DM the VVS events and VVS recurrence. Intriguingly, different studies proposed the HRV as a simple, reproducible and well-recognized method for evaluating sympatho vagal activity and autonomic dysfunction [8–9]. However, in this study we evaluated the entity of autonomic dysfunction by HRV alterations, and its relevance to cause VVS and the VVS recurrence in T2DM vs. non T2DM patients (controls) at 12 months of follow up.

2. Methods

In the prospective multicenter Carvas study conducted at University of Campania "Luigi Vanvitelli", Naples, Catholic University of Sacred Heart, Campobasso, and John Paul II Research and Care Foundation, Campobasso, Italy, we investigated from January 2010 to January 2017 a population of 1567 consecutive patients who had reported at least two syncopal episodes of unknown origin during the previous 6 months, and who had a recurrence of syncope during Head Up Tilt Test (HUT). VVS was defined as a loss of consciousness whose cause had not been determined by the following series of examinations performed in affected patients: clinical history, clinical examination (auscultation, carotid sinus massage, blood pressure measurement in supine, and upright position), electrocardiogram (ECG), chest X-ray, echocardiography, 24 h Holter ambulatory monitoring, late potentials, and a complete neurological examination, according to last international guidelines [1]. Therefore, patients with neuropathy, arterial hypertension, indications of heart failure and coronary heart disease or depression of left ventricle ejection fraction (LVEF < 55%) were excluded. All enrolled patients were in stable sinus rate before to perform the HUT, and they performed a 24 h ECG Holter, to asses sinus rhythm, heart rate, and HRV before to receive a HUT. The patients were then divided in diabetics (n 250, 15.9%) vs. non diabetics (n 1317, 84%), accordingly to diagnostic criteria for T2DM [10]. However, finally we performed a propensity score matching (PSM) analysis to evaluate 121 diabetics vs. 121 non diabetics. Fig. 1. The study complies with the Declaration of Helsinki, the research protocol was approved by the locally appointed ethics committee, and informed consent was obtained from all the subjects.

2.1. *Patients monitoring*

The enrolled patients were regularly followed by clinical visits 10 days, and at 3th, 6th and 12th months after clinical discharge by the treating physician. These patients had diagnosis of VVS in accordance to HUT result [1]. All the patients gave their written informed to participate in the trail. Clinical evaluations included physical examination, vital signs, and review of adverse events, fasting blood (at least 12 h from last meal) have been performed for glycemia, lipid profile (total cholesterol (TC), triglycerides, HDL-C, and LDL-C) at every visit. Syncope recurrence and other clinical events were collected during patients interview, visits, and by hospital discharge schedules.

2.2. *Cardiovascular autonomic neuropathy evaluation*

In all patients we performed the autonomic function test evaluation by classical Ewing cardiovascular autonomic function tests, heart and pulse rate variability. The autonomic function measurements were performed in the morning (08:00–10:30) in a room with a quiet ambiance and temperature of 19–22 °C, in fasting condition from midnight and refraining from smoking and caffeine-containing beverages for almost 12 h before the evaluations. However, the cardiovascular autonomic reflex function tests were divided in parasympathetic and sympathetic tests [3,5]. The parasympathetic cardiovascular reflex tests included the heart rate response during deep breathing, the Valsalva maneuver and quick standing, whereas the sympathetic tests included the blood pressure response during the sustained handgrip test and quick standing [5]. The deep breathing test consisted of

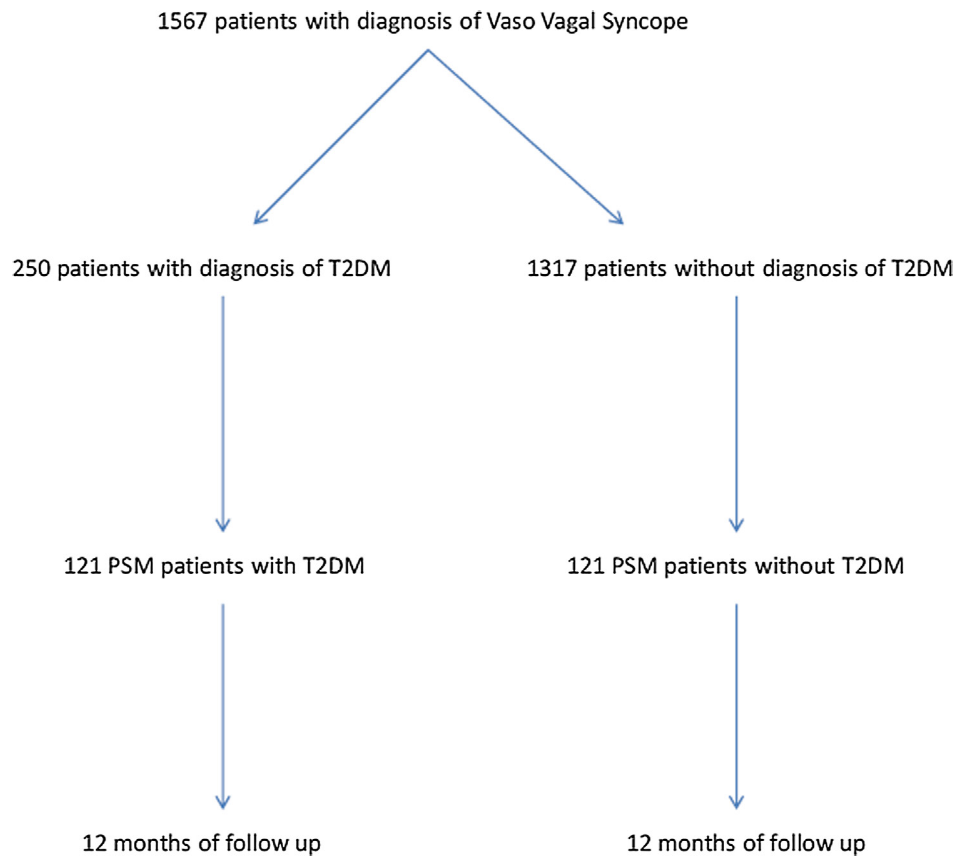


Fig. 1 – Study flow diagram. PSM: propensity score matched; T2DM: Type 2 Diabetes Mellitus.

six deep breaths in 1 min to determine the maximum and minimum R–R intervals during each breathing cycle [5]. The R–R intervals during inspiration and expiration were expressed as the R–R inspiration/R–R expiration ratio [5]. The Valsalva maneuver consisted of a forced expiration in a manometer against 40 mmHg for 15 s [5]. The Valsalva ratio was calculated as the division of the longest R–R interval by the shortest R–R interval [5]. During the quick standing test, the heart rate response after standing was derived from the R–R intervals at 15 and 30 beats after standing, and reported as the ratio of the longest vs. the shortest R–R interval [5]. The sympathetic component of the standing test was based on the systolic blood pressure response 2 min after standing [5]. The last test consisted of the diastolic blood pressure response during the sustained handgrip by a dynamometer to establish a maximum developed force, followed by a handgrip squeeze of 30% of the maximum force for 5 min [5]. Therefore, we defined Cardiovascular autonomic neuropathy (CAN) as a composite CAN index that included HR ratio < 1.30 plus Valsalva ratio < 1.5 or a decrease of > 10 mm Hg in diastolic blood pressure (DBP) upon standing (CAN+) [6–7].

2.3. HRV measurements

Before to perform HUT, all enrolled patients underwent 24 h ambulatory ECG Holter monitoring using a Marquette Laser Holter analyser (Software version 5.8), which provides the capability of classification of QRS complexes and the rejection of technical errors and interference as well as systoles arising outside the sinus node. However, authors interpreted HRV data obtained by an automatical analysis by time domain and frequency domain [8]. The time domain measures analyzed were the following: the mean NN (mean of all coupling intervals between normal beats), the standard deviation about the mean of all coupling intervals between normal beats (SDNN), the mean of all 5 min standard deviations of NNs (SD), the proportion of adjacent normal RR intervals differing by 50 ms (pNN50), the root-mean-square of the difference between successive RRs (rMSSD), and the standard deviation of 5 min mean NN intervals (SDANN), [8]. In addition, the HRV variability analysis records the component of low-frequency range (LF, 0.04–0.15 Hz) and the component of the high-frequency range (HR, 0.15–0.4 Hz), and the LF/HF ratio as index of the autonomic system dysfunction [8].

2.4. Head up tilt test (HUT)

HUT was always performed in the morning in a quiet room with the lights slightly dimmed, after overnight fasting. Authors used a motorized tilt table with foot support according as previously reported [2]. Therefore, after a 5-minute supine control phase, patients were moved to the 60°upright position for a maximum duration of 45 min or until syncope developed [2]. At 20 min, 400 mg of nitroglycerin spray was administered sublingually [2]. At the time of syncope, patients were immediately tilted back to the horizontal position.

Syncope was defined as an abrupt, transient loss of consciousness, and a loss of postural tone associated to bradycardia, hypotension, or both [1–2]. Therefore, authors divided the patients into three groups according to whether were present during the onset of syncope [1–2]. A vasodepressor response was defined as a decrease in systolic blood pressure to <60 mmHg without a decrease in heart rate during symptoms [1–2]. A cardioinhibitory response was defined as an abrupt decrease in heart rate by ≥20% without any antecedent decrease in systolic blood pressure [1–2]. A mixed response was defined as a concurrent decrease in systolic blood pressure to <60 mmHg and a decrease in heart rate by ≥ 20% compared with averages 4 min before the onset of symptoms [1–2].

2.5. *Study endpoints*

To assess HRV alterations in patients with T2DM vs. patients without T2DM (controls), and to study VVS recurrence after HUT diagnosis in and their correlation with autonomic dysfunction in patients with T2DM vs. controls.

2.6. *Statistical analysis*

All data have been analyzed by two different physicians, and analyzed by a physician experienced in statistical analysis. The patients divided before in diabetics and non diabetics, and during follow up visits and controls in patients with syncope recurrence vs. patients without syncope recurrence. We postulated that the number of patients with alterations in the study endpoints were significantly different between diabetics and non diabetics. Normally distributed variables were tested by two-tailed Student *t* test for paired or unpaired data, as appropriate, or by one-way analysis of variance (ANOVA) for more than two independent groups of data. The categorical variables were compared by chi-square or Fisher exact test where appropriate. The statistical significance was set at $p < 0.05$ (two-sided tests) and for multiple testing we used a statistical significance of $p < 0.01$. A multivariable logistic regression analysis was conducted to predict syncope recurrence at follow up. Among all risk factors and all clinical and angiographic parameters evaluated (age, sex, resting heart rate, systolic and diastolic blood pressure, diabetes etc), only the variables presenting a $p \leq 0.25$ at univariable analysis were included in the model. The stepwise method with backward elimination was used and Odds Ratios (OR) with 95% confidence intervals was calculated. Sample size calculation was done using a computer software by a established confidence interval, and a confidence level of 95% for the study population, resulting in the study population of 180 patients. For comparison among diabetics and non diabetics, a propensity score matching (PSM) was developed from the predicted probabilities of syncope recurrence by a multivariable logistic regression model. Diabetics (n 121) were matched to non diabetics (n 121) on the basis of PSM. In all matched patients, the balancing property was satisfied. The power of the study was assessed using $\pi = 0.80$ as a standard for adequacy. Statistical analysis was performed using the SPSS software package for Windows 17.0 (SPSS Inc., Chicago Illinois). Statistical analysis was performed using the SPSS software package for Windows 17.0 (SPSS Inc., Chicago Illinois).

3. Results

Patient characteristics of all study population, and for 121 PSM diabetics vs. 121 PSM non diabetics are reported in Table 1 and 2. The T2DM vs. non T2DM patients had higher rate of HRV dysfunction, lower values of LF/HF ratio, and higher rate of syncope recurrence at follow up ($p < 0.05$). Table 3. In detail, syncope recurrence was: vasodepressor type (19 (15.7%) vs. 10 (8.3%), $p < 0.05$), mixed type (37 (30.6%) vs. 9 (7.4%), $p < 0.05$), and cardioinhibitory type (5 (4.1%) vs. 3 (2.5%), $p > 0.05$), comparing T2DM vs. non T2DM patients. Table 2. At linear regression analysis, hypertension was linked to higher rate of all cause of syncope, vasodepressor, cardio inhibitory, and mixed syncope recurrence ($p < 0.05$). Table 3. However, heart rate increased syncope recurrence and mixed syncope recurrence events ($p < 0.05$), while lower values of the LF/HF ratio increased the number of syncope recurrence, and specifically of vasodepressor, cardio inhibitory and mixed syncope recurrence ($p < 0.05$). Table 3. Finally, T2DM increased the rate of syncope recurrence ($p < 0.05$). Table 3. At multivariate analysis, T2DM predicted syncope recurrence (HR 1.322, CI 95%: 1.169–1.616, $p < 0.05$), vaso depressor syncope recurrence (HR 1.101, CI 95%: 1.019–1.518, $p < 0.05$), and mixed syncope recurrence (HR 1.010, CI 95%: 1.001–4.984, $p < 0.05$). Table 4. At multivariate analysis, the autonomic dysfunction as assessed by HRV alterations (lower LF/HF ratio) predicted syncope recurrence (HR 2.567, CI 95%: 1.322–4.985, $p < 0.05$), vasodepressor syncope recurrence (HR 5.302, CI 95%: 1.151–12.422, $p < 0.05$), and mixed type of syncope (HR 5.151, CI 95%: 1.090–29.334, $p < 0.05$). Table 4. Similarly, hypertension (HR 0.165, CI 95% (0.076–0.358), $p < 0.05$), ACE inhibitors (HR 2.340, CI 95% (1.021–5.361), $p < 0.05$), and ARS blockers (HR 2.250, CI 95% (1.059–4.782), $p < 0.05$) predicted syncope recurrence. Table 4. In the dispersion curves, we reported a statistical significant difference in the syncope recurrence in diabetics vs. non diabetics by LF/HF ratio values ($p < 0.05$). Fig. 2. In addition, the Kaplan curves showed the cumulative survival free from syncope recurrence events (and for any type of syncope recurrence) in T2DM vs. non T2DM patients ($p < 0.05$). Fig. 3 (see. Fig. 4.).

4. Discussion

Main findings of our study are that, T2DM patients vs. no T2DM patients (controls) present a major degree of autonomic dysfunction, and a higher rate of VVS recurrence at 12 months of follow up. The VVS recurrence in T2DM patients is more frequently vasodepressor and mixed. In addition, hypertension is linked to higher rate of syncope, and predicted syncope recurrence at 12 months of follow up in T2DM patients vs. controls ($p < 0.05$). To date, also antihypertensive drugs (ACE inhibitors and ARS blockers drugs) predicted syncope recurrence at 12 months of follow up in T2DM patients vs. controls ($p < 0.05$). Furthermore, a higher heart rate increased syncope recurrence and mixed syncope recurrence events in T2DM patients vs. controls ($p < 0.05$).

Table 1 – Characteristics of study population at baseline.

Study variables at baseline	Study population (n 242)	121 PSM Diabetics	121 PSM Non Diabetics	P
Clinical characteristics				
Age (years)	55.54 ± 23.8	55.14 ± 21.2	55.95 ± 26.27	n.s.
Gender (male %)	123 (48.4)	61 (50.4)	62 (51.2)	n.s.
Smokers	33 (13)	18 (14.9)	15 (12.4)	n.s.
BMI > 30 kg/m2	19 (7.5)	10 (8.3)	9 (7.4)	n.s.
Dyslipidemia (%)	88 (34.6)	45 (37.2)	43 (35.5)	n.s.
Hypotiroidism (%)	25 (9.8)	14 (11.6)	11 (9.1)	n.s.
Systolic BP (mmHg)	122 ± 18	124 ± 18	120 ± 17	n.s.
Diastolic BP (mmHg)	73 ± 9	73 ± 9	72 ± 9	n.s.
Systemic Hypertension	140 (57.8)	71 (58.7)	69 (57)	n.s.
LVEF (%)	58 ± 5	57 ± 5	59 ± 4	n.s.
Prodromes (%)	131 (54.1)	70 (57.8)	61 (50.4)	n.s.
N Episodes/year (n)	2.5 ± 1.9	2.9 ± 1.2	2.2 ± 1.5	n.s.
Fasting glucose (mmol)	6.94 ± 2.17	8.89 ± 1.44	6 ± 0.67	<0.05*
HB1Ac (%)	6.5 ± 0.8	7.4 ± 0.6	6.1 ± 0.6	<0.05*
Autonomic dysfunction				
Resting heart rate (bpm)	70 ± 13	74 ± 14	65 ± 10	<0.05*
Deep breathing; HR (ratio)	1.23 ± 0.1	1.12 ± 0.08	1.28 ± 0.08	<0.05*
Valsalva manoeuvre (ratio)	1.14 ± 0.05	1.04 ± 0.01	1.19 ± 0.02	<0.05*
Lying to standing (30:15 ratio)	1.15 ± 0.02	0.97 ± 0.01	1.22 ± 0.02	<0.05*
Postural BP changes (mmHg)	10 ± 3	9 ± 3	12 ± 2	n.s.
Sustained handgrip test (mmHg)	15 ± 4	16 ± 3	15 ± 5	n.s.
LF/HF ratio	5.09 ± 0.68	4.46 ± 0.29	5.72 ± 0.58	<0.05*
LF, normalized units	84.4 ± 3.9	80.3 ± 2.1	87.68 ± 2.1	n.s.
HF, normalized units	18.2 ± 1.2	18.1 ± 1.4	18.2 ± 1.1	n.s.
ECG Holter parameters				
Mean NN	816.62 ± 70.18	860.84 ± 58.9	772.40 ± 49.8	<0.05*
SDNN	180.94 ± 32.47	203.88 ± 25.87	158.01 ± 19.68	<0.05*
SDANN	172.19 ± 38.17	194.17 ± 30.74	142.21 ± 31.73	<0.05*
SD	85.41 ± 18.12	105.15 ± 17.85	75.66 ± 12.20	<0.05*
RMSSD	64.47 ± 15.88	82.11 ± 16.08	58.83 ± 13.55	<0.05*
pNN50	25.32 ± 9.09	27.69 ± 9.43	22.95 ± 8.12	<0.05*
Echocardiographic parameters				
IVS (mm)	10 ± 2	11 ± 2	10 ± 3	n.s.
LVEDv (ml/m^2)	49.8 ± 12.1	50.2 ± 10.6	49.8 ± 12.5	n.s.
LVESv (ml/m^2)	19.9 ± 5.6	21.2 ± 5.5	19.2 ± 5.4	n.s.
LVEF (%)	60 ± 5	59 ± 4	60 ± 5	n.s.
LAV (ml/m^2)	26.3 ± 2.7	26.6 ± 2.9	26.2 ± 2.7	n.s.
Cardiovascular medications				
Beta blockers (%)	63 (26.1)	32 (26.5)	31 (25.6)	n.s.
Calcium blockers (%)	42 (16.7)	21 (16.7)	21 (16.7)	n.s.
ACE inhibitors (%)	103 (42.6)	53 (43.8)	50 (41.3)	n.s.
ARS blockers (%)	37 (15.3)	17 (14)	20 (16.5)	n.s.
Loop diuretics (%)	51 (21)	24 (19.8)	27 (22.3)	n.s.
Tiazides diuretics (%)	67 (27.7)	35 (28.9)	32 (26.4)	n.s.
Statins (%)	97 (40)	50 (41.3)	47 (38.8)	n.s.
Class 1 anti arrhythmic drugs (%)	25 (10.3)	13 (10.7)	12 (9.9)	n.s.
Class 3 anti arrhythmic drugs (%)	7 (2.8)	3 (2.5)	4 (3.3)	n.s.
Digitalis (%)	20 (8.3)	10 (8.3)	10 (8.3)	n.s.
Anti platelets (%)	75 (31)	40 (33)	35 (29)	n.s.
Anticoagulants (%)	19 (7.9)	9 (7.4)	10 (8.2)	n.s.
Hypoglycemic drugs				
Metformin	65 (53.7)	65 (53.7)	/	/
Sulfonylureas	15 (12.4)	15 (12.4)	/	/
Glinides	6 (5)	6 (5)	/	/
Glitazones	5 (5)	5 (5)	/	/
Incretins	16 (13.2)	16 (13.2)	/	/
Insulin therapy	26 (21.5)	26 (21.5)	/	/

ACE: angiotensin converting enzyme; ARS: angiotensin receptors; BMI: body mass index; BP: blood pressure; HB1Ac: glycated hemoglobin 1 Ac; HF: high frequency; HR: heart rate;
IVS: inter ventricular septum; LF: low frequency; LAV: left atrium volume; LVEDv: left ventricle end diastolic volume; LVESv: left ventricle end systolic volume; LVEF: left ventricle ejection fraction; pNN50: the proportion of adjacent normal RR intervals differing by 50 ms; N: number; NN: mean of all coupling intervals between normal beats; RMSSD: the root-mean-square of the difference between successive RRs; SDNN: the standard deviation about the mean of all coupling intervals between normal beats; SDANN: the standard deviation of 5 min mean NN intervals; SD: the mean of all 5 min standard deviations of NNs.

Table 2 – Characteristics of study population at 12 months of follow up.

STUDY VARIABLES AT 12th MONTH	Study population (n 242)	121 PSM Diabetics	121 PSM Non Diabetics	P
Clinical characteristics				
Smokers	31 (12.8)	16 (13.2)	15 (12.4)	n.s.
BMI > 30 kg/m^2	17 (7.4)	9 (7.4)	8 (6.6)	n.s.
Dyslipidemia (%)	79 (31.3)	41 (33.8)	38 (31.4)	n.s.
Hypotiroidism (%)	25 (9.8)	14 (11.6)	11 (9.1)	n.s.
Systolic BP (mmHg)	121 ± 13	123 ± 15	120 ± 12	n.s.
Diastolic BP (mmHg)	70 ± 5	70 ± 8	70 ± 6	n.s.
Systemic Hypertension	131 (54.1)	68 (56.2)	63 (52)	n.s.
Fasting glucose (mmol)	7.44 ± 0.88	8.15 ± 1.21	6 ± 0.59	<0.05*
HB1Ac (%)	6.5 ± 0.5	7.0 ± 0.4	6.1 ± 0.6	<0.05*
Echocardiographic parameters				
IVS (mm)	10 ± 2	11 ± 2	10 ± 3	n.s.
LVEDv (ml/m^2)	49.8 ± 12.1	49.6 ± 11.2	49.5 ± 11.9	n.s.
LVESv (ml/m^2)	19.9 ± 5.6	20.2 ± 4.9	18.9 ± 5.8	n.s.
LVEF (%)	60 ± 6	59 ± 6	60 ± 6	n.s.
LAV (ml/m^2)	26.0 ± 2.4	26.1 ± 2.4	25.8 ± 2.3	n.s.
Syncope recurrence events (%)				
Total number of events (%)	86 (35.5)	64 (52.9)	22 (18.2)	<0.05*
Vasodepressor (%)	29 (12)	19 (15.7)	10 (8.3)	<0.05*
Cardio-inhibitory (%)	11 (4.5)	6 (4.9)	5 (4.1)	n.s.
Mixed (%)	46 (19)	37 (30.6)	9 (7.4)	<0.05*
Cardiovascular medications				
Beta blockers (%)	67 (27.7)	35 (26.4)	31 (25.6)	n.s.
Calcium blockers (%)	35 (13.9)	19 (15.7)	16 (13.2)	n.s.
ACE inhibitors (%)	107 (44.2)	55 (45.4)	52 (43)	n.s.
ARS blockers (%)	42 (17.3)	22 (18.2)	20 (16.5)	n.s.
Loop diuretics (%)	51 (21)	24 (19.8)	27 (22.3)	n.s.
Tiazides diuretics (%)	71 (29.3)	37 (30.5)	34 (28.1)	n.s.
Statins (%)	103 (42.5)	53 (43.8)	50 (41.3)	n.s.
Class 1 anti arrhythmic drugs (%)	25 (10.3)	13 (10.7)	12 (9.9)	n.s.
Class 3 anti arrhythmic drugs (%)	7 (2.8)	3 (2.5)	4 (3.3)	n.s.
Digitalis (%)	20 (8.3)	10 (8.3)	10 (8.3)	n.s.
Anti platelets (%)	75 (31)	40 (33)	35 (29)	n.s.
Anticoagulants (%)	19 (7.9)	9 (7.4)	10 (8.2)	n.s.
Hypoglycemic drugs				
Metformin	65 (53.7)	65 (53.7)	/	/
Sulfonylureas	15 (12.4)	15 (12.4)	/	/
Glinides	6 (5)	6 (5)	/	/
Glitazones	5 (5)	5 (5)	/	/
Incretins	16 (13.2)	16 (13.2)	/	/
Insulin therapy	26 (21.5)	26 (21.5)	/	/

ACE: angiotensin converting enzyme; ARS: angiotensin receptors; BMI: body mass index; BP: blood pressure; HB1Ac: glycated hemoglobin 1 Ac; HF: high frequency; HR: heart rate;
IVS: inter ventricular septum; LF: low frequency; LAV: left atrium volume; LVEDv: left ventricle end diastolic volume; LVESv: left ventricle end systolic volume; LVEF: left ventricle ejection fraction; pNN50: the proportion of adjacent normal RR intervals differing by 50 ms; N: number; NN: mean of all coupling intervals between normal beats; RMSSD: the root-mean-square of the difference between successive RRs; SDNN: the standard deviation about the mean of all coupling intervals between normal beats; SDANN: the standard deviation of 5 min mean NN intervals; SD: the mean of all 5 min standard deviations of NNs.

Consequently, altered values of LF/HF ratio increased the number of syncope, and of any type of syncope recurrence comparing T2DM vs. controls ($p < 0.05$). Moreover, in the CARVAS study the autonomic dysfunction assessed by HRV alterations and LF/HF ratio, independently predicted syncope recurrence, and in detail vasodepressor and mixed type of VVS in T2DM patients vs. controls. Finally, T2DM increased the rate of VVS recurrence, and it was predictive of VVS recurrence, and specifically of vasodepressor and mixed syncope recurrence in T2DM patients vs. controls ($p < 0.05$).

The ECG Holter records the HRV variability, and the LF/HF ratio as index of the autonomic system dysfunction [8–11]. However, the LF is the component of the low-frequency range (0.04–0.15 Hz), and the HF is the component of the high-frequency range (0.15–0.4 Hz), [11]. Intriguingly, LF is modulated by both the sympathetic and parasympathetic nervous system and associated with baroreceptor activity [11]. On the contrary, HF is modulated by the parasympathetic nervous system, and connected with respiration and blood pressure changes [11]. Moreover, the LF/HF ratio is an index reflecting the interactions of both types of autonomic modulation with the regulation of heart rate and blood pressure [11]. From this, the LF/HF ratio is a complex marker of autonomic dysfunction, used to stratify and to predict VVS

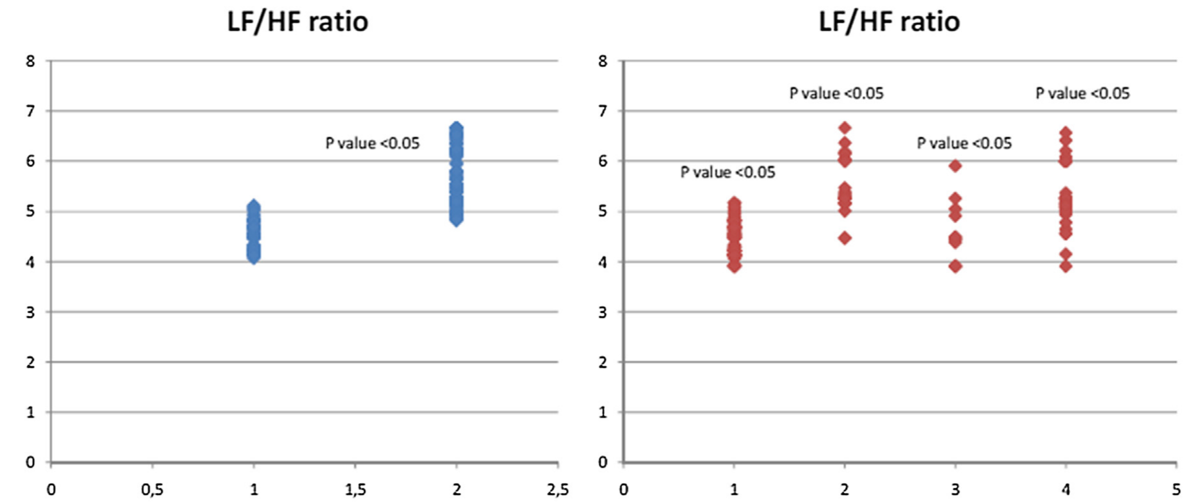
Table 3 – Linear regression analysis between study variables and syncope recurrence. Confidence interval (CI) 95%, p < 0.05 (*). ACE: angiotensin converting enzyme; ARS: angiotensin-renin receptors; BP: blood pressure; LF/HF: low frequency/high frequency ratio; LVEF: left ventricle ejection fraction; T2DM: type 2 diabetes mellitus.

Variables	Syncope recurrence		Vasodepressor syncope		Cardio inhibitory syncope		Mixed syncope	
	R value	P	R value	P	R value	P	R value	P
Age	0.010	0.750	0.012	0.644	0.015	0.851	0.005	0.754
Smoking	−0.050	0.490	0.005	0.938	0.092	0.024	0.040	0.538
Hypertension	0.264	0.001*	0.152	0.001*	0.087	0.004*	0.215	0.001*
Dyslipidemia	0.041	0.763	0.166	0.215	−0.236	0.062	0.041	0.763
Systolic BP	0.002	0.259	0.160	0.230	0.229	0.083	0.162	0.223
Diastolic BP	−0.003	0.391	0.232	0.080	0.162	0.224	0.180	0.176
LVEF	−0.008	0.110	−0.001	0.793	−0.001	0.678	−0.001	0.822
Beta blockers	0.145	0.118	−0.036	0.640	0.023	0.663	−0.107	0.201
ACE inhibitors	−0.137	0.077	−0.002	0.972	0.055	0.208	−0.026	0.714
diuretics	0.090	0.186	0.086	0.142	0.020	0.611	0.094	0.133
ARS blockers	−0.118	0.056	−0.076	0.151	−0.020	0.565	−0.043	0.441
Nitroderivates	−0.034	0.561	−0.007	0.887	−0.005	0.895	−0.042	0.451
Anti arrhythmics	0.099	0.431	−0.022	0.832	0.057	0.414	−0.048	0.667
digitalis	−0.042	0.657	−0.226	0.085	−0.027	0.614	0.270	0.072
Alpha litics	0.112	0.772	−0.023	0.943	−0.136	0.532	0.366	0.065
Calcium blockers	0.215	0.128	−0.111	0.141	−0.041	0.413	−0.115	0.150
Sex	0.009	0.750	0.212	0.110	0.201	0.133	0.009	0.949
Heart rate	0.162	0.032*	0.081	0.545	0.007	0.951	0.449	0.001*
LF/HF ratio	0.166	0.007*	0.112	0.003*	0.088	0.001*	0.268	0.001*
T2DM	0.362	0.001*	0.057	0.276	−0.039	0.252	0.074	0.062

recurrence in overall population and in T2DM patients [11–12]. Indeed, in our study we found that, T2DM patients vs. controls had lower LF/HF values ($p < 0.05$), as the result of an increased vagal sinus modulation [13]. To date, lower LF/HF values increased the risk for future syncope recurrence in T2DM patients vs. controls ($p < 0.05$), and specifically lower LF/HF values predicted vasodepressor and mixed VVS recurrence in T2DM vs. controls patients. Therefore, the vasodepressor VVS is due to the fall and the collapse of the blood pressure, while in the mixed VVS there are the pressure drop and the fall of the heart rate blood pressure together during the clinical event [1]. Moreover, the blood pressure fall might be seen as a common mechanism to cause VVS in both vasodepressor and mixed forms [14]. However, a clear association exists between syncopal episodes and altered blood pressure values [15]. To date, we found that altered blood pressure values linked to a higher number of VVS, and they predicted VVS recurrence in T2DM vs. controls patients ($p < 0.05$). Similarly, in our study the vaso-active therapy with ACE inhibitors and ARS blockers drugs predicted VVS recurrence at 12 months of follow up in T2DM vs. controls patients ($p < 0.05$). Thereafter, in line with other authors not only a history of hypertension but also a treatment with anti-hypertensive drugs increased the risk to have future VVS recurring events [1,14–17]. Indeed, during the clinical VVS event we have a pathological response of the autonomic system, caused by sympathetic tone withdrawal secondary to hypotensive stimuli [1,14–17]. In detail, the anti-hypertensive therapy with ACE inhibitors and ARS blockers drugs might specifically act by lowering the blood pressure, with modulation and block of the ACE functions and of the ARS pathways, without interfering with sympathetic tone function [1,14–17]. However, ACE inhibitors and ARS blockers vs. other anti-hypertensive drugs have not a regulative effect on sympathetic tone functions, and on the autonomic dysfunction as observed during the VVS event [1,14–17]. Consequently, for all these reasons the anti-hypertensive treatment with ACE inhibitors and ARS blockers might cause in our study a higher rate of VVS recurrence at 12 months of follow up in T2DM vs. controls patients. To date, the simultaneous assessment of blood pressure and heart rate during a diagnostic tilt-test in VVS patients is relevant and applied in risk-guided tilt training and in the implanted devices technologies to trigger pacing intervention [17]. Therefore, a lower LF/HF ratio might evidence in T2DM vs. controls patients a higher degree of sympathetic and parasympathetic tone dysfunction, with abnormal blood pressure response during the VVS events. In conclusion, the marked autonomic system dysfunction leads to higher rate of VVS, and this might explain also the higher risk for VVS recurrence in T2DM vs. controls. Moreover, the T2DM is predictive of future VVS, and specifically of vasodepressor and mixed VVS recurrence at 12 months of follow up. Intriguingly, we might speculate that, the autonomic dysfunction and the existing link between autonomic dysfunction and increased number of VVS events and VVS recurrence in T2DM patients, might be the result of a persistent form of induced cardiac autonomic neuropathy [6]. Indeed, the cardiac autonomic neuropathy in T2DM patients looks to be the resulting form of complex sympathetic and vaso vagal cardiovascular abnormalities, that impact on blood pressure regulation and heart rate response to vaso-active stimuli [1,6,14–17]. The cardiac autonomic neuropathy might be seen as a distinctive factor in T2DM, that influences the number of VVS events, and the VVS recurrence in T2DM patients vs. controls. Finally, in the CARVAS study the CAN by the sympathetic/parasympathetic

Table 4 – Multivariate analysis for any type of syncope recurrence, vasodepressor syncope recurrence, cardiac inhibitory syncope, and mixed syncope. Confidence interval (CI) 95%, $p < 0.05$ (*). ACE: angiotensin converting enzyme; ARS: angiotensin-renin receptors; LF/HF: low frequency/high frequency ratio; LVEF: left ventricle ejection fraction; T2DM: type 2 diabetes mellitus.

Syncopre recurrence	Multivariate analysis HR (95% CI)	*p*	Vasodepressor syncope recurrence	Multivariate analysis HR (95% CI)	*p*
LF/HF ratio	2.567 (1.322–4.985)	0.005*	LF/HF ratio	5.302 (1.151–12.422)	0.032*
T2DM	1.322 (1.169–1.616)	0.001*	T2DM	1.101 (1.019–1.518)	0.006*
Age	0.999 (0.989–1.009)	0.835	Age	0.998 (0.980–1.015)	0.786
Smoking	1.260 (0.643–2.469)	0.510	Smoking	1.150 (0.377–3.503)	0.806
Hypertension	0.165 (0.076–0.358)	0.001*	Hypertension	0.197 (0.038–1.013)	0.052
LVEF	0.955 (0.910–1.002)	0.063	LVEF	1.016 (0.927–1.115)	0.731
Beta blockers	0.733 (0.278–1.931)	0.529	Beta blockers	0.747 (0.123–4.552)	0.752
Calcium blockers	1.087 (0.450–2.627)	0.853	Calcium blockers	0.967 (0.189–4.946)	0.967
diuretics	0.6 (0.294–1.227)	0.162	diuretics	0.621 (0.229–1.679)	0.348
ACE inhibitors	2.340 (1.021–5.361)	0.044*	ACE inhibitors	2.407 (0.625–9.268)	0.202
ARS blockers	2.250 (1.059–4.782)	0.035*	ARS blockers	2.532 (0.682–9.4)	0.165
nitroderivates	0.784 (0.418–1.471)	0.784	nitroderivates	0.595 (0.199–1.779)	0.353
Anti arrhytmics	0.667 (0.182–2.441)	0.541	Anti arrhytmics	0.650 (0.060–7.010)	0.723
digitalis	0.767 (0.307–1.918)	0.570	digitalis	0.323 (0.098–1.069)	0.064
Alpha litics	0.186 (0.010–1.674)	0.974	Alpha litics	6.132 (0.121–9.251)	0.991
Cardiac inhibitory syncope recurrence	Multivariate analysis HR (95% CI)	*p*	Mixed syncope recurrence	Multivariate analysis HR (95% CI)	*p*
LF/HF ratio	6.676 (0.963–46.307)	0.055	LF/HF ratio	5.151 (1.090–29.334)	0.039*
T2DM	0.473 (0.084–2.673)	0.473	T2DM	1.010 (1.001–4.984)	0.009*
Age	0.994 (0.972–1.017)	0.634	Age	0.988 (0.970–1.006)	0.180
Smoking	0.243 (0.071–1.839)	0.075	Smoking	0.731 (0.253–2.112)	0.563
Hypertension	0.010 (0.001–5.216)	0.922	Hypertension	0.157 (0.020–1.262)	0.082
LVEF	0.946 (0.848–1.055)	0.318	LVEF	1.082 (0.977–1.198)	0.129
Beta blockers	0.901 (0.068–11.934)	0.901	Beta blockers	1.322 (0.110–15.874)	0.826
Calcium blockers	2.628 (0.287–24.044)	0.392	Calcium blockers	0.730 (0.165–3.236)	0.679
diuretics	0.358 (0.085–1.498)	0.159	diuretics	0.728 (0.240–2.205)	0.574
ACE inhibitors	0.435 (0.091–2.068)	0.295	ACE inhibitors	0.901 (0.178–4.549)	0.899
ARS blockers	1.664 (0.272–10.179)	0.582	ARS blockers	1.517 (0.352–6.533)	0.576
nitroderivates	1.090 (0.262–4.541)	0.906	nitroderivates	1.903 (0.460–7.862)	0.374
Anti arrhytmics	0.289 (0.014–6.059)	0.424	Anti arrhytmics	0.274 (0.015–5.096)	0.386
digitalis	3.944 (0.391–39.808)	0.245	digitalis	0.627 (0.159–2.475)	0.505
Alpha litics	0.186 (0.010–1.674)	0.099	Alpha litics	0.167 (0.001–5.214)	0.889



Study variables	Study population (n 242)	121 PSM Diabetics	121 PSM Non Diabetics	P value
LF/HF ratio	5.12±0.69	4.46±0.29	5.72±0.58	<0.05*

Study variable	SYNCOPE RECURRENCE (1)		VASO DEPRESSOR SYNCOPE (2)		CARDIO INHIBITORY SYNCOPE (3)		MIXED SYNCOPE (4)	
	R	P	R	P	R	P	R	P
LF/HF ratio	0.166	0.007*	0.112	0.003*	0,088	0,001*	0.268	0.001*

Fig. 2 – A, LF/HF ratio (values on y axis) in diabetics vs. non diabetics (p < 0.05). On × axis, 1 is for diabetics, 2 is for non diabetics. B, LF/HF ratio (values on y axis) in patients without syncope recurrence (1 on × axis), in patients with vasodepressor syncope recurrence (2 on × axis), in patients with cardio inhibitory syncope recurrence (3 on × axis), and in patients with mixed syncope recurrence (4 on × axis).

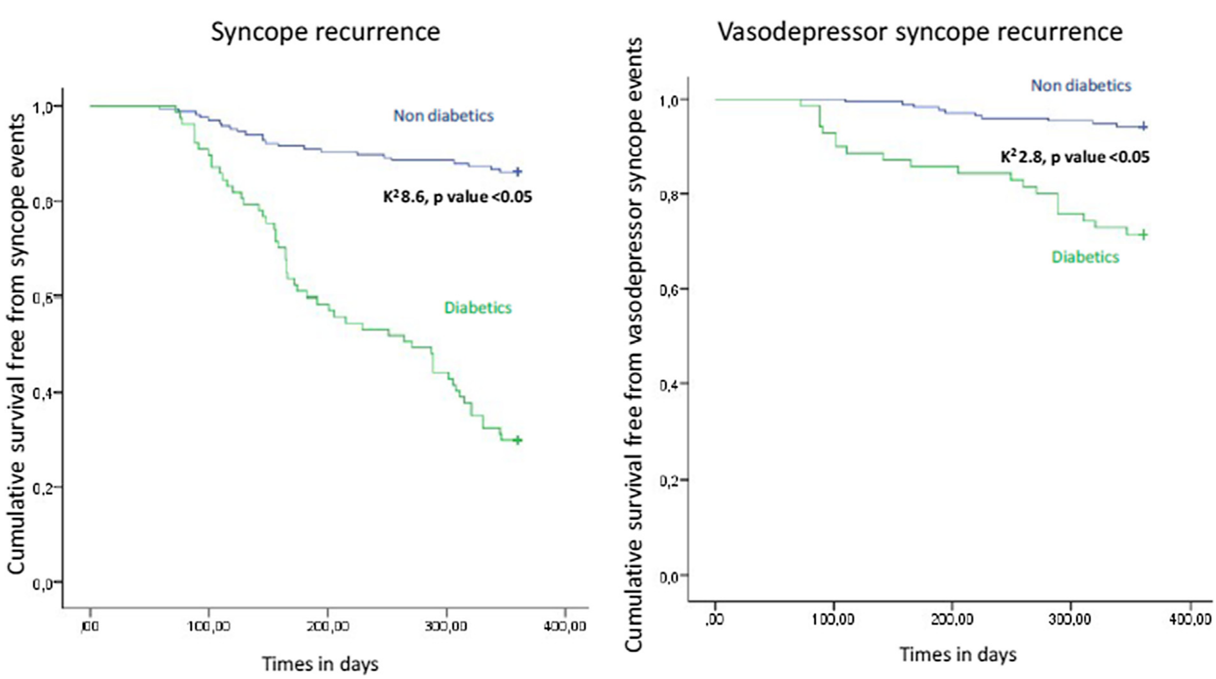


Fig. 3 – Kaplan curve for syncope recurrence, and vasodepressor syncope recurrence comparing diabetics (green color) vs. non diabetics (blue color), (*p < 0.05). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

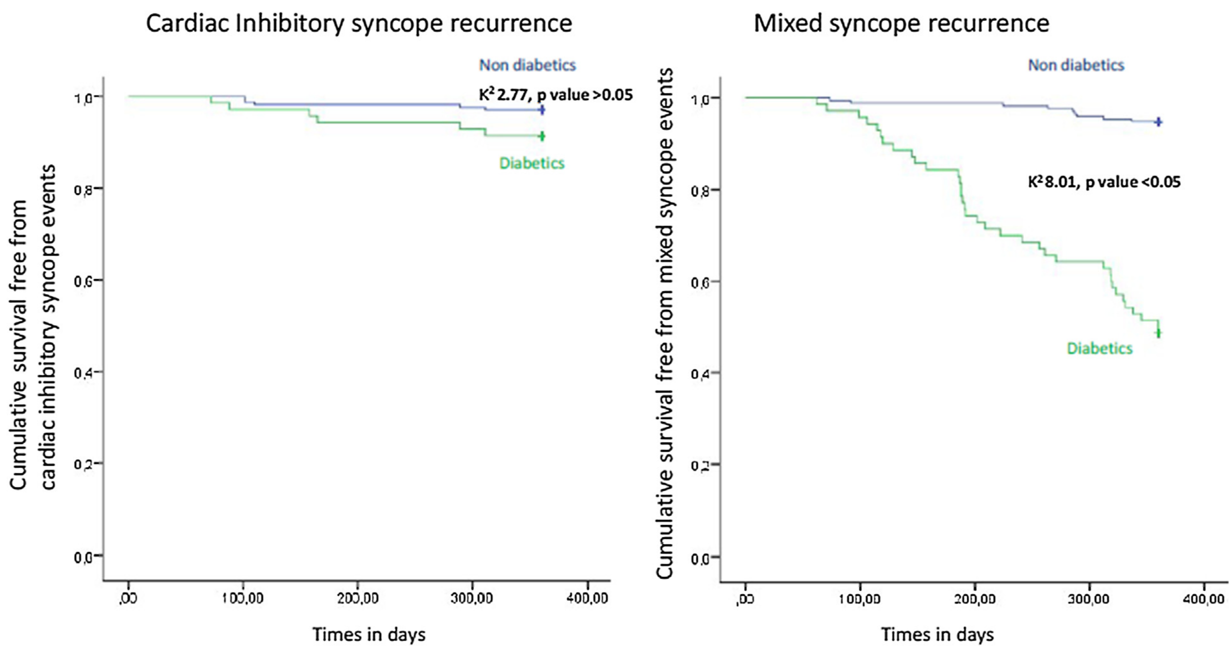


Fig. 4 – Kaplan curve for cardiac inhibitory syncope recurrence, and mixed syncope recurrence comparing diabetics (green color) vs. non diabetics (blue color), ($^*p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

nervous system influences both the modulation and control of blood pressure and the heart rate in stable and dynamic conditions [11]. In fact, the adrenergic system shows a relevant role in the regulation of cardiovascular and metabolic function. However, the β Adrenoceptors (ARs) pathways, such as the kinase of the G protein coupled receptor type 2 (GRK2), are clearly involved in regulation of cardiac contractility, remodeling and metabolism [18]. To date, adrenergic system shows adaptive dynamic processes in metabolic unfavorable conditions and in aging, altering the baroreflex sensitivity to adrenergic signaling, and affecting its ability to regulate countless physiological functions [19]. Indeed, a relevant link exists between the β-ARs over stimulation and the development of insulin resistance progression in "in vivo and ex vivo" experiments in β2-AR-null mice [20]. Moreover, the CAN in T2DM patients vs. controls might cause the higher rate of vasodepressor and mixed VVS recurrence at 12 months of follow up. In this setting, a lower LF/HF ratio in T2DM patients vs. controls with VVS looks to be a valid index and a measure of the decline in parasympathetic tone resulting by diabetic neuropathy. At the end, taken together our study results might propose the LF/HF ratio (lower values) as a key factor to explain the increase number of VVS recurrence, and specifically a predictor index of future vasodepressor VVS events in T2DM patients vs. controls.

5. Study limitations

In this study, not all centers contributed with the same amounts of patients, but no significant differences were observed among patients enrolled by the different centers. The information provided by patients' diary was used to categorize for the absence or presence of symptom (syncope or palpitation) at the time of syncope. The events reported in the diary by each patient were referred at physician at each clinic visit. The follow-up was 360 days, and the short term duration of follow up may affect long-term clinical outcomes. After HUT, and clinical discharge, patients' arrhythmias recurrence was detected by surface ECG registration, ECG Holter monitoring, and this may represent another study limitation. This study population was too small to draw any definitive conclusion on the possible correlation between T2DM and syncope recurrence findings. The capability of T2DM to cause VVS event and its recurrence, and to influence therapeutic decisions or to improve clinical outcomes was beyond of the scope of this study, and it remains to be demonstrated by an appropriately designed study conducted on higher number of VVS patients with T2DM confirmed diagnosis.

Acknowledgments

Ce.S: study design, data analyses and interpretation, study writing and editing; P.P: study interpretation; M.S., G.P., and Co.S: data collection; M.B., M.R.R., G.N., and G. P.: manuscript revision; R.M: manuscript revision and editing.

Conflicts of interest

None to declare.

Research support founds

None.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2019.04.015.

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