Clinical Update

Diabetic Cardiovascular Autonomic Neuropathy: Risk Factors, Clinical Impact and Early Diagnosis

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Summary

Cardiovascular autonomic neuropathy (CAN) is one of the most clinically significant complications of diabetes mellitus (DM), but one of the least frequently diagnosed. In this review, we discuss the major risk factors for the development and progression of CAN in patients with DM, the natural history of autonomic neuropathy and its impact on cardiovascular disease in DM, as well as the tests for the early diagnosis and staging of CAN in the clinical practice. The bibliographic research was based on two databases: Medline and Tripdatabase, with the following descriptors: *diabetic cardiovascular autonomic neuropathy* and *cardiovascular autonomic neuropathy and diabetes*. We selected English and German articles, written between 1998 and 2007. In its initial stages (early and intermediate), CAN may be diagnosed and reversed. However, in advanced cases (severe stage), the only treatment that remains is a symptomatic one. CAN is associated with higher cardiovascular morbidity and mortality rates and poor quality of life in diabetic individuals.

Introduction

Cardiovascular autonomic neuropathy (CAN) occurs when peripheral autonomic fibers (sympathetic and parasympathetic) of the cardiovascular system (CVS), are affected, thus resulting in neurohumoral regulation disturbances. The sympathetic-vagal balance (both tonic and phasic) modulates the function of three of the main CVS structures: the sinus node (heart rate), the ventricles (end-systolic and end-diastolic volumes) and the blood vessels, including microcirculation (total peripheral resistance).

The autonomic nervous system (ANS), thus, plays a key role in the modulation of the CVS dynamics by means of an interaction between the sympathetic and vagal tonus which, in physiologic conditions, act in a negative feedback manner. In other words, the activation of the former is followed by the inhibition of the latter. In clinical practice,

Key words

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this modulation is usually assessed by the well-known study of heart rate variability (HRV), which means an analysis of spontaneous and induced fluctuations that occur in HR (or in the electrocardiographic RR interval) as a result of ANS sympathetic and parasympathetic activities on sinus node automaticity.

Most patients with diabetic polyneuropathy (PNP) show some degree of autonomic dysfunction. Patients with predominant autonomic signs and symptoms are considered as having diabetic autonomic neuropathy (DAN). Although this may affect any organ system, it usually starts in the skin neurovascular system (foot microcirculation) and in the cardiovascular system (CAN). Furthermore, it affects the gastrointestinal system (gastroparesis, constipation and diarrhea) and genitourinary system (urinary incontinence, neurogenic bladder and erectile dysfunction).

CAN is one of the major complications of DM since its presence is associated with worsening prognosis and patient's poorer life quality. The following clinical manifestations are associated with CAN: resting tachycardia, severe orthostatic hypotension (OH), syncope, exercise intolerance (due to chronotropic and inotropic response block), perioperative instability, asymptomatic myocardial ischemia and infarction, left ventricular (LV) diastolic and systolic dysfunction, and increased risk of renal diseases, chronic renal failure (CRF), stroke, and sudden cardiac death (SCD).

Despite the potential negative impact on the quality of life of patients with CAN, this disease falls among the least understood and least frequently diagnosed complications of individuals with DM. This type of neuropathy can usually be found in approximately 25% of the patients with type-1 diabetes mellitus (DM1) and in 34% of those with type-2 diabetes mellitus (DM2). The prevalence of CAN progressively increases in a direct proportion to age, duration of DM and poor glycemic control[1].

CAN may be subdivided into *subclinical* (in which functional and reversible alterations are predominant) and *clinical* (when structural neuronal alterations are already present): the first one is only diagnosed by tests and may occur as soon as the diagnosis of certain types of DM are diagnosed, or in the first years of the disease; the second form, as the name suggests, is symptomatic and occurs in more advanced stages[2].

Autonomic fibers are compromised in the several clinical subtypes of diabetic neuropathies. The most common type (classical PNP: symmetric, distal, and predominantly sensitive), shows a strong correlation between the progressive lesion of both somatic and autonomic fibers, i.e., nowadays we know that 50% of the diabetic patients with PNP have asymptomatic

CAN, whereas 100% of those with symptomatic CAN present classical PNP[3,4].

This review is aimed at finding and discussing the best evidence available in the recent literature (from 1998 to 2007) in order to clarify three key questions:

1) Which are the major risk factors (RF) for the development and progression of CAN in individuals with DM? Is there any difference between CAN of DM1 and that of DM2?

2) What is CAN natural history and its clinical impact on DM or, in practical terms, what is the significance of this diagnosis for both physician and diabetic patient?

3) How should an early diagnosis and staging be made in the clinical practice, and which are currently the best tests in terms of specificity, sensitivity and reproducibility?

For this purpose, the following strategy was used: bibliographic survey in two databases (Medline and Tripdatabase) with the descriptors *diabetic cardiovascular autonomic neuropathy* and *cardiovascular autonomic neuropathy and diabetes*. We selected articles written in English and German from 1998 to 2007 (a total of 106 references). Nevertheless, articles published before 1998 were also included, provided they were relevant or had been frequently cited (19 references).

Risk factors for CAN development and progression in patients with diabetes mellitus. Differences between CAN in DM1 and in DM2

The risk of developing autonomic dysfunction in DM depends on several factors. However, two of them are well established in the literature and are common to both DM1 and DM2: Duration of disease and the degree of glycemic control[2,5-7]. In both DM types, poor blood glucose control (chronic hyperglycemia) plays an important role both in the initial pathophysiology (oxidative stress, microcirculation dysfunction due to nitric oxide loss and Schwann cell lesion due to accumulation of free radicals) as well as in its progression (axonal degeneration and neuronal apoptosis)[8-10]. Although diabetic neuropathies are classified among DM microangiopathic complications, the pathophysiological mechanism is multifactorial, and there is enough evidence that small-fiber PNP and even CAN may precede DM (PNP related to glucose intolerance or prediabetes)[19]. Although it falls outside the scope of this review, the final common pathway of the different mechanisms already described to explain the pathophysiology of PNP seems to be oxidative and nitrative stress[4,9].

The presence of CAN, however, is not only associated with the duration and degree of hyperglycemia. In the past years, several studies[6,11-15] consistently showed that cardiovascular risk factors (CVRF), such as systolic blood pressure (SBP), body mass index (BMI), triglycerides, and smoking, play an important role in the development of CAN. It is noteworthy that these factors share at least two points in common: they are potentially modifiable and are associated with insulin resistance, once again suggesting that CAN may arise with a metabolic syndrome.

Even more important, however, were the results of the Steno 2 study, in which the intensified multifactorial intervention (hyperglycemia, hypertension, dyslipidemia and microalbuminuria) in patients with DM2 reduced the risk of CAN progression by 68%[13,14]. The role of intensive control (three doses of NPH insulin) in preventing and slowing the progression of CAN in patients with DM1 is also well-known: in the classical DCCT study, its prevalence was reduced by 53%[16].

Regarding the differences between CAN in DM1 and in DM2, it is worth pointing out that both signs and symptoms appear later in DM1 than in DM2, i.e., the great majority of DM1 patients with CAN remain totally asymptomatic for years and, when the symptoms appear, CAN is usually in advanced and irreversible stages[17].

CAN seems to be more prevalent in DM2 patients, appearing earlier and causing higher mortality rates [8,18]. This is likely due to the longer duration of the metabolic abnormalities (dysglycemia), which occur even prior to the diagnosis of DM2, in the prediabetes and metabolic syndrome stages[19,20]. These differences suggest that the natural history of CAN presents different progression routes in DM1 and in DM2, and moreover, that pathophysiological mechanisms also seem to be different. During the past decade, several studies demonstrated that anti-sympathetic ganglion, anti-vagus nerve or anti-myelin-associated-glycoprotein autoantibodies occurr in a significant proportion of patients with DM1, and many of them presented severe CAN[21].

Also worthy of attention is the fact that CAN symptoms are inexplicably uncommon and, when present, they oscillate and do not always progress, as is the case with OH. As a matter of fact, the great majority of diabetic patients with CAN are currently known to remain asymptomatic for decades, and less than 1% develops symptomatic OH[1,2]. Since clinical history and physical examination are ineffective for its early detection, it is of crucial importance to perform quantitative tests in order to diagnose CAN in its initial and still reversible stages[22].

In the past two decades, there was a great advance in our knowledge of RF for CAN initiation and progression, thanks to the development of more sensitive and specific methods for its diagnosis. It is the case of the HRV study, which allows a non-invasive, selective and longitudinal assessment of autonomic function by mathematically analyzing the magnitude of HR variations[23].

In DM2, the following RF are associated with a reduced HRV: age, obesity, hyperinsulinemia, DM duration, hypertension, retinopathy, PNP and smoking[1,2,24,25]. In DM1, the factors that increase the risk of developing CAN are: HbA1c, SBP, age, retinopathy, albuminuria, PNP, hypertriglyceridaemia, dyslipidemia, gender (female), and time of exposure to hyperglycemia[6,11,26-28].

Current data that differentiate CAN in DM1 and in DM2 in terms of risk factors and natural history are summarized in Table 1. We can conclude that, in relation to RF, there are modest differences between CAN in DM1 and in DM2 and, regarding its natural history, CAN seem to progress more rapidly in DM2 than in DM1.

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Table 1 - CAN in DM1 and DM2: differences in relation to risk factors and natural history

Risk factors	Type-I DM	Type-II DM
Age	+	+
Gender (female)	+	-
Obesity	-	+
Hyperinsulinemia	na	+
Duration of DM	++	++
Smoking	+	+
HbA1c	++	++
Hypertension	++	+
Retinopathy	++	+
Hypertrygliceridemia	+	+
Classical PNP	++	++
Microalbuminuria	++	++
Dyslipidemia (> LDL and < HDL)	+	(+)
Natural history		
Prevalence at diagnosis of DM	7.7%	5%
Prevalence after 10 years	38%	65%
Prevalence (random)	25%	34%

Strong association: ++; moderate: +; not found: -; controversial: (+); na: not applicable; inverse association: 1/+.

Natural history of cardiovascular autonomic neuropathy and its impact on diabetes mellitus cardiovascular disease

Although the natural history of CAN in DM remains partially obscure, great advances, regarding its prognosis, have been achieved in the past decade. Despite difficulties such as the lack of standardization and of a single methodology in the diagnosis among the different epidemiological studies, the prevalence of CAN is known to range from 7.7% in patients with newly diagnosed DM1 (although this can be related to transient hemodynamic changes) to 90% in DM1 patients eligible for pancreas transplantation[2].

A Finnish study[29] followed patients with DM2 for ten years and found that the prevalence of CAN, as assessed by HRV, increased from 5% to 65%, whereas in the control group (non-diabetic patients) it increased from 2% to 28%. The prevalence of OH, in turn, (SBP decrease by 20mmHg or more after three minutes in the standing position) increased, after ten years, from 7% to 24% in the group studied (DM2), and from 6% to 9% in the control group. Additionally, the presence of CAN after five years was an independent risk factor for stroke[30,31].

Since early 1980's, several studies had already been demonstrating increased mortality in diabetic patients with CAN, as can be observed in Table 2[5,7,29,32-41]. However, only in a recent meta-analysis[42] a strong association between the degree of autonomic dysfunction and death risk was established. Fifteen studies involving a total of 2900 diabetic patients with

and without CAN were reviewed in this meta-analysis. During the follow-up period, which ranged from 0.5 to 16 years, mortality rates were consistently higher in patients with CAN (30%) than in those without CAN (13%). However, a major criticism currently directed to all of these studies is the fact that coronary artery disease (CAD) frequently coexists with CAN in diabetic patients, thus CAN may not actually be the single factor accounting for higher cardiovascular mortality in DM[43]. Currently there is strong evidence that CAN is an independent risk factor for CV mortality in diabetic patients[44], and it has already been demonstrated that patients with CAN associated with liver diseases and alcohol use (non-diabetic individuals) also present a higher mortality risk[45].

The mechanisms proposed to account for this increased mortality are: difficulty in recognizing angina (atypical manifestations such as nausea, shortness of breath and tiredness are common), asymptomatic ischemia or MI, dysfunction of the coronary flow autoregulation, increased heart rate, LV systolic and diastolic dysfunction, increased risk of arrhythmias (prolonged QT interval), decreased nocturnal protection against MI, changes in BP circadian cycle, increased cardiac mass, increased risk of microalbuminuria and, lastly, apnea[1,2]. The death in bed syndrome, in which the triad CAN + sympathetic-adrenergic discharge + nocturnal hypoglycemia plays a key role, is also well-known[46].

Several clinical studies[42,43,47,48] suggest an important participation of CAN in the etiopathogenesis of asymptomatic myocardial ischemia (AMI) found in diabetic individuals. In a large prospective study[47], 434877 patients with MI were evaluated, and 33% of them did not present chest pain. The percentages of patients with DM were 32.6% among those without chest pain, *vs* 25.4% in the group with chest pain. Moreover, several studies have shown an independent association between CAN and asymptomatic CAD[49-51]. Recently, AMI was found to be an independent predictor of hard CV events (SCD and MI) in asymptomatic type-2 diabetic patients[52].

On the contrary, in a recent review article[53], a researcher raised an old controversy: the accelerated atherosclerosis seen in diabetics patients, and not CAN, would be responsible for the higher incidence of asymptomatic ischemia and infarction. Either way, both hypotheses are compatible with a third possibility: that CAN may exert a direct effect in accelerating even further[46] the atherosclerotic process in DM.

In fact, there is currently both experimental and clinical evidence that peripheral arteries undergoing sympathectomy present severe atherosclerosis (macroangiopathy), due to a double mechanism:

1) *Hemodynamic effect* - in which the faster systolic volume ejection itself (resulting from the resting tachycardia seen in CAN) is independently associated with vascular lesion[53,54].

2) *Phenotypic effect* - greater migration of vascular smooth muscle cells (medium layer) to the inner layer and further differentiation of these cells, regardless of nitric oxide activity[55].

In a recent prospective study[56,57] for detecting the prevalence and predictors of silent coronary disease in patients with DM2, the strongest predictor of a positive pharmacological stress

Table 2 - Mortality in diabetic patients with (CAN+) and without (CAN-) cardiovascular autonomic neuropathy

Authors	Ref.	Follow-up (years)	Number of tests	Mortality				P value
				NAC+		NAC-		
				n	%	n	%	
Ewing et al (1980)	5	5	3+S	21/40	53	5/33	15	<0.05
Hasslacher et al (1983)	32	5	1	3/16	19	3/42	7	NS
Navarro et al (1990)	33	3.3 (1 – 7.3)	2	41/175	23	2/57	4	<0.05
Sampson et al (1990)	7	10	1+S	18/49	37	4/38	11	<0.05
O'Brien et al (1991)	34	5	4	23/84	27	21/422	5	<0.05
Ewing et al (1991)	35	3	5+QTc	10/32	31	3/39	8	<0.05
Jermendy et al (1991)	36	5	4+QTc	12/30	40	1/23	4	<0.05
Rathmann et al (1993)	37	8	2+QTc	8/35	23	1/35	3	<0.05
Luft et al (1993)	38	8 (6 – 10)	4	7/34	21	1/19	5	NS
Navarro et al (1996)	39	1 – 11.5	2	101/359	28	6/128	5	<0.05
Orchard et al (1996)	40	2	1	8/88	9	9/399	2	<0.05
Töyry et al (1996)	29	10	1	3/23	13	3/99	3	<0.05
Veglio et al (2000)	41.	5	2+QTc	10/76	13	10/240	4	<0.05
Total		Mean: 5	–	265/1041	25	69/1574	4	–

NS - non significant; Qtc - corrected QT interval; S - autonomic symptoms; Ref. - reference.

(adenosine) myocardial perfusion imaging (SPECT) was the presence of CAN (assessed by the abnormal Valsalva ratio). This study also revealed that 22% (n = 113) of the patients with type-2 diabetes already presented AMI at enrollment. The CVRF were then more intensively treated in these patients (statins, aspirin and angiotensin-converting enzyme inhibitors), and after three years 71 of them underwent a repeat stress imaging which demonstrated resolution of the myocardial ischemia in 79% (n = 56)[57].

By showing a significant correlation between the severity of CAN and the degree of impairment of the left ventricular function, other studies using Doppler echocardiography suggested that CAN plays an important role in the pathogenesis of diabetic cardiomyopathy. LV diastolic dysfunction, in particular, seems to occur early and be asymptomatic in young diabetic individuals with CAN[58].

Finally, there is a well established association between CAN and diabetic nephropathy which contributes to the high mortality rates found in diabetic patients[8]. Ewing et al[5] reported a mortality rate of 53% after five years in a cohort of diabetic patients with CAN, *vs* 15% in the control group (diabetic patients without CAN). However, the individuals in the first group had long-standing symptomatic CAN and already presented macroangiopathy. As a matter of fact, 50% died of CRF. Sampson et al[7], in turn, excluded diabetic patients with CRF or with clinical CAN for more than two years from their cohort and found, during the follow-up period a mortality rate of 27% *vs* 11% in the control group (diabetic patients without CAN) after a period of 10 to 15 years.

Lurbe et al[59] correlated nondipping of nocturnal BP (due to a loss of the circadian rhythm of blood pressure that typically occurs in diabetic patients with CAN) with higher incidence

of nephropathy (microalbuminuria) and macroangiopathy. Interestingly, in this study the increased SBP during sleep (nondippers) preceded the development of microalbuminuria in young type-1 diabetic individuals, thus linking CAN (earlier) to the deterioration in glomerular filtration rate (later).

Tests for the early detection and staging of cardiovascular autonomic neuropathy in current clinical practice

For the diagnosis and staging of CAN in clinical practice, noninvasive autonomic tests that are, simple, reproducible and sensitive both for the sympathetic (later impairment) and parasympathetic (earlier impairment) systems, should be used, so as to allow a longitudinal follow-up of the alterations[23,60,61]. There are currently two standardized methods for this purpose:

Ewing tests[5,62]

Of the five tests for outpatients classically described by Ewing[62], three (deep breathing test, Valsalva maneuver and orthostatic test) are currently recommended by the American Diabetes Association and American Academy of Neurology[8,61] for DM2 at the time of diagnosis, and for DM1 five years after the diagnosis. After the first survey, these tests should be yearly repeated. These three tests have a good reproducibility, specificity higher than 91% and sensitivity from 93% (deep breathing and orthostatic test) to 98% (Valsalva).

Computerized heart rate variability (hrv) study[1,2,8]

In the past years, earlier detection of autonomic

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dysfunction[63,64] became feasible thanks to the spectral analysis study of HRV. This modern technology uses a mathematical algorithm (fast Fourier transform) to turn a complex biological signal, such as HRV (result of the sympathovagal balance in the sinus node), into its causing components, presenting them according to the frequency with which they alter the HR[2,23]. The result (spectral amplitude) is presented in an Amplitude (Y axis) *vs* Frequency (X axis) diagram as shown in Figure 1A. We can observe that the spectral amplitude does not only reflect the magnitude of HRV (Y axis), but also the oscillations in different frequencies, i.e., the number of HR fluctuations per second (X axis). This allows us to better distinguish the relative impact of the sympathetic and vagal modulation on HRV.

It has been demonstrated that the total spectral amplitude (total power or TP) of HRV consists of three key frequency bands, as represented in Figure 1:

1) very low-frequency component or VLF (from 0.01 to 0.04 Hz) which is related to fluctuations in the vasomotor tonus linked to thermoregulation and sweating (sympathetic control);

2) low-frequency component or LF (from 0.04 to 0.15 Hz) associated with the baroreflex (sympathetic control with vagal modulation);

3) high-frequency component or HF (from 0.15 to 0.5 Hz) which is related to sinus arrhythmia (parasympathetic control).

In diabetic patients with predominantly vagal (but early) dysfunction, HF amplitude is reduced or absent, whereas in the presence of predominantly sympathetic (but late) dysfunction, LF and VLF amplitudes are reduced. The more advanced cases are characterized by the absence of all frequency bands. In addition to its high sensitivity (99%) and specificity (100%), this method[2,23,62-65] has the advantage of not requiring active cooperation from the patient (it is performed at rest). However, it requires computerized equipment and coupled mathematical software.

Currently, seven parameters are used for the early detection of CAN: the three spectral analysis frequency bands (VLF, LF and HF) plus four Ewing tests (the three previously described plus the OH test described in Attachment 1).

The protocol presented at the end of this review, in Attachment 1, is based on recommendations of different ANS study laboratories[10,24,28,63-65], as well as on European and American consensus[1,2,23,60,61]. According to this protocol, the diagnosis of CAN is made when at least three of the seven tests are abnormal with a specificity of 100%. On the other hand, when only two tests are abnormal, the diagnosis of *incipient CAN* is made with a specificity of 98%[1,2].

In the absence of a computerized system for HRV spectral analysis, the four Ewing tests should be performed. The diagnosis of *established CAN* requires at least two abnormal tests. When only one of them is abnormal (usually the deep breathing test or the orthostatic one), the diagnosis of *early* or *incipient CAN* is made. Later, with the progression of CAN, the Valsalva maneuver also becomes abnormal, characterizing the diagnosis of *intermediate CAN*. Finally, when OH is present, *severe CAN* is diagnosed[1,2,8].

Deep breathing (or E:I), and orthostatic tests (or 30:15 ratio) as well as spectral analysis in the HF band assess only the parasympathetic component (vagal afference of the heart) of cardiovascular ANS which is the most precociously affected in DM (Notice that, although the patient does not present CAN, the HF band in Figure 1B is already abnormal). The spectral amplitude in VLF band and the OH test, in turn, assess the sympathetic component of the ANS. The LF band spectral power and the Valsalva maneuver assess both the sympathetic and parasympathetic components of CV autonomic function, and are the most sensitive.

Conclusions

Although CAN is the most extensively studied and the most hazardous among the clinical manifestations of diabetic autonomic neuropathy, its pathophysiology remains one of the most obscure and controversial topic in current diabetology. For instance, some researchers consider CAN an additional component of metabolic syndrome[19,20,66]. Several questions remain unanswered, such as:

1) What are the mechanisms responsible for triggering early CAN and where is the point of no return, i.e., the point from which morphological and pathophysiological alterations become irreversible?

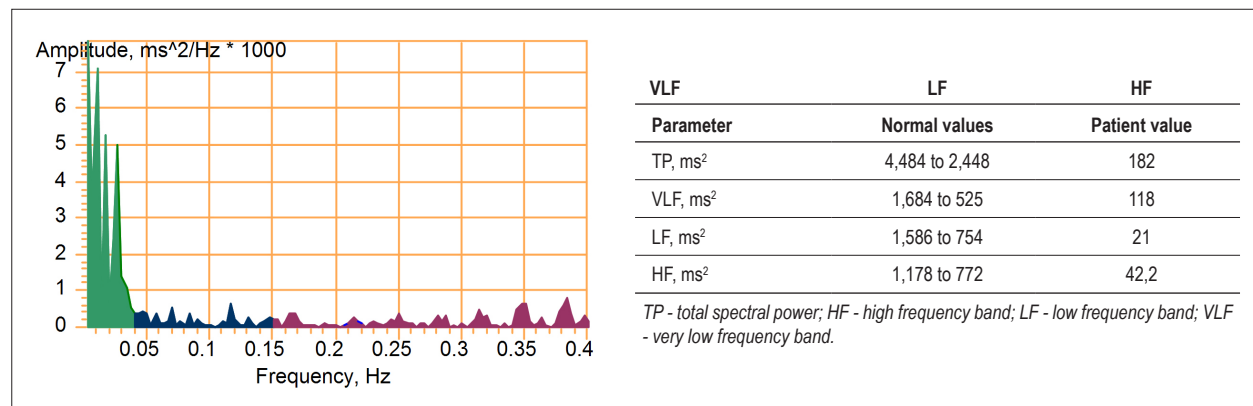2) What is the best method to detect silent coronary disease

Parameter	Normal values	Patient value
	VLF	LF	HF
TP, ms^2	4,484 to 2,448	182
VLF, ms^2	1,684 to 525	118
LF, ms^2	1,586 to 754	21
HF, ms^2	1,178 to 772	42,2

TP - total spectral power; HF - high frequency band; LF - low frequency band; VLF - very low frequency band.

Figure 1A - *Spectral power of a 52-year-old female patient with DM2 for 12 months and CAN+.*

Parameter	Normal values	Patient value
TP, ms^2	4,484 to 2,448	2,658
VLF, ms^2	1,684 to 525	895
LF, ms^2	1,586 to 754	1,085
HF, ms^2	1,178 to 772	678

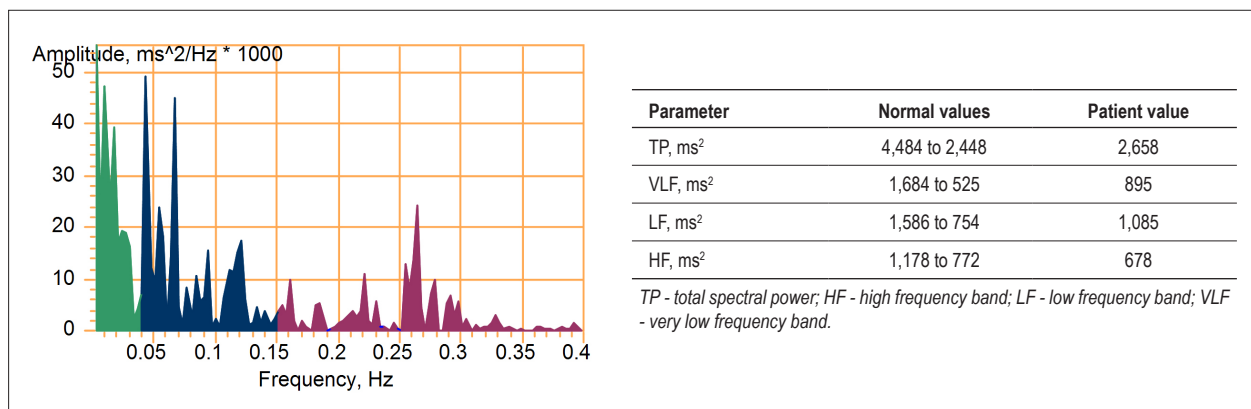*TP - total spectral power; HF - high frequency band; LF - low frequency band; VLF - very low frequency band.*

Figure 1B - *Spectral power of a 34-year-old patient with DM1 for 23 years and using continuous insulin infusion pump for eight years.*

in diabetic individuals[67], and when should it be performed?

3) What is the relationship between hyperinsulinemia[68], CAN and metabolic syndrome? Which comes first: parasympathetical dysfunction or insulin resistance[69]?

It is fundamental to point out that CAN is currently known as an early complication of DM, and its progression, both insidious and silent, is associated with considerable morbidity and mortality, as well as with serious impairment of the quality of life of diabetic individuals.

Objective detection of CAN in its early stages (*incipient and intermediate forms*) via CV tests and computerized systems (spectral analysis of HRV) is both feasible and mandatory in the current state of art for four reasons:

1) Since CAN may be delayed (DCCT and Steno 2 studies), and since nowadays there is a real possibility of reverting its natural course, the early diagnosis and staging of CAN is crucial, in order to stop further progression to advanced and irreversible stages (*severe CAN*). Moreover, all studies on clinical treatment show that *incipient or intermediate* CAN may be reverted or improved, whereas severe cases cannot be either reverted or improved [1,70].

2) Symptomatic manifestations occur very late in the course of DM and do not always progress. This stresses the importance of quantifying autonomic deficit by means of objective and prospective tests, i.e., diagnosis should not be made only on the basis of clinical history or physical examination.

3) The more severe CAN is (late stages), the higher CV risk and mortality seems to be[71], since we now know that, once the symptoms develop, mortality in five to seven years is of the order of 50%[5]. In addition, CAN is a marker of poor prognosis for microangiopathy, especially nephropathy, as demonstrated by a prospective Swedish study with an eleven-year follow-up[72]. Therefore, early detection and staging may identify diabetic individuals at risk for silent CAD, CHF, CRF and premature death. It is fundamental to warn and alert these patients when they initiate to physical training and exercise programs.

4) Timely identification of DM autonomic dysfunction may improve the prophylaxis of target-organ injury – both macroangiopathy (CAD, SCD and stroke) and microangiopathy (CRF and retinopathy), with the use of specific drugs for the CVRF associated with CAN: hypertension and albuminuria (ACE inhibitors and angiotensin II receptor blockers) and dyslipidemia (statins). A more intensive control of DM and hypertension in individuals with incipient and intermediate CAN is also currently recommended[70,73].

No less important is the fact that, in the past years, we have learned that the CVRF traditionally associated with macroangiopathy in DM2 (BMI, triglycerides, smoking and SBP) also play a pathophysiological role in the natural history of microangiopathic complications of DM1, in particular CAN[11,26]. Even more interestingly, the intensive treatment of these factors prevents the progression of CAN in DM2[13,14].

In conclusion, the presence of CAN in diabetic patients is associated with a mortality rate, caused by CV events, two to three times higher, and total mortality rate up to five times higher in relation to diabetic patients without CAN[74]. Thus, CAN is an important risk marker for macroangiopathy, in general, and for CAD, in particular.

Potential Conflict of Interest

No potential conflict of interest relevant to this manuscript was reported.

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Attachment 1

Protocol for yearly CAN screening of the Universidade Federal de São Paulo (UNIFESP).

A) Questionnaire for symptoms

A.1) When standing up: dizziness, visual disorders, presyncope.

A.2) When exercising: shortness of breath, nausea, diaphoresis, pain.

A.3) Dysphagia, nausea, early satiety, anorexia.

A.4) Diarrhea, fecal incontinence, constipation, postprandial vomiting.

A.5) Sexual dysfunction: erectile, vaginal lubrication.

A.6) Incontinence, pollakiuria, urgency, retention, recurrent UTI.

A.7) Anhidrosis, hyperhidrosis, heat intolerance, gustatory sweating.

Clinical Update

B) Seven parameters

B.1) Valsalva Maneuver (Valsalva ratio)

The patient remains in the supine position (SP) at 30 degrees and, after a 15-minute rest, forcibly exhales to keep a 40-mmHg pressure for 15 seconds. At approximately the 14th second, the patient shows a peak physiological tachycardia. After this effort, the sphygmomanometer valve is released and the patient is monitored by EKG tracing for 30 to 45 seconds, when a peak physiological bradycardia occurs. The Valsalva ratio is the relation between tachycardia and bradycardia or between the longest and shortest RR intervals.

B.2) Orthostatic test (30:15 ratio)

The test consists of performing EKG with the patient in SP under the same conditions as described above. After the patient stands up (orthostasis), the relation between the heart rates or between the RR-intervals corresponding to the maximum tachycardia at approximately the 15th beat and the maximum bradycardia at approximately the 30th beat is analyzed.

B.3) Deep breathing test (E:I ratio)

The test consists of performing EKG during deep inspiration and expiration with a minimum duration of 5 seconds each and obtaining the maximum heart rate (inspiration) divided by the minimum heart rate (expiration), or longest RR (E) divided by the shortest RR (I) ratio.

B.4) Orthostatic hypotension test (OH)

Patient in SP at 30 degrees for a 15-minute rest. Blood pressure is measured at baseline and three minutes after standing. A drop in systolic blood pressure higher than or equal to 20 mmHg is considered abnormal and a drop in systolic blood pressure between 10 and 19 mmHg is considered borderline.

B.5) Spectral analysis of HRV (spectral amplitude in the three bands: VLF, LF and HF)

Patient at rest in SP at 30 degrees and breathing spontaneously. The ECG is recorded in a computer or laptop for 300 seconds and is further analyzed by a mathematical algorithm and expressed in a diagram of oscillation amplitude (HR variations per second) *vs* frequency (hertz).

Figures 1A and 1B show the three frequency bands, from left to right: VLF, LF and HF of the spectral amplitude. The values expressed in square milliseconds reflect the area under the curve of each band (green, blue, and red, respectively).

Note

All CV tests should be performed in the morning, under fasting conditions, with a capillary blood glucose level lower than 180 mg/dl and all cardiovascular medication, anxiolytics, antidepressants, caffeine and decongestants discontinued for at least eight hours and optimally 24 hours before (because it will depend on the half-life of each drug in particular). Normal values always depend on the age range of the patient and are standardized[23,65].