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REVIEW ARTICLE

Methods of investigation for cardiac autonomic dysfunction in human research studies

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Summary

This consensus document provides evidence-based guidelines regarding the evaluation of diabetic cardiovascular autonomic neuropathy (CAN) for human research studies; the guidelines are the result of the work of the CAN Subcommittee of the Toronto Diabetic Neuropathy Expert Group. The subcommittee critically reviewed the limitations and strengths of the available diagnostic approaches for CAN and the need for developing new tests for autonomic function.

It was concluded that the most sensitive and specific approaches currently available to evaluate CAN in clinical research are: (1) heart rate variability, (2) baroreflex sensitivity, (3) muscle sympathetic nerve activity, (4) plasma catecholamines, and (5) heart sympathetic imaging. It was also recommended that efforts should be undertaken to develop new non-invasive and safe CAN tests to be used in clinical research, with higher sensitivity and specificity, for studying the pathophysiology of CAN and evaluating new therapeutic approaches.

Keywords diabetic neuropathy; heart rate variability; baroreflex sensitivity; microneurography; catecholamines; cardiac imaging

Abbreviations: BRS – baroreflex sensitivity; CAN – cardiovascular autonomic neuropathy; CARTs – cardiovascular autonomic reflex tests; DHPG – 3, 4-dihydroxyphenylglycol; HED – [^{11}C]-metahydroxyephedrine; HRV – heart rate variability; MIBG – [^{123}I]-metaiodobenzylguanidine; MSNA – muscle sympathetic nerve activity.

Introduction

This consensus document provides evidence-based guidelines regarding the evaluation of diabetic cardiovascular autonomic neuropathy (CAN) for human research studies; the guidelines are the result of the work of the CAN Subcommittee of the Toronto Diabetic Neuropathy Expert Group.

The most sensitive and specific diagnostic tests currently available to evaluate CAN in clinical research are: (1) heart rate variability (HRV), (2) baroreflex sensitivity (BRS), (3) muscle sympathetic nerve activity (MSNA), (4) plasma catecholamines, and (5) heart sympathetic imaging.

This article briefly reports the rationale for each CAN diagnostic test, reviews critical evidence regarding the sensitivity and specificity of each test in diabetes, and provides succinctly final recommendations.

A detailed description of the technical methods, their use in diabetes, and additional references are reported in the online supplement attached to this article (see Supporting information).

The methodology adopted for rating the quality of evidence and strength of recommendations was that suggested by the American Academy of Neurology [1] for diagnostic studies.

Heart rate variability

Rationale

Heart rate is never completely stable. Continuous tonic, phasic, and transient external and internal stimuli of multiple origins affect heart rate to a variable but measurable extent. Five different mechanisms have been described: (1) sympathetic and parasympathetic efferences to the sinus node; (2) neurohumoral influences (e.g. catecholamines, thyroid hormones); (3) stretch of the sinus node; (4) changes in local temperature; and (5) ionic changes in the sinus node. Under resting conditions, it can be assumed that the short-term HRV is essentially determined by the first and third factors. The sympathetic and parasympathetic stimuli directly influence heart rate and are responsible for a physiologic variation in the heart rate, or HRV. The HRV can be evaluated in the time and frequency domains.

Time domain measures of the normal R-R intervals include the difference between the longest and shortest R-R intervals, the standard deviation of 5-min average of normal R-R intervals (SDANN), and the root-mean square of the difference of successive R-R intervals (rMSSD). Longer recordings (e.g. 24-h) allow the calculation of additional indices, as the number of instances per hour in which two consecutive R-R intervals differ by more than 50 ms over 24 h (pNN50). Essentially, all these indices explore the parasympathetic activity.

In the frequency domain, the use of spectral analysis of R-R interval (and other cardiovascular and respiratory signals) allows a precise description of the different fluctuations (see Supporting Information for technical details) of these signals. The components of the HRV obtained by spectral analysis provide information about both the sympathetic and parasympathetic influences on the heart [2,3].

It is traditionally accepted that the parasympathetic system affects the overall variability (e.g. variance, total power) and the sympathetic activity essentially influences a rather narrow band around 0.1 Hz (low frequencies) equivalent to a fluctuation of approximately 6 cycles/min.

However, the influence of these two factors on HRV is markedly different. While the parasympathetic activity influences the total amount of variability (the total power of the spectrum), the sympathetic activity rather than increasing or decreasing the fluctuations seems to act like a low-pass filter. When the sympathetic activity predominates (e.g. tilting, physical exercise), to a large extent only the fluctuations at lower frequency can influence the HRV, whereas the faster perturbations (e.g. those determined by respiration) cannot [4–8]. Accordingly, although respiration [which normally generates heart rate fluctuations at higher frequency, around 0.25 Hz, (high frequencies)] normally increases in depth and frequency during sympathetic activation, its influence on HRV is progressively smaller, and consequently the low frequencies predominates in the spectrum. However, the power in all frequency components is reduced, as an effect of the global reduction in HRV and in total power, induced by the parasympathetic withdrawal and the increase in heart rate. This explains the rather paradox phenomenon whereby the low frequencies predominates (in relative term) over the high frequencies during sympathetic activation, but the amplitude (or the 'power') in low- and high-frequency fluctuations actually decreases. With extreme sympathetic activation and parasympathetic withdrawal (that occurs in conditions like submaximal exercise and severe heart failure), the overall variability (or total power) is so small that the low-frequency component can no longer be measured. Accordingly, it is not surprising that the low-frequency power (when expressed in absolute values) neither correlates with direct measures of sympathetic activity (e.g. those provided by microneurography) nor reflects sympathetic changes, and it is now universally accepted that the low frequencies absolute power does not reflect the sympathetic activity.

Conversely, when measured in relative terms (i.e. as a percentage of the global HRV), the relative proportion of the low- over the high frequencies, provides a relative and approximate indication of the sympathetic modulation to the heart [9,10]. Thus, the sympathetic influences on HRV can only be evaluated on the relative proportion of HRV components [2]. Contributors to these low-frequency oscillations in blood pressure and heart rate include baroreflex activity and activity of an endogenous oscillator in the brainstem or spinal chord [2–5].

The respiratory component (normally at high frequency) is traditionally attributed to the parasympathetic activity (respiratory sinus arrhythmia, between 12 and 18 breaths/min, or approximately at an average of 0.25 Hz). Although the low-frequency fluctuations should not be influenced by respiration, respiration is highly variable and slow-breath-induced low frequencies are very frequent, particularly during spontaneous breathing. These spurious low frequencies explain the poor correlation between direct measures of sympathetic activity and the low frequencies, even when measured in relative terms, during spontaneous breathing (at rest or during different interventions) [11]. Conversely, by increasing or regularizing the breathing rate the correlation between the normalized low frequencies and the sympathetic activity increases [11] as a result of the elimination of the respiratory artefacts on HRV. The simultaneous analysis of respiration allows identification of periods without slow breaths during spontaneous breathing. Only the analysis performed on these data segments can be free from artefacts.

The direct stretch of the sinus node has a very small influence on the HRV of a healthy subject at rest (2–4% of HRV) [12], but accounts for nearly 100% of HRV in denervated hearts [13], severe CAN (due to a permanent loss of autonomic modulation), or also during transient withdrawal of autonomic modulation as it occurs during submaximal exercise. This can be seen

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as a small respiration-linked fluctuation that correlates positively with the increase in ventilation during physical exercise. During extreme sympathetic activation and the consequent reduced autonomic modulation of HRV, the direct stretch of the sinus node remains the only evident fluctuation [12]. This component should be taken into account in severe CAN or during submaximal exercise.

Other fluctuations in lower frequencies (e.g. very-low frequency components) are essentially caused by 'external' factors (changes in activity and posture of ambulant subjects [14]), and probably reflect parasympathetic activity, similar to the absolute power of the other frequency components, the total power, or the time-domain indices.

To avoid important bias in the interpretation of HRV it is recommended to perform spectral analysis with control for respiration; to include adequate beat editing capability to avoid the influence of artefacts and ectopic beats; to understand the different significance of absolute and relative components of HRV, to have clear indications as regards to the different methodological approaches and mathematical algorithms. Failing this, spectral analysis cannot provide additional information as compared with the simpler indices of global variability (standard deviation of R-R intervals, variance, total power) and results may be incorrect. This can occur when using some commercially available equipments developed for Holter 24-h electrocardiogram recordings for short-term and experimental data [15]. Conversely, when applied with appropriate methodology, the spectral analysis provides additional information to the time-domain indices, such as information about sympathetic activation (though in relative terms). Additionally, when beat-to-beat blood pressure recording is obtained simultaneously with HRV, it is possible to obtain an index of sympathetic activation from the low-frequency power of blood pressure [2,4–6,9]. The increase in low-frequency power of blood pressure is particularly evident during sympathetic activation induced by tilting [2,9] or physical exercise [6].

Based on studies using acceptable techniques, there is evidence of reduced parasympathetic modulation of heart rate in diabetes and also reduced modulation of systolic blood pressure in the low-frequency region [16–18] particularly after sympathetic stimulation in response to tilting, or in the microcirculation [19]. As most of the cardiovascular autonomic reflex tests (CARTs) essentially explore the parasympathetic activity (as strongly suggested in the other paper on CAN in this issue), there is no other simple test of sympathetic activity capable of identifying early (functional or anatomic) autonomic sympathetic abnormality [20]. CARTs are considered the gold standard for CAN testing. Impaired HRV time- and frequency-domain indices have been reported in diabetic patients before CARTs abnormalities arise. However, the few studies that assessed the diagnostic accuracy against the reference standard of CARTs found only fair results (see online supplement for details). Time- and frequency-domain analysis of 24-h electrocardiogram recordings has documented an abnormal nocturnal sympathetic predominance in diabetic patients that was linked to blood pressure non-dipping. In obese patients weight loss was associated with an improvement in global HRV and in parasympathetic HRV indices [21].

Highlights

- HRV testing is a clinically relevant measure in addition to CARTs and provides key information about autonomic – parasympathetic and sympathetic – modulation of the cardio-vascular system.
- Analysis of HRV can be done using statistical indices in the time and frequency domains.
- Time-domain indices of global HRV and total spectral power of HRV represent the index of parasympathetic activity, as well as the HRV spectral power in the high-frequency region, while the relative proportion (not the absolute power) in the low frequencies of HRV provides a relative measure of sympathetic modulation. This interpretation should be made with cautions if respiratory artifacts (slow breaths) cannot be excluded.
- The parasympathetic nervous system also modulates HRV in the high- and low-frequency regions, and low-frequency power decreases or does not change during sympathetic activation. Thus, the absolute power in the low-frequency region should not be used as an index of sympathetic activity.
- Application of the technique is critically dependant upon understanding of the underlying physiology, the mathematical analyses used, and the many confounders and possible technical artefacts.

Confounders

- Misinterpretation of power spectrum due to irregular respiratory pattern and verbalization during breathing, creating artefactual low frequencies and false 'sympathetic overactivity'.
- Lack of spectral decomposition algorithm when using autoregressive methodology.
- Use of the absolute power of R-R interval low-frequency spectral data as evidence of sympathetic activity.
- In case of very low HRV (2–4% of total variability found in healthy subjects) the interpretation of spectral components is affected by the presence of non-autonomic components in the respiratory range.
- Other confounding factors (such as drugs) similar as those reported for CARTs.

Recommendations

- The best approach to HRV testing involves the analysis of electrocardiogram recordings in

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conjunction with respiration and beat-to-beat blood pressure recordings (level C).
- When respiration cannot be recorded, breathing rate should be controlled (15 breaths/min), and hyperventilation or slow deep breathing avoided (level B).
- The subjects must not speak during recordings (level C).
- The optimal recording time is 4–5 min during well controlled rest. Longer times (7 min) may be preferable if fast Fourier transform methods are used and if frequent ectopics are to be edited. Long uncontrolled recording times should be avoided (level C).
- When testing is done under stable conditions, autoregressive or fast Fourier transform methods can be used.
- When fast changes are to be expected (e.g. during interventions) autoregressive algorithms are preferred, or alternatively special time-varying techniques.
- Age-related reference curve should be obtained for the healthy population in the same environment and using the methodology adopted, construct 95% confidence limits (level B).
- Other recommendations on confounding factors are similar as those reported for CARTs.
- Used with the appropriate methodology HRV has an increasingly important role in clinical research and therapeutic trials.

During 24-h recordings:

- If the goal is to define the circadian pattern of autonomic activity, long-duration spectra (e.g. 1 h) and autoregressive algorithms are preferable.
- If the goal is to define relatively faster modifications, shorter time windows (e.g. 5 min) are preferable. Special time-varying techniques can provide beat-to-beat autonomic changes.

Baroreflex sensitivity

Rationale

Continuous changes in blood pressure are sensed in different pressure-sensitive areas (particularly carotid bifurcations and aortic arch) of the arterial tree. The afferent fibres meet at the brainstem and elicit a double response, vagal and sympathetic. An increase in blood pressure reduces the firing of sympathetic vascular and cardiac efferents and increases the firing of vagal cardiac efferents, resulting in a rapid reduction in heart rate and in blood pressure. The reduction in blood pressure is due to both a reduction in cardiac output, which in turn is caused by bradycardia, and to a slower direct vasodilation secondary to sympathetic withdrawal. A reduction in blood pressure induces opposite responses. Thus, to correctly define the baroreflex function, one has to consider both the vagal efferent activity (evidenced by changes in heart rate in response to changes in blood pressure), and the sympathetic efferent activity (mainly directed to the arterial vessels). The latter response cannot be easily studied in clinical environment, but can be obtained for research purposes with simultaneous recordings of MSNA [22] or, indirectly, by neck suction [7]. In practice, the term 'baroreflex sensitivity' normally applies to the cardiac-vagal arm, and to methods measuring changes in heart rate in response to changes in (systolic) blood pressure. The BRS is an interesting approach as it combines information derived from both heart rate and blood pressure.

The measurement of the cardiac-vagal arm BRS can be done with several methods: drugs or physical manoeuvres can be applied to modify blood pressure; alternatively, spontaneous blood pressure variations can be used. In all cases the response in heart rate to the changes in blood pressure is quantified. These methods have been described in detail in the online supplement section of this article (see Supporting Information). None of the BRS tests available today – based on drug-induced or physically induced changes in blood pressure, spontaneous blood pressure fluctuations with the sequences technique or spectral analysis – have shown so far a definite advantage over the others, or a clinically relevant difference.

Longitudinal studies have demonstrated that BRS has important independent prognostic value in cardiac patients [23–25] and in diabetic patients [26].

Although some observations in diabetic patients support an early impairment of BRS before CARTs abnormalities [27,28], very few studies have evaluated so far the diagnostic accuracy of BRS measures as compared with the reference standard of CARTs with inconsistent results. Thus, no definite conclusion is possible on the diagnostic characteristics for CAN of BRS assessment, in particular on its sensitivity. In patients without CAN an early stage of functional BRS abnormalities [29] still responsive to life-style intervention – physical training [30] or dietary improvement and weight reduction [31] – has been documented. BRS assessment may warrant use for identifying subjects at risk for CAN and also in clinical trials.

Highlights

- Cardiac vagal BRS assessment is an important component of autonomic testing as it combines information derived from both heart rate and blood pressure.
- Cardiac vagal BRS is a widely recognised independent prognostic index for cardiovascular mortality and morbidity in the general – mainly cardiac – and the diabetic population (class II).
- No definite conclusion is possible on the diagnostic characteristics of BRS assessment (classes III–IV).
- The presence of early abnormalities with respect to CARTs and their reversibility with appropriate treatments warrant the clinical use of BRS in

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identifying subjects at risk for CAN and to test potential therapeutic approaches (classes II–III).
- Pharmacological methods allow assessment of BRS across a range of physiologically relevant blood pressure and – when used with microneurography – measurement of the sympathetic baroreflex. But this invasive technique is limited to research purposes.
- The methodology of BRS (in particular spontaneous BRS) is simple and fast
- All BRS techniques require a dedicated beat-to-beat non-invasive blood pressure monitor.
- None of the BRS tests today available have shown a definite advantage over the others, nor a clinically relevant difference (class II).

Confounders

- Fluctuations induced by drifts of the non-invasive blood pressure monitors.
- Most methods need a large number of arbitrary constraints imposed by the calculations that may affect the results.
- Respiratory pattern: although BRS measures in general do not need a strict control of respiratory pattern, slow breathing increases BRS and reduces sympathetic efferent drive [29,32]; therefore, some feedback from respiration is necessary to correctly interpret the results.
- Age-related reduction in BRS [33].
- Other confounding factors (e.g. drugs) are similar as those for CARTs.

Recommendations

- If the spontaneous approach is adopted, it is suggested to use a battery of methods based on the simplest single 5 min recording procedure (spontaneous BRS) and present the results in terms of a central measure (average or median) (level C).
- Recording should be performed during spontaneous breathing for 4–5 min, under monitored respiration, or during controlled breathing at 15 breaths/min (level C).
- Pre-filtering of the data improves the agreement between methods and provides a more robust estimate of BRS (level C).
- The recording time should be kept between 4 and 5 min of well-controlled rest. Avoid long uncontrolled recording times (level C).
- The subjects must not speak during recordings (level C).
- Age-related reference curves should be obtained for the healthy population of in the same environment and for the methodology adopted, and construct 95% confidence limits (level B).
- Other recommendations on confounding factors are similar as those reported for CARTs.

Muscle sympathetic nerve activity

Rationale

MSNA, i.e. bursts of efferent sympathetic activity in the skeletal muscle at rest or in response to various physiological perturbations, can be directly recorded and measured via microelectrodes inserted into a fascicle of a distal sympathetic nerve to the skin or muscle (microneurography) more commonly at the level of the peroneal nerve. MSNA bursts are related to an inhibitory effect of systole on the arterial baroreceptors, and the burst frequency increases during reductions in blood pressure and vice-versa.

Owing to its invasiveness and the time-consuming nature of the procedure, MSNA is not indicated for routine autonomic assessment. However, by being the most direct measure of sympathetic activity it is an essential research tool.

Increased resting MSNA and blunted responsiveness to physiological hyperinsulinaemia or glucose ingestion have been described in type 2 diabetic patients having neuroadrenergic autonomic dysfunction, and resembles insulin-resistant states and obesity. MSNA abnormalities in these conditions reverse with weight loss [20,34]. In contrast, type 1 diabetes is associated with a significant decrease in the number of bursts, by about half [35]. Although reproducibility is similar to non-diabetic subjects, obtaining good quality recordings is much more difficult in patients with diabetic polyneuropathy than in non-diabetic subjects [20,36], presumably as a result of a reduction in the conducting sympathetic nerve fibres.

Highlights

- The MSNA is the only method allowing direct and continuous measurement of sympathetic nerve traffic (class I).
- MSNA is the only method that can directly assess the sympathetic vascular arm of the arterial or cardiopulmonary baroreflex (class I).
- Type 1 diabetes appears to be associated with a reduction of MSNA (class IV).
- In early type 2 diabetes, resting MSNA might be increased, possibly due to hyperinsulinaemia (class IV).
- The technique is difficult, invasive, time-consuming, requires specialized trained operator and cannot be repeated often in the same subject (class II).

Confounders

- Blood pressure variation
- Large inter-individual variations
- Food intake
- Age
- Posture

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- Hypoxia
- Hydration
- Exercise
- Female reproductive hormones
- Arousal
- Sleep
- Mental stress
- Ethnicity

Recommendations

- MSNA should not be routinely employed for the diagnosis of CAN (level C).
- MSNA should be employed with standard CARTs or for specific tests aimed at measuring vascular sympathetic modifications (e.g. glycaemic clamps) (level C).

Catecholamine assessment and cardiovascular sympathetic tests

Rationale

The most important catecholamines in human plasma are norepinephrine and epinephrine, both reflecting sympathetic nervous activity: norepinephrine is released from sympathetic nerve endings by exocytosis, a small proportion reaching the systemic circulation [37]. Thus, circulating norepinephrine mirrors whole-body sympathetic activity when measured in systemic venous plasma. Epinephrine is derived from sympathetic (preganglionic) stimulation of the adrenal medulla and circulating epinephrine therefore reflects the degree of sympathetic activation of the adrenal medulla. Plasma norepinephrine and epinephrine levels can respond differentially in response to stressors; larger plasma norepinephrine responses than epinephrine responses are found upon exposure to cold, and larger plasma epinephrine responses are found in response to glucoprivation and fainting. Other catechols comprise the catecholamine precursor, 3,4-dihydroxy-L-phenylalanine and the main neuronal metabolite of norepinephrine, 3,4-dihydroxyphenylglycol (DHPG) [38].

Norepinephrine plasma appearance rate is in principle the biochemical equivalent of MSNA. Norepinephrine plasma appearance rate and clearance have been determined in idiopathic autonomic neuropathy as well as in diabetic CAN. While norepinephrine clearance is low in idiopathic autonomic neuropathy, this was not the case in CAN, and accordingly in diabetic CAN no additional diagnostic power was added by the inclusion of [^{3}H]-norepinephrine kinetic studies [39]. Thus, catecholamine kinetics is an interesting technique which may give more information about catecholamine production and clearance across different regions – but is unsuitable to be used as a diagnostic tool yet. Plasma DOPA is not related to sympathetic neuropathy and has a mixed neuronal and non-neuronal origin. Plasma DHPG may be a more sensitive marker of overall sympathetic innervation than supine plasma norepinephrine [40], and simultaneous measurement of norepinephrine and DHPG yields more information than measurement of either alone. Catecholamine assessment in diabetes showed in general lower than normal responses to postural changes [41], exercise [42,43], hypoglycaemia [44], and CARTs [45–47]. A subnormal orthostatic increment in plasma norepinephrine is a specific but not sensitive index of baroreflex–sympathoneural failure or sympathetic noradrenergic denervation.

Highlights

- Clinical investigations including catecholamine determinations have contributed significantly to the understanding of the pathophysiology of CAN (class III). In the diagnostic context, the significance has been less prominent, partly due to the limited inclusion of the assays in clinical evaluations.
- Plasma catecholamine concentrations can indicate sympathetic noradrenergic and adrenomedullary hormonal system activity. Because levels of catechols are extremely responsive to lifestyle factors such as posture, temperature, dietary intake, medications, distress, and comorbidities, the clinical diagnostic value of plasma levels of catechols depends importantly on controlling or monitoring these factors (class III).
- Whole-body plasma norepinephrine and epinephrine respond rather slowly (minutes) to different physiological manoeuvres.
- During turnover studies, different regional norepinephrine and epinephrine activities are 'diluted' into a large plasma pool, contributing to blunted responses. Standardization of experimental conditions is to a large extent prohibitive for clinical routine purposes. In general, there is no neurochemical index that specifically assesses cardiac sympathetic innervation or function. This requires measurement of rates of entry of norepinephrine into the venous drainage of the heart, in turn requiring right heart catheterization, measurement of coronary sinus blood flow, and infusion of tracer-labelled norepinephrine.

Confounders

- Plasma norepinephrine concentrations increase with age. Thus, age matching is mandatory for comparisons.
- Smoking increases sympathetic nervous activity and catecholamine concentrations – 24 h tobacco abstention is required for comparisons. Posture, emotional stress, and ambient temperature all affect catecholamine concentrations and should thus be standardized.

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Recommendations

- In a number of experimental conditions, plasma catecholamine measurements are mandatory. For clinical routine diagnosis and staging of CAN the usefulness of plasma catecholamine concentrations is less obvious (level C).
- Plasma norepinephrine, epinephrine, and DHPG concentrations should be measured when whole-body sympathetic activity is assessed together with other relevant physiological parameters (heart rate, blood pressure, cardiac output, hormonal and metabolic events).

Heart sympathetic imaging and heart function tests

Rationale

Direct assessment of cardiac sympathetic innervation is possible using radiolabelled catecholamines or sympathomimetic amines that are actively taken up by sympathetic nerve terminals. The attraction of this technique is that it allows direct characterization of the pattern of target organ dysinnervation in diabetes. It is unclear whether this modality can directly assess nerve terminal function. An important limitation is that the imaging depends on delivery of the agent by coronary perfusion. In patients with coronary arterial or arteriolar narrowing, decreased innervation can be difficult or impossible to distinguish from decreased perfusion, without concurrent perfusion imaging.

Although in principle, it is possible to directly assess the integrity of both the parasympathetic as well as the sympathetic nervous system, there has been a paucity of research on parasympathetic imaging of the heart. Cardiac sympathetic neuroimaging, before and after administration of particular pharmacologic probes, can assess specific aspects of neuronal function. This combination has rarely been used.

Four tracers have been utilized to visualize the sympathetic nervous innervation of the heart: [^{123}I]-*meta*-iodobenzylguanidine (MIBG), [^{11}C]-*meta*-hydroxyephedrine (HED), 6-[^{18}F] dopamine, and [^{11}C]-epinephrine.

The washout rates from the myocardium of [^{11}C]-epinephrine or 6-[^{18}F]-dopamine can give information on vesicular integrity. In subjects with type 1 diabetes and CAN, the washout rates of [^{11}C]-epinephrine parallels those of [^{11}C]-HED, suggesting regional differences in vesicular uptake or retention [48]. Causes of defective tracer uptake or increased washout from the heart are a matter of current research.

The interpretation of findings using sympathetic neurotransmitter analogues is complicated by the fact that alterations in sympathetic nervous system tone may also affect the retention of these tracers, and this fact is often not considered as an explanation for the clinical findings. In the isolated rat heart model, elevated norepinephrine concentrations in the perfusion increased neuronal HED clearance rates consistent with the concept that neuronal 'recycling' of HED can be disrupted by increased synaptic norepinephrine levels [49]. Alternatively at high norepinephrine concentrations, non-neuronal uptake of HED into myocardial cells and impaired retention may be an interfering factor.

Additionally, interpretation of early myocardial [^{123}I]-MIBG retention is complicated by increased body mass index and diastolic blood pressure which have been reported to reduce myocardial MIBG uptake [50]. Moreover, difficulties and delays in acquisition of utilizable images can complicate the interpretation of the measurement obtained. The delivery of tracers is critically influenced by myocardial perfusion, so myocardial retention of tracers should be performed with a quantitative analysis of myocardial blood flow. This can be performed using positron emission tomography in order to derive a myocardial retention index [51]. However, although regional perfusion deficiencies can be excluded using single photon emission computed tomography, quantitative analysis of regional myocardial perfusion cannot be performed. Additionally, myocardial ischaemia or damage is also known to result in cardiac denervation which may occur in the absence of alterations in CARTs [52], whereas CAN is associated with impaired vasodilatory capacity in response to adenosine [53]. Anoxic ischaemia severely decreases the efficiency of vesicular sequestration and thus accelerates the loss of radioactivity, giving the false impression of denervation. Left ventricular dysfunction in diabetes has also been reported to reduce [^{123}I]-MIBG retention and increased washout rate [54].

Highlights

- Scintigraphic tracers directly assess the structural integrity of the sympathetic nervous system supply to the heart (class III).
- [^{123}I]-MIBG scanning and single photon emission computed tomography are widely used and available at most secondary care institutions; however, MIBG scanning is approved and reimbursed for evaluation of pheochromocytoma and so far not for evaluation of cardiac sympathetic innervation.
- Most data relate to the evaluation of cardiac sympathetic integrity; few studies evaluate the respiratory system.
- The relationships of deficits in tracer uptake/washout to sympathetic neuronal integrity and function are poorly understood: current tracers may not be the most optimum. Combined neuroimaging-pharmacologic approaches are required.
- Scintigraphic data correlates with HRV testing, but have greater sensitivity to detect changes in sympathetic neuronal structure and/or function [55,56] (class III).

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- Scintigraphic data correlate with indices of myocardial perfusion and left ventricular dysfunction in type 1 diabetes [57] (class III).
- Limited studies demonstrate that decreased 'uptake' and excessive 'washout' of MIBG-derived radioactivity is an adverse prognostic finding in a spectrum of conditions including diabetes and that scintigraphic data are affected by the quality of glucose control [58–60] (class III).
- Cost of scintigraphic studies is considerable.

Confounders

- Parasympathetic tracers are not yet generally available.
- [^{11}C]-HED and 6-[^{18}F]-dopamine positron emission tomography have limited availability and are not reimbursed.
- Damage to the myocardium and left ventricular dysfunction interferes with tracer uptake and washout independently of changes in CARTs.
- Regional myocardial [^{123}I]-MIBG 'uptake' is semi-quantitative and not a clean index of neuronal uptake, which occurs extremely rapidly.
- [^{123}I]-MIBG retention is affected by body mass index, diastolic blood pressure, and local factors which influence the tracer uptake and retention.
- Delivery of tracers is critically influenced by myocardial perfusion (myocardial retention of tracers should be performed with quantitative analysis of myocardial blood flow).
- The effects of the following on the kinetics of myocardial tracer retention are poorly understood:
  - age (*except for 6-[^{18}F]-dopamine*)
  - gender
  - glucose
  - insulin
  - dyslipidaemia
  - hypertension
  - vasoactive agents
- Methodology for the assessment of sympathetic integrity is not standardized.
- Normative values have not been developed.

Recommendations

- Scintigraphic studies should not be routinely employed for the diagnosis of CAN and should be utilized in concert with standard CARTs (level C).
- Scintigraphic studies are extremely valuable in the identification of sympathetic noradrenergic denervation as a mechanism of neurogenic orthostatic hypotension (level B).
- [^{123}I]-MIBG single photon emission computed tomography offers semi-quantitative assessment and [^{11}C]-HED, 6-[^{18}F]-dopamine, and [^{11}C]-epinephrine positron emission tomography offer quantitative assessment of cardiac sympathetic integrity (level B).
- There is no standardized methodology for scintigraphic assessment of cardiac sympathetic integrity and only limited data on the reproducibility exist (level C).
- Scintigraphic tracer uptake is affected by myocardial perfusion, and tracer retention is affected by available energy for the active neuronal and vesicular uptake transporters (level C).
- The results of scintigraphy should be compared with an appropriate control population (level C).
- Scintigraphic studies offer good sensitivity to detect sympathetic neuronal loss in the heart (level C).
- Scintigraphy is appropriate to explore the effects of sympathetic denervation on cardiac physiology, metabolism, and function (level C).
- Scintigraphy is useful as a marker of cardiac sympathetic denervation in cross-sectional and longitudinal research studies (level C).

Conclusions

Assessment of HRV and BRS are the most widely used and readily available diagnostic tests for CAN in clinical research. They offer the possibility to provide new information about the pathophysiology of autonomic dysfunction in diabetes, to clarify the natural history of CAN with regard to the early autonomic abnormalities observed in diabetes and pre-diabetes, and to obtain more sensitive and comprehensive end-points in clinical trials in CAN. They might also be used in clinical practice in secondary care institutions to provide additional early and prognostic information to current CARTs. To obtain meaningful results, however, they need control of confounding factors, strict standardization with regard to respiration and blood pressure recording and to comply with various technical requirements (in particular for HRV testing).

Scintigraphic investigations may be available at most secondary care institutions and are potentially useful in longitudinal research studies. The role of MSNA and catecholamine assessment as end-points in clinical trials, as already applied in life-style intervention trials in obesity, needs to be further elucidated. Conversely, these techniques are the gold reference for assessing the role of the sympathetic nervous system in quantitative terms.

Supporting information

Supporting information may be found in the online version of this article.

Conflict of interest

None declared.

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Appendix

The Toronto Consensus Panel on Diabetic Neuropathy

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