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ASSESSMENT OF CARDIOVASCULAR AUTONOMIC FUNCTION

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INTRODUCTION

Autonomic nervous system integrates and regulates the function of visceral organs to provide logistic support for survival enabling the body to cope up with exercise, stress, and other physical and mental activities. Autonomic failure results in mild-to-severe degree of life-threatening situation depending on the degree of dysfunction. Autonomic failure is encountered in many clinical conditions as primary disorder or secondary disorders such as diabetes mellitus, alcoholism, amyloidosis etc. In these conditions clinical assessment may not be sufficient to quantify the degree of autonomic failure. Clinical symptoms of autonomic failure may appear late in the course of disease. In these conditions, early detection, and quantification of degree of loss of autonomic function can be made by a series of autonomic function tests. In some cases, such as myocardial infarction, quantification of autonomic impairment holds a promise to be of predictive value for the survival. Thus clinically, the value of autonomic functions testing is being increasingly recognized in the evaluation and management of several disorders where the autonomic function is compromised.[1]

Table 1: Indications for Cardiovascular Autonomic Function Testing.

Indications[2]
1. Peripheral Neuropathy - Diabetes, Amyloidosis, Sjogren's syndrome
2. Neurodegenerative Disorders - Parkinsonism, Alzheimers disease
3. Recurrent Syncope
4. Orthostatic Hypotension
5. Anhidrosis
6. Leprosy
7. Autonomic Neuropathy
8. Multiple Systems Atrophy
9. Postural Orthostatic Tachycardia syndrome
10. Hereditary Neuropathies
11. Baroreflex Failure
12. Riley Day Syndrome

Table 2: Contraindications for Cardiovascular Autonomic Function Testing.

Contraindications
Absolute
1. Acute Myocardial infarction - Within 48 hours may aggravate infarction and induce ventricular arrhythmias.
2. Unstable Angina - There is risk of Myocardial infarction and can induce ventricular arrhythmias.
3. Uncontrolled Cardiac Arrhythmias - May cause circulatory collapse
4. Active Endocarditis - May result in embolization.
5. Severe Aortic Stenosis - May result in arrhythmias, syncope and ischemia.
6. Decompensated Heart Failure - Circulatory collapse and arrhythmias may result.

7. Pulmonary Embolism and Infarction - Acute pulmonary embolism may get aggravated. 8. Acute non cardiac disorders that get aggravated with exercise - Diseases like renal failure, thyrotoxicosis, infections can get aggravated with exertion. 9. Acute Pericarditis/ Myocarditis - Arrhythmias may result. 10. Physical Disability
Relative 1. Coronary Stenosis 2. Stenotic Valvular Disease - Moderate to severe aortic stenosis may aggravate arrhythmias, syncopes and ischemias. 3. Electrolyte Abnormalities - Severe electrolyte abnormalities can result in electrolyte imbalance. 4. Tachy/ Brady Arrhythmias - If the arrhythmia is poorly controlled, it may result in hemodynamic compromise. 5. Atrial fibrillation with increased ventricular rate 6. Obstructive Hypertrophic Cardiomyopathy 7. Severe Hypertension (Systole Blood Pressure > 200 mmHg, Diastolic Blood Pressure > 110mmHg) 8. Severe Hyperthyroidism 9. Stroke within 1 month 10. Mental Impairment

Procedure[3]

The patient should relax and sit or lie down as instructed. Any stimuli or disturbance may affect the test results. The tests are relatively easy to perform, but the interpretation is difficult. This requires standardized patient preparation, procedures, evaluation using standard values and algorithms, for laboratories.

Patient Preparation

Patients should discontinue the following before examination

48 hours before

- Anticholinergics (e.g., antihistamines, antidepressants)
- Sympathomimetics (α- and β-agonists)
- Parasympathic mimetics
- Mineralocorticoids (e.g., 9-α-Fludrocortisone)
- Diuretics

24 hours before

Sympathicolytics (α-antagonists, β-antagonists)

12 hours before

- Alcohol
- Analgesics

On the day of test

- No wearing of confining clothing
- No support stockings

3 hours before the test

- Coffee, tea
- Food

Assessment of cardiovascular autonomic tone

Assessment of cardiovascular autonomic tone is done by measuring Heart rate Variability (HRV). Heart rate variability (HRV) is the variation in heart rate (i.e. in R-R intervals) that differs from one beat to the next at rest.[4,5] Variations in the sympathetic and parasympathetic influences on the heart cause these changes.[6] Autonomic tone can be quantified by using HRV and it has a high predictive value in many diseases.[7,8] To quantify autonomic tone, 5 minutes ECG recording is sufficient according to the guidelines of Task Force (1996).[9]

For short term analysis of HRV, Electrocardiogram (ECG) is recorded in supine position for 5 min after 10 min of rest in supine position. Room temperature is maintained at 24 ^{o}C. Subjects are instructed to close the eyes and to avoid talking, moving hands, legs, and body, coughing and sleeping. The ECG signals are acquired using lead-2 at sample rate of 1000/s and gain X 2000 from power lab data recording system (AD Instruments, Australia). HRV analysis is based on a 5-minute period of ECG signal which is artifact free using Lab Chart software (AD Instrument, Australia).

Quantification of HRV

HRV analysis is done by three methods: Time domain, Frequency domain and Non-Linear methods.

Time Domain Methods

In this method, statistical tools are applied to quantify the variations in RR intervals and the following parameters are computed.

SDRR - Standard deviation of the R-R intervals

SDSD- Standard deviation of differences between adjacent RR intervals.

RMSSD-The Root square of the mean of the sum of the squares of differences between adjacent RR intervals.

RR50 – Number R-R interval differences ≥ 50 ms.

pRR50 – Percentage of NN50.

Most of the conventional time domain parameters (i.e. SDRR, SDSD, RMSSD, RR50 and pRR50) are marker of parasympathetic activity.

Frequency Domain methods

The frequency components of HRV are analysed by using many methods. Fast Fourier Transform (FFT) is one of the commonly employed methods. The power spectrum is subsequently divided into three frequency bands: VLF- (0.001 to 0.04) Hz, LF- (0.040 to 0.15) Hz and HF- (0.15 to 0.4) Hz (Table 2). Power spectral densities (PSD) are plotted in ms2/Hz against pre-set frequencies. Power of the spectral bands are calculated in ms2 (absolute power) and in normalized units (nu). For example, normalize unit of LF is calculated by the formula: [LF/total power-VLF] x 100. Power of LF and HF are established in short term analysis of HRV. However, physiological explanation for VLF in short term recording is not well defined.

Table 3: Frequency domain parameters.

Frequency bands	Frequency	Mediated by
Very low frequency (VLF)	0.001-0.04 Hz	Possibly renin - angiotensin system
Low frequency (LF)	0.04-0.15 Hz	Parasympathetic and sympathetic influences
High Frequency (HF)	0.15-0.4 Hz	Parasympathetic influence
LF/HF ratio		Sympatho-vagal balance

Nonlinear Methods (Poincare plot)

The Poincare plot is a scatter plot of the current R-R interval against the R-R interval immediately preceding it (R-R*n* vs R-R*n+1*). The R-R interval Poincare plot typically appears as an elongated cloud of points oriented along the line-of-identity at 45° to the normal axis. The conventional parameters of Nonlinear methods are enumerated in Table.

Table 4: Non-linear parameters.

Parameters	Description	Mediated by
SD1	Dispersion of points perpendicular to the line of identity	Vagal influences
SD2	Dispersion along the line of identity	Sympathetic influences
SD2/SD1	Ratio of SD2/SD1	Sympatho-vagal balance

Assessment of cardiovascular autonomic reactivity

1. Deep breathing test

Procedure

The patient is instructed that breathing should be smooth, slow and deep. The investigator gives the hand signal to maintain the rate and timing of the breathing. For 6 cycles per min, the inspiration is done for 5 sec and expiration for 5 seconds. If cycles are not appropriately done, it is repeated after 2 minutes in order to get 6 complete cycles (i.e. each cycle consists of inspiration for 5 seconds followed by expiration for 5 seconds).

Stimulus: Deep and regular breathing at the rate of 6 breaths per minute
Afferents: Central
Efferent: Parasympathetic (cardiovagal, cholinergic)
Normal Response: Increase in heart rate with inspiration and decrease in heart rate with expiration.

Calculations

- Delta HR - difference between the maximal and minimal heart rate during inspiration and expiration respectively, averaged for 6 cycles.
- E:I ratio: ratio of the longest R-R interval and shortest R-R interval averaged over 6 cycles.

Normal values

- Delta HR

≥15 bpm normal
11 -14 bpm borderline
≤10 bpm abnormal

- E:I ratio - ≥1.21 normal

Physiological basis of the test

The variation of heart rate with respiration known as sinus arrhythmia is primarily mediated by the vagal innervation of the heart. The neuronal output from the respiratory centre influences the gain of the afferent and efferent outputs at the nucleus tractus solitarius. Pulmonary stretch receptors as well as cardiac mechanoreceptors and possibly baroreceptors contribute in the regulation of heart rate variations.

Factors known to affect the DBT

Age, hyperventilation, hypocapnia, increasing resting heart rate, cardiac failure, pulmonary disease, and CNS depression are known to affect DBT.

Abnormal DBT is seen in 80-85% of patients with autonomic failure syndromes such as multiple system atrophy and progressive autonomic failure.

Precautions

1. Signal should be given properly to maintain inspiratory and expiratory phase of each respiratory cycle for 5 sec.
2. Breathing of the subject should be slow, smooth and deep which can be confirmed from the respiratory tracing during the test.
3. If cycles are not appropriately done, it is repeated after 2 minutes in order to get 6 complete cycles (i.e. each cycle consists of inspiration for 5 seconds followed by expiration for 5 seconds).

Contraindication
Test should not be conducted in patients with acute respiratory disease.

2. Valsalva Maneuver
Procedure
The patient blows into a mouthpiece attached to sphygmomanometer. The expiratory pressure is kept at 40 mmHg for 15 seconds. A small air leak in system is useful to prevent the closure of glottis during the maneuver. At the end of 15 seconds the pressure is released. Due care is taken to prevent deep breathing before and after the maneuver.

Stimulus: Forced expiration through open glottis
Afferents: Baroreceptors and cranial nerves IX and X.
Efferent: Parasympathetic (Cardiovagal cholinergic), Sympathetic (adrenergic)

Normal Response
- Phase I: rise in blood pressure, decrease in heart rate
- Phase II: fall in blood pressure with rise late in the Phase II, increase in heart rate (HR)
- Phase III: fall in blood pressure
- Phase IV: increase in blood pressure, decrease in HR

Calculations
Valsalva Ratio: Longest RR interval during phase IV/ shortest RR interval during phase II.

Normal values: VR > 1.21

Physiological basis of the test
Valsalva Maneuver involves forced expiration through open glottis. When the forced expiration is started (Phase I), the blood pressure rises for a few beats along with decrease in heart rate (increase in RR interval). With continued strain (Phase II) at 40 mmHg, the pressure in the thorax is higher than the pressure in the great veins. As a result, the venous return becomes very low. This leads to drop in the blood pressure. The drop in the pressure is sensed by the baroceptors in the aortic arch and carotid sinus. The baroreceptor reflex is initiated leading to vagal withdrawal and sympathetic stimulation. As a result, the heart rate increase (RR interval decreases). The blood pressure keeps falling during the early phase II despite increase in heart due to falling stroke volume. However, in the late phase II, the blood pressure may show increase. On the release of the respiratory strain (Phase III), the blood pressure drops suddenly for few beats and then rises again (Phase IV). The rise in blood pressure is due to sudden increase in the venous return leading to overshoot above the baseline values. Due to baroreflex, this rise is associated with the decrease in heart rate (increase in the RR interval).

It is important to note that changes in the blood pressure during Phase I and Phase III are purely mechanical events. Rise in heart rate during the phase II is mediated initially with vagal withdrawal and subsequently by increase in sympathetic outflow. The decrease in the heart rate in response to overshoot in Phase IV is mediated by baroreflex (vagal).

Factors known to affect the Valsalva ratio
1. Age: with increase in age, the VR becomes lower
2. Position of the patient
3. Expiratory pressure: Decrease in expiratory pressure decreases the VR
4. Duration of the strain : decrease in duration lesser than 15 seconds decreases VR
5. Medications

Precautions
1. Valsalva apparatus should be kept at the level of subject's eye so that subject can see the pressure level during the test.
2. Subject should be instructed properly for how to blow through mouthpiece of the apparatus.
3. The blow should be forceful in order to increase mercury level up to 40 mmHg and should be maintained for 15 sec.
4. Subjects should avoid deep breath before start of the maneuver and just after the maneuver and avoid body movements.
5. If the maneuver is not done appropriately it should be repeated again at least after two minutes of rest.

Contraindication
Diabetic retinopathy, proliferative degenerative retinopathy and papilloedema

3. Lying to Standing Test
The patient is instructed to attain the standing posture from supine position within 3 seconds and recordings are taken. They are told to inform the investigator if he/ she feel dizziness or are uncomfortable during standing. The blood pressure and heart rate are recorded at baseline and serially at 0.5th, 1st, 2nd, 2.5th and 5th min. 30:15 ratio is calculated from ECG.

Stimulus: Change of posture from lying to standing
Afferents: Baroreceptors and cranial nerve IX & X
Efferent: Sympathetic (adrenergic), Parasympathetic (cardiovagal, cholinergic)
Normal Response: Initially increase in heart rate followed by decrease in heart rate. Fall in blood pressure

Calculations
30:15 ratio – it is calculated as the ratio between the longest R-R interval at or around the 30th beat and the shortest R-R interval at or around the 15th beat (Ewing et al, 1978).

Normal values
- Fall of systolic BP
  - ≤ 10 mm Hg normal
  - 11 -29 mm Hg borderline
  - ≥30 mm Hg abnormal

- 30:15 ratio
  - ≥ 1.04 normal
  - 1.01 – 1.03 borderline
  - ≤1.0 abnormal

Physiology of the test
The change of posture from lying to standing puts hydrostatic stress on the venous return. The venous decrease due to pooling of blood in the lower limbs results in decrease in the blood pressure. The decrease in blood pressure activates the baroreflex resulting in rise of the heart rate (between 10 – 20 seconds). The rise of heart rate raises the blood pressure towards the resting values. The recovery of blood pressure results in the decrease in the heart rate later (25 – 35 seconds).

Precautions
1. It should be ensured that subjects stand-up in 3 sec. Proper instructions and signal should be given in this respect.
2. Subject should also be told to inform the investigator if he/ she feels dizziness or is uncomfortable during standing.
3. Subject should not hold anything during standing.
4. The time interval for the BP measurement during the test should be appropriately followed.
5. If subject feels dizzy and not able to stand more, subject should be comfortably requested to sit/lie down.

Contraindications
Severe orthostatic hypotension (fall in SBP > 35 - 40 mmHg), unstable cardiovascular disease, pregnancy

4. Isometric Grip Test
The baseline blood pressure is recorded. The patient is asked to grip the handgrip dynamometer using maximum force with the dominant hand for a few seconds. The value is noted down, and the procedure is repeated thrice. The maximum value of the three readings is considered as the maximal voluntary contraction (MVC). The patient is instructed to maintain sustained grip on the dynamometer at 30% of MVC for 4 minutes. After the patient starts the contraction, the blood pressure is measured on the contra-lateral arm at 1st, 2nd, 4th minute. One more reading is taken 2 minutes after the release of the grip.

Calculation
- Change in DBP is calculated as Highest DBP during the test minus Baseline DBP

Normal values
- Increase in diastolic BP
- ≥ 16 mmHg normal
- 11 – 15 mmHg borderline
- ≤ 10 mmHg abnormal

Physiology of the test
Voluntary muscle activity is associated with sympathetic outflow to the cardiovascular system to increase the heart rate and blood pressure. The rise in heart rate is also due to parasympathetic withdrawal and activation of other central command. The accumulation of metabolites during the isometric contraction initiates the exercise reflex resulting in sustained sympathetic activity. Isometric exercise is associated with rise in diastolic pressure.

Precautions
1. Subject should grip on the dynamometer with maximal effort for the measurement of MVC.
2. Investigator should monitor the subject properly throughout the HGT for the sustained contraction at 30% of MVC.
3. The time interval for the BP measurement during the test and after the test should be appropriately followed.
4. The subject should avoid the body movement during and after the test.

1. Cold pressor test
Cold water of 10°C is prepared. The patient immerses the hand in water up to the wrist for 1 minute. After the hand is removed from the water, it is covered by a towel.

The baseline blood pressure is taken, and the blood pressure is taken just before the hand is taken out of water (i.e. at the end 1 minute of immersion). The blood pressure is taken again at 1.5 min and 4 min after the hand is withdrawn from the cold water.

Stimulus: Immersion of hand in cold water (10°C)
Afferents: Nociceptive and cold receptor, pain and temperature pathways
Efferent: Sympathetic (adrenergic)
Normal Response: Rise in blood pressure

Calculation
- Change in DBP is calculated as Highest DBP during the test minus Baseline DBP

Normal values
Increase in diastolic BP ≥10 mmHg

Physiology of the test
The cold water causes stimulation of cold receptors and pain receptors in the hand. The information is carried to the brain through spinothalamic pathways. The reflex involves, rise in sympathetic outflow to the vasculature and heart resulting in rise in blood pressure. In patients with afferent small-fibre neuropathy or spinothalamic tract dysfunction or with central or efferent sympathetic lesions, such as occur in diabetic or alcoholic autonomic neuropathies, the responses to CPT are diminished or even absent

Precautions

1. Subject should immerse hand in cold water up to the wrist and should not touch the bottom of the cold-water bath.
2. Subject should be monitored properly for the immersion of hand for 1 min.
3. The time interval for the BP measurement during the test and after the test should be appropriately followed.
4. If subject feels severe pain after immersion of hand in cold water hand should be taken out before 1 min.
5. Subject's hand should be properly covered with a towel after he takes his hand out from cold water.

Contraindication

Coronary artery disease, severe hypertension (SBP > 180, DBP > 110)

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