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# Differences in neurohumoral and hemodynamic response to prolonged head-up tilt between patients with high and normal standing norepinephrine forms of postural orthostatic tachycardia syndrome

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ABSTRACT

*Objective:* To investigate the optimal timing for blood sample collection of catecholamines and the possible correlations between neurohumoral and hemodynamic responses to prolonged head-up tilt (HUT) in postural orthostatic tachycardia syndrome (POTS).

*Methods:* Nineteen patients underwent a 30-minute, 70° HUT test. Blood samples (norepinephrine (NE), epinephrine and dopamine) were taken in the 10th minute of supine, and 10th, 20th and 30th minutes of HUT.

*Results:* There were no significant differences in the proportion of high and normal standing NE patients in the different time points. Mean NE (nmol/L) values in 10th, 20th and 30th minute of HUT were 4.37, 4.87, and 4.35 in the high standing NE, and 2.49, 2.59 and 2.88 in the normal standing NE group. High standing NE patients had higher blood pressure (BP) during the first 6 min of HUT (2nd minute after the HUT systolic BP (sBP): 118.29 ± 15.65 vs. 95.70 ± 13.43, $p = 0.004$; diastolic BP (dBP): 78.71 ± 6.68 vs. 65.10 ± 9.04, $p = 0.003$), while normal standing NE patients exhibited a drop in BP compared to resting values during the same time period. The normal standing NE group exhibited a progressive increase in norepinephrine values during the HUT.

*Conclusion:* One blood sample taken at the 10th minute of HUT correctly identifies high and normal standing NE POTS patients, but a small number of patients (1 out of 19, 5.2%) can be misidentified. High and normal standing NE POTS patients display distinctly different neurohumoral and hemodynamic responses to HUT.

## 1. Introduction

Postural orthostatic tachycardia syndrome (POTS) is one of the most common orthostatic intolerance syndromes usually affecting young, predominantly female adults. It is defined as ≥ 30 bpm increment in heart rate (HR) upon standing or head-up tilt (HUT) in absence of orthostatic hypotension, with an associated history of orthostatic intolerance symptoms (Freeman et al., 2011). The most common orthostatic intolerance symptoms associated with POTS include light-headedness or dizziness, weakness, palpitations, pre-syncope and tremulousness. A relatively high proportion of patients are also chronically fatigued and suffer from sleep disturbances (Thieben et al., 2007). More than a few underlying pathophysiological mechanisms have been proposed, some of which include autoimmune autonomic neuropathy (Vernino et al., 2000), blood volume and renin-angiotensin-aldosterone system perturbation (Stewart et al., 2006), abnormal norepinephrine (NE) clearance (Jacob et al., 1999) and physical deconditioning (Parsaik et al., 2012). It is believed that the most common type of POTS is caused by peripheral sympathetic dysfunction resulting in gradual venous blood pooling in the lower extremities when standing, which leads to compensatory tachycardia. This type of POTS is referred to as neuropathic and these patients have indirect signs of peripheral sympathetic dysfunction, such as abnormal responses on quantitative sudomotor axon reflex test (Benarroch, 2012). The second most common type of POTS is high standing NE which is thought to be a result of centrally mediated, exaggerated sympathetic activation. These patients usually have higher plasma norepinephrine values during standing (> 3.5 nmol/L). Some of these patients may also have signs of peripheral autonomic neuropathy (Thanavaro and Thanavaro, 2011).

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Although POTS is a heterogeneous and multifactorial disorder, distinguishing patients with standing NE of 3.5 nmol/L (i.e. 600 pg/mL) or higher seems to be of clinical use, especially with regards to treatment choice. It has been shown that patients with standing NE > 3.5 nmol/L have a better response to treatment with β-blocker medications (Thieben et al., 2007). Therefore, the main goal of this study was to investigate the relevance of time of blood sample collection on determining NE values induced by 70° HUT, with respect to the cut-point of 3.5 nmol/L. Furthermore, we wanted to investigate possible correlations between neurohumoral and hemodynamic response to prolonged HUT in POTS patients.

## 2. Methods

### 2.1. Patients

Nineteen patients diagnosed with POTS from January 2015 to March 2016 were enrolled. Criteria for diagnosing POTS were based on sustained increment of heart rate (HR) ≥ 30 bpm or an average HR of ≥ 120 bpm within 10 min of assuming an upright posture, in the absence of orthostatic hypotension (Freeman et al., 2011) on HUT test and a history of orthostatic intolerance symptoms lasting at least 3 months (Thieben et al., 2007). After signing the informed consent approved by the Ethical committee of the University Hospital Center Zagreb, patients were invited for additional HUT test and plasma catecholamine level analysis within one week. None of the patients used medications which could affect blood pressure or heart rate. In order to check for possible deconditioning we confirmed that patients did not suffer from systemic illnesses or other medical issues which led to prolonged bed rest, and were able to carry out their usual daily activities during at least two months prior to testing. Depending on the plasma norepinephrine (NE) levels during HUT, patients were categorized as either high standing NE (≥ 3.5 nmol/L) or normal standing NE (< 3.5 nmol/L). Since multiple blood samples were taken during the HUT, patients who had plasma NE ≥ 3.5 nmol/L during at least one of the studied time points were considered to be high standing NE.

### 2.2. Head-up tilt table test

All tests were performed in a quiet and dimly lit room. Patients were instructed not to drink coffee or smoke before the testing. After the patient laid down on the testing table, a pressure cuff and ECG electrodes were adjusted at appropriate sites. A peripheral vein catheter was installed in the antecubital or radial vein of the right arm and 20 min of settling period was given before recording. Based on our previous study which investigated differences in hemodynamic profile during a 30-min HUT, the HUT test consisted of 10-minute supine phase followed by a 30-minute, 70° head-up tilt (Crnosija et al., 2016). Beat-to-beat (B2B) HR and blood pressure values were recorded using Task Force Monitor (TFM) (CNSystems Medizintechnik AG, Austria). Blood samples for plasma catecholamine level analysis were collected from the peripheral vein catheter in the 10th minute of the supine phase and in the 10th, 20th and 30th minutes of HUT. Some patients could not finish the entire test due to development of vasovagal response (i.e. a sudden drop in HR and blood pressure) or other intolerable symptoms. In cases where HUT had to be ended before the 10th minute, blood samples were collected just before or during returning to supine position. If a patient developed vasovagal response or requested test termination within 5 min from prior blood sampling during the HUT, an additional blood sample was not collected.

### 2.3. Plasma catecholamine levels

Catecholamines of interest were norepinephrine (NE), epinephrine (E) and dopamine (DA). The blood samples were collected directly into chilled tubes containing EGTA and reduced glutathione for determination of plasma catecholamine levels during the supine and tilted phases of HUT test (Kabevette® N, Kabe Labortechnik GmbH). Plasma levels of cateholamines were measured on high pressure liquid chromatography (HPLC Prominence; Shimadzu GmbH) with an electrochemical detector CLC 100 (Chromsystems GmbH, Germany) using a commercially available HPLC kit and a reverse phase analytical column for HPLC analysis of catecholamines in plasma (Chromsystems GmbH, Germany).

### 2.4. Data preparation and statistical analysis

Several data were extracted by hand from the recorded B2B dataset. Values for the supine HR, systolic (sBP) and diastolic (dBP) blood pressure were calculated as the respective average values for each patient from 10 min of the recorded B2B data prior to the time of blood sample collection. All standing HR, systolic (sBP) and diastolic (dBP) blood pressure values were calculated as the respective average values for each patient from 1 min of the recorded B2B data prior to the time of blood sample collection. These values were used for all further analyses. Changes (Δ) in catecholamines, HR, sBP and dBP values in the 10th, 20th and 30th minutes of HUT were calculated with respect to respective supine values (e.g. $\Delta sBP_{20\ min} = sBP_{20min} - sBP_{sup}$). Furthermore, average HR, sBP and dBP values were calculated from the B2B dataset for each of the first 10 min of HUT. Also, Δ values were calculated for each of the first 10 min with respect to supine values (e.g. $\Delta sBP_{1\ min} = sBP_{1\ min} - sBP_{sup}$). Subscript "$_{sup}$" identifies values pertaining to supine phase of testing and subscripts "$_{min}$" denote values pertaining to specified time point in HUT.

Statistical analysis was performed using the IBM SPSS software, version 20. The Kolmogorov-Smirnov test was applied to test whether the data have a normal distribution. Differences in the distribution of qualitative variables were determined with the $\chi 2$ test and McNemar test, while the differences in quantitative variables, with respect to the distribution, were determined with the use of the parametric *t*-test, paired *t*-test and ANOVA with repeated measures and non-parametric Mann-Whitney and Wilcoxon test. Correlations between variables were determined with Pearson's and Spearman's coefficients, without corrections for the multiple comparisons. p values < 0.05 were considered as significant.

## 3. Results

### 3.1. Descriptive analysis

We included 19 POTS patients into the study of which 14 (73.7%) were female. Three patients fulfilled the POTS criteria by exhibiting average HR of ≥ 120 bpm during HUT. Clinical characteristics of the studied cohort is presented in Table 1. Seven patients were identified as high standing NE (group HIGH) and 12 were normal standing NE (group NORM). There was no difference in gender proportions between the two groups (in the high NE group 5 females and 9 females in normal standing NE group). Patients in high NE group were older

**Table 1**
Frequency and differences in clinical symptoms between patients with high and normal standing NE POTS.

| Symptom | High standing NE group (N, %) | Normal standing NE group (N, %) | p value |
|---|---|---|---|
| Lightheadedness | 7 (100%) | 8 (69.2%) | 0.245 |
| Cognitive dysfunction | 3 (42.9%) | 1 (8.3%) | 0.117 |
| Presyncope/syncope | 6 (85.7%) | 11 (91.7%) | 1.000 |
| Palpitations | 2 (28.6%) | 3 (25.0%) | 1.000 |
| Chest tightness | 0 | 1 (8.3%) | 1.000 |
| Fatigue | 1 (14.3%) | 1 (8.3%) | 1.000 |
| Gastrointestinal (diarrhea, abdominal pain) | 1 (14.3%) | 2 (16.7%) | 1.000 |