Heart rate variability in pulmonary hypertension with and without sleep apnea

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ABSTRACT

Objectives: Our aims were to evaluate HRV in pulmonary hypertension (WHO Group 1 and 4) compared to control subjects, and to assess whether the presence of sleep apnea in those with pulmonary hypertension would be deleterious and cause greater impairment in HRV.

Methods: This retrospective case-control study analyzed electrocardiogram segments obtained from diagnostic polysomnography.

Results: Forty-one pulmonary hypertension patients were compared to 41 age, sex and apnea-hypopnea index matched healthy controls. The pulmonary hypertension group had decreased high frequency, very low frequency, low frequency, and percentage of normal R-R intervals that differ by > 50 ms compared to control subjects. Moderate to severe right ventricle dysfunction on echocardiography was a predictor of lower high frequency in pulmonary hypertension patients.

Conclusions: There were no differences in any HRV measures in pulmonary hypertension patients with or without sleep apnea. Impaired HRV was demonstrated in pulmonary hypertension patients however, the presence of sleep apnea did not appear to further reduce vagal modulation.

1. Introduction

Heart rate variability (HRV), the fluctuation in time intervals between consecutive heartbeats, is regulated by both the sympathetic and parasympathetic divisions of the autonomic nervous system at the sino-atrial node and provides an assessment of autonomic nervous system modulation (Pagani et al., 1986; Task Force of ESC/NASPE, 1996). Decreased HRV portends a worse prognosis in the general population (Hillebrand et al., 2013; Maheshwari et al., 2016) and in particular in those with heart failure (Danilowicz-Szymanowicz et al., 2016). Moreover, the clinical importance of low HRV has been recognized for risk stratification for sudden arrhythmic death in the general population (Maheshwari et al., 2016).

Pulmonary artery hypertension (PAH) is a progressive pulmonary vascular disorder characterized by progressive narrowing of the pulmonary vessels (Galie et al., 2016) producing an increase in the afterload to the naïve right ventricle (RV) with subsequent RV failure and death. Due to comorbid conditions including obesity and increased age PAH patients are predisposed to sleep apnea (SA) with high prevalence rates demonstrated in small cohort studies (Minic et al., 2014). Increased sympathetic activation occurs in both PAH (McGowan et al., 2009; Velez-Roa et al., 2004) and obstructive sleep apnea (OSA) (Somers et al., 1995). In PAH it is an independent predictor of clinical deterioration (Ciarka et al., 2010). Furthermore, impaired HRV has been demonstrated in PAH (McGowan et al., 2009; Wensel et al., 2009; Yi et al., 2014) and OSA (Narkiewicz et al., 1998; Wang et al., 2008; Wiklund et al., 2000).

The pathophysiological impact of SA on PAH is uncertain, although evidence from studies on those with other cardiac conditions suggests that it would have both an acute and chronic adverse hemodynamic impact (Bradley and Floras, 2003). The long-term prognosis of PAH was dismal prior to the availability of recent therapeutic options for PAH (D’Alonzo et al., 1991). However, with both the increased recognition of, and superior survival in PAH determining the true impact of SA in PAH may be important (Sitbon et al., 2016). HRV provides a non-invasive...
assessments of autonomic modulation and in light of its prognostic value in the general population it may also be of importance in the PAH group. Our hypothesis was that SA would be deleterious to those with pulmonary hypertension (PH) and therefore, greater sympathovagal modulation would occur in those with SA and PH compared to those with isolated PH. The objectives of our study were to evaluate HRV from polysomnographic data 1) in patients with PH compared to healthy individuals, and 2) in PH and control subjects between those with and without SA.

2. Methods

2.1. Study design and population

This is a retrospective case-control study of adults with PH (WHO Group 1 or 4) (Galie et al., 2016) and matched healthy control patients referred as part of their routine care for diagnostic polysomnography ( PSG) at the University Health Network, Toronto, Canada, from January 1, 2008 to November 27, 2016. PH was defined as per World Health Organization (WHO) guidelines (Galie et al., 2016). Healthy subjects were matched for age, sex, and apnea-hypopnea index (AHI) to the PH group, and were derived from consecutive PSG performed over the study period. Exclusion Criteria included a) pregnancy, b) pulmonary or cardiac disease c) patients on treatment for SA, d) those on amiodarone, beta-blocker or non-dihydropyridine calcium channel blocker medications, e) subjects with atrial fibrillation, excessive premature beats on the PSG electrocardiogram (ECG) (>5% beats) or cardiac pacemaker and f) a total sleep time <1.5 h on the PSG.

The clinical and medical information was collected within 6 months of the PSG. For those with PH, information collected also included the right heart catheterization data, two-dimensional echocardiography, and six-minute walk distance. PH subjects were stratified into low, intermediate and high risk (Leuchte et al., 2018). The study was approved by the University Health Network Research Ethics Board (15–9401).

2.2. Polysomnography

A diagnostic PSG was performed in all patients. Thoracoabdominal motion was monitored by respiratory inductance plethysmography; nasal airflow by nasal pressure cannula; oxyhemoglobin saturation (SaO2) by oximetry; and cardiac rhythm was monitored by a 2-lead ECG. Signals were recorded on a computerized sleep recording system (Sandman, Nellcor Puritan Bennett Ltd, Ottawa, ON, Canada). Standard techniques and scoring criteria were used to evaluate subjects for sleep stages and arousal from sleep (Iber et al., 2007).

The severity of SA was assessed by the number of apneas and hypopneas per hour of sleep (AHI). Apnea scoring required a greater than 90% signal drop for at least 10 seconds, and hypopnea scoring required a greater than 30% reduction in nasal pressure signal excursions from baseline and an associated ≥3% desaturation or arousal (Berry et al., 2012). Apneas and hypopneas were scored as central or obstructive in the absence or presence of out-of-phase thoracoabdominal motion. The oxygen desaturation index (ODI) was defined as the number of oxygen desaturations per hour ≥3% below baseline. The scoring criteria were consistent over time. Subjects were divided into NSA (AHI <5 events/h) and SA (AHI ≥5 events/h) groups. Sleep apnea was deemed obstructive (OSA) if ≥50% of events were obstructive and central (CSA) if >50% were central in nature. The Epworth Sleepiness Scale (ESS), a subjective measure of sleepiness, was completed on the night of the PSG (Johns, 1991).

2.3. Heart rate variability

Lead II ECG data (sampled at 256 Hz) from the PSG with at least 1 segment ≥5 min of stage N2, with no apnea or hypopnea events (AHI = 0) was used to determine the HRV. Each ECG segment had signal quality verified calculating the difference between the R peaks and the mean energy of a normalized filtered ECG signal. Those that did not meet the minimum score (0.1) were excluded. The degree of relationship between respiration (from respiration) and HRV at a specific frequency was determined by coherence analysis. Only ECG segments with coherence >0.5 were included (MatLab R2015a, The MathWorks Inc., Natick, MA, US).

HRV was then evaluated by Kubios HRV 2.2 (Kubios, Kuopio, Finland) using both time and frequency domains analysis (Taskinen et al., 2014). Time analysis involved calculation of the average of R-R intervals (Mean R-R), Standard deviation of R-R intervals (SDNN) the average of heart rate, the root mean square of successive R-R interval differences (RMSSD), the percentage of normal R-R intervals that differ by >50 ms (pNN50), and the integral of density distribution (histogram) (that is, number of all R-R intervals) divided by the maximum of the density distribution (R-R triangular index) (Lahiri et al., 2008; Stein and Pu, 2012; Task Force of ESC/NASPE, 1996). Frequency spectrum of ECG segments was calculated using the Fast Fourier Transform, from which 3 frequency bands were derived: very low frequency (VLF) from 0.003 to 0.04 Hz, low frequency (LF) from 0.04 to 0.15 Hz, and high frequency (HF) from 0.15 to 0.4 Hz (Lahiri et al., 2008; Stein and Pu, 2012; Task Force of ESC/NASPE, 1996). The total power of frequencies ranges and the LF/HF ratio were also calculated.

2.4. Statistical analysis

PH and SA groups were analyzed as dichotomous variables (i.e., PH or Control and NSA or SA, respectively). Continuous variables were compared using Student t test or one-way analysis of variance (ANOVA), and Mann-Whitney or Kruskal-Wallis test as appropriate. Discrete data was examined by Chi squared or Fisher exact test (for frequencies <5). Correlations were examined by Pearson’s or Spearman’s rank for normally and non-normally distributed variables, respectively. Univariate analysis for predictors of HF and LF/HF included age, sex, body mass index (BMI), hypertension, smoking, ESS, indicators of SA (arousals, AHI, cumulative percentage time at SaO2 <90% (CT90)), indicators of PH (mean pulmonary artery pressure, right atrial pressure, mixed venous oxygen saturation, cardiac index, diastolic pressure gradient, right atrial area, RV function (normal-mild dysfunction vs moderate-severe dysfunction), no-or-trivial vs ≥ mild parciardial effusion (Klein et al., 2013), B-type natriuretic peptide (BNP), six-minute walk distance, WHO functional class), as independent variables. Any independent variable with an alpha value <0.1 was included in the multivariable models of analysis of covariance (ANCOVA). Possible confounders (age, BMI, hypertension, AHI, mean pulmonary artery pressure) were also tested. Logarithmic transformation was used to convert HF and LF/HF to satisfy the normal distribution assumption. A p-value of <0.05 was considered significant. Analyses were performed by SPSS 20.0 (SPSS Inc, Chicago, IL).

Sample size was calculated assuming an effect size of 0.88 in HF difference between PH and control groups. A two-tailed α of 0.05 and β of 95% were used. The resulting sample size was 35 patients for each group. A p-value of <0.05 was considered significant. Additionally, after the study competition, we calculated the effect size considering the number of PH patients with sleep SA (n = 25) and without SA (n = 16), using a two-tailed α of 0.05 and β of 95%. The resulting effect size was 1.18.

3. Results

Of 66 PH patients initially screened, 25 were excluded (Fig. 1). Group 1 PH was present in 38 and Group 4 PH in 3 subjects. The mean time from diagnosis of PH was 1.8±3.5 years. Of the 41 PH subjects included 58.5% were on pulmonary vasodilators, 17.1% on calcium channel blockers, 39.0% on loop diuretics and 29.3% on anti-coagulation medications. The 41 PH patients included were matched for age, sex, and AHI to the control group. Subjects in both groups were predominantly female,
middle aged, and overweight (Table 1). PH patients had a higher prevalence of dyslipidemia and hypertension compared to the control group. Subjects were not subjectively sleepy as measured by the ESS (7.1 ± 4.2).

Compared to controls, the PH subjects had a significantly greater frequency of CSA and lower mean nocturnal SaO2.

3.1. HRV in PH compared to control subjects

In PH patients, there were significantly lower mean R-R, SDNN, pNN50, R-R triangular index, VLF, LF, HF, total power, and higher heart rate compared to control subjects (Table 2). LF/HF did not differ between groups.

3.2. Effect of SA on HRV

When subjects were stratified according to the presence of SA, the control subjects with SA (control-SA, n = 21) had similar demographics

### Table 1
Baseline characteristics.

|                      | Control N = 41 | PH N = 41 | p-value |
|----------------------|----------------|-----------|---------|
| Age, years           | 48 (13)        | 47 (15)   | 0.745   |
| Female sex n (%)     | 30 (73)        | 31 (76)   | 0.800   |
| Body mass index, kg/m² | 29 (6)        | 30 (7)    | 0.319   |
| Dyslipidemia n (%)   | 2 (5)          | 8 (19)    | 0.043   |
| Hypertension (%)     | 0              | 8 (19)    | 0.005   |
| Smoking n (%)        | 0              | 1 (2)     | 0.500   |
| Epworth Sleep Scale  | 7 (4)          | 7 (4)     | 0.524   |
| Apnea-hypopnea index, n/h | 15 (21)   | 15 (21)   | 0.985   |
| Central index, n/h   | 1 (1)          | 4 (12)    | 0.556   |
| Obstructive index, n/h | 14 (20)    | 10 (15)   | 0.584   |
| Central sleep apnea n (%) | 0       | 6 (15)    | 0.025   |
| Total sleep time, min | 337 (56)  | 307 (65)  | 0.27    |
| Stage N1, %          | 9 (7)          | 9 (8)     | 0.933   |
| Stage N2, %          | 61 (10)        | 65 (11)   | 0.091   |
| Stage N3, %          | 13 (10)        | 13 (10)   | 0.963   |
| REM Stage, %         | 18 (6)         | 13 (7)    | 0.003   |
| Arousal index, n/h   | 22 (14)        | 22 (19)   | 0.481   |
| Mean SaO2, %         | 95 (2)         | 90 (4)    | <0.001  |
| CT90, %              | 3 (9)          | 45 (39)   | <0.001  |
| ODI, n/h             | 12 (18)        | 16 (17)   | 0.036   |
| Supplemental oxygen use n (%) | 0       | 4 (10)    | 0.116   |

Abbreviations: PH = pulmonary hypertension, REM = rapid-eye movement, SaO2 = Oxygen saturation, CT90, cumulative percentage time at SaO2 < 90%, ODI = Oxygen desaturation index. Results are shown as Mean (SD) or n (%).

### Table 2
Heart rate variability in PH and control subjects.

|                      | Control N = 41 | PH N = 41 | p-value |
|----------------------|----------------|-----------|---------|
| Mean R-R, ms         | 972 (138)      | 831 (108) | <0.001  |
| SDNN, ms             | 50 (18)        | 35 (23)   | 0.001   |
| Mean heart rate, 1/min | 63 (9)        | 74 (11)   | <0.001  |
| RMSSD, ms            | 41 (22)        | 31 (29)   | 0.091   |
| pNN50, %             | 18 (19)        | 9 (14)    | 0.001   |
| R-R triangular index | 12 (4)         | 8 (4)     | <0.001  |
| VLF Power, ms²       | 1075 (858)     | 569 (614) | <0.001  |
| LF Power, ms²        | 801 (691)      | 382 (614) | <0.001  |
| HF Power, ms²        | 723 (774)      | 467 (1003)| <0.001  |
| Total Power, ms²     | 2600 (1849)    | 1395 (1825)| <0.001  |
| LF/HF ratio          | 2 (2)          | 4 (8)     | 0.581   |

Abbreviations: PH = pulmonary hypertension, Mean R-R = average of R-R intervals, SDNN = standard deviation of R-R intervals, RMSSD = root mean square of successive R-R interval differences, pNN50 = percentage of normal R-R intervals that differ by > 50 ms, R-R triangular index = number of all R-R intervals divided by the maximum of the density distribution, VLF = very low frequency, LF = low frequency, HF = high frequency. Results are shown as Mean (SD).
to the control subjects without SA (control-NSA, n = 20) (Table 3). Polysomnographic characteristics were similar other than an elevated AHI (26 vs 3 events/h, p < 0.001), ODI (25 vs 3/h, p < 0.001) and arousal index (29 vs 16/h, p = 0.047) in the control-SA compared to the control-NSA group. In the control subjects no significant differences between groups were demonstrated for HRV (Fig. 2). However, when control subjects with moderate to severe SA (AHI ≥15 events/h) were compared to control-NSA patients, the mean R-R, RMSSD, pNN50, and HF were significantly lower (Table 4). LF/HF was similar among all groups.

The 25 PH patients with SA (PH-SA) were older, had higher systolic blood pressure (123 ± 14 vs 112 ± 17 mmHg, p = 0.024) and lower BNP (70 ± 94 vs 275 ± 317 ng/L, p = 0.020) compared to those without SA (PH-NSA) (Table 5). Although, the PH risk stratification score was similar between groups. A mild degree of SA was present in the PH–SA group and they had a significantly higher arousal index than the PH–NSA group. However, the mean SaO2, ODI, CT90 and sleep architecture were similar between those with PH–SA and PH–NSA (Table 5). HRV parameters were similar in PH patients with or without SA, irrespective of SA severity (Fig. 2).

3.3. Predictors of HRV

In control subjects, SA severity represented by the AHI and CT90 were negatively correlated with both the RMSSD (r = -0.324, p = 0.039; r = -0.450, p = 0.003) and pNN50 (r = -0.316, p = 0.044; r = -0.473, p = 0.002), respectively. HF was inversely correlated to arousals (r = -0.321, p = 0.041) and CT90 (r = -0.477, p = 0.002). LF was associated with CT90 (r = -0.373; p = 0.016), while LF/HF was associated with arousals (r = 0.311, p = 0.048), but not with CT90 (r = 0.151, p = 0.345).

Among PH subjects, there were a number of weak associations of uncertain significance (Table 6). Diastolic pressure gradient was inversely correlated with HRV parameters including the pNN50 (r = -0.456, p = 0.005). Of the available markers of PH severity the presence of pericardial effusion (r = -0.373; p = 0.016) compared to Control-SA.

4. Discussion

Our study demonstrates that the polysomnographic HRV was significantly decreased in PH compared to control subjects. Secondly, the influence of SA on HRV among PH patients was explored, and contrary to our expectations no additional autonomic modulation was demonstrated.

HRV has been utilized as a tool for the non-invasive assessment of the autonomic nervous system regulation of the sinoatrial node (Shaffer and Ginsberg, 2017; Task Force of ESC/NASPE, 1996). A pathological state is usually indicated by reductions in HRV. However, biological systems are complex and this is reflected by the dynamic interplay between the sympathetic and parasympathetic nervous systems. In the usual healthy state at rest the balance of activity is parasympathetic. The time domain measure of RMSSD and pNN50 are influenced by parasympathetic activity as is HF, the frequency domain which reflects respiratory sinus arrhythmia. Both parasympathetic and sympathetic activity contribute to SDNN, however, in short duration recordings the variability primarily reflects parasympathetic activity. LF is modulated by both the parasympathetic and sympathetic outflow, while the LF/HF is considered to reflect the sympathovagal balance (Shaffer and Ginsberg, 2017). However, increased sympathetic nervous activity may both suppress and augment parasympathetic nervous activity to maintain homeostasis (Tulppo et al., 2005). Understanding this interplay is important when interpreting HRV.

Sleep is a period of quiescence and in non-rapid eye movement sleep there is usually autonomic stability with a reduction in sympathetic nervous activity and a predominance of parasympathetic nervous activity

| Table 3 | Clinical Characteristics and Polysomnography of Both Control and Pulmonary Hypertension subjects stratified according to the presence of Sleep Apnea. |
|---|---|
| | C-NSA N = 20 | C-SA N = 21 | p-value | PH-NSA N = 16 | PH-SA N = 25 | p-value | Between group p-value |
| Age, years | 44 (15) | 52 (11) | 0.395 | 39 (12) | 52 (15) | 0.004 | 0.006 |
| Male sex n (%) | 5 (25) | 6 (29) | 0.796 | 2 (12) | 8 (32) | 0.265 | 0.585 |
| Body mass index, kg/m² | 26 (4) | 31 (7) | 0.056 | 27 (8) | 32 (6) | 0.068 | 0.003 |
| Diabetes n (%) | 1 (5) | 0 | 0.488 | 2 (12) | 5 (20) | 0.685 | 0.099 |
| Dyslipidemia n (%) | 2 (9) | 0.488 | 4 (25) | 4 (16) | 0.689 | 0.109 |
| Hypertension n (%) | 2 (10) | 0 | 0.488 | 1 (6) | 6 (24) | 0.448 | 0.003 |
| Smoking n (%) | 0 | 0 | 0 | 1 (4) | 0.390 | 0.195 |
| Coronary artery disease n (%) | 0 | 0 | 1 (6) | 3 (12) | 0.488 | 0.191 |
| Stroke/TIA n (%) | 0 | 0 | 0.056 | 0.055 | 0.150 |
| Epworth Sleepiness Scale | 8 (5) | 7 (4) | 0.999 | 7 (3) | 7 (4) | 0.999 | 0.760 |
| Apnea-hypopnea index, n/h | 3 (2) | 26 (24) | -0.001 | 2 (1) | 23 (24) | <0.001 | <0.001 |
| Central index, n/h | 1 (1) | 1 (2) | 0.869 | 0 | 7 (15) | 0.176 | 0.022 |
| Obstructive index, n/h | 4 (8) | 24 (22) | 0.003 | 2 (1) | 16 (17) | 0.003 | <0.001 |
| Central sleep apnea n (%) | 0 | 0 | 0 | 6 (24) | 0.025 |
| Total sleep time, min | 341 (66) | 333 (46) | 0.999 | 325 (83) | 295 (48) | 0.692 | 0.057 |
| Stage 1, % | 8 (6) | 10 (8) | 0.999 | 9 (6) | 9 (9) | 0.999 | 0.907 |
| Stage 2, % | 60 (9) | 61 (11) | 0.999 | 65 (11) | 64 (11) | 0.999 | 0.379 |
| Stage 3,% | 15 (11) | 10 (9) | 0.607 | 13 (9) | 13 (11) | 0.999 | 0.432 |
| REM stage, % | 17 (6) | 18 (6) | 0.999 | 13 (7) | 13 (6) | 0.999 | 0.024 |
| Arousal index, n/h | 16 (7) | 29 (17) | 0.047 | 14 (7) | 28 (21) | 0.025 | 0.003 |
| Mean SaO2, % | 96 (2) | 95 (2) | 0.307 | 90 (4) | 90 (4) | 0.999 | <0.001 |
| CT90, % | 1 (1) | 6 (12) | 0.972 | 45 (39) | 45 (39) | 0.999 | <0.001 |
| ODI, n/h | 3 (2) | 25 (21) | <0.001 | 8 (6) | 22 (20) | 0.149 | <0.001 |
| Supplemental oxygen use n (%) | 0 | 0 | 0 | 2 (12) | 2 (8) | 0.637 | 0.170 |

Abbreviations: C = Control, PH = pulmonary hypertension, NSA = no sleep apnea, SA = sleep apnea, TIA, Transitory ischemic attack, REM = rapid-eye movement, SaO2 = Oxygen saturation, CT90 = cumulative percentage time at SaO2 < 90%, ODI = Oxygen desaturation index.

Results are shown as Mean (SD) or n (%).

* p < 0.05 compared to Control-NSA.

† p < 0.01 compared to Control-No-SA.

‡ p < 0.01 compared to Control-SA.

§ p < 0.05 compared to Control-No-SA.
This delicate balance is disrupted by OSA, which causes intermittent hypoxia, the generation of negative intrathoracic pressure and cortical arousals. In our study, HRV in control subjects with SA did not differ significantly from those without. This unexpected failure to demonstrate an impact of SA on the autonomic nervous system may due to the small cohort and/or the overall mild degree of OSA. The importance of OSA severity was demonstrated by the significant attenuation of parasympathetic outflow in those with moderate-severe OSA compared to those without. Furthermore, in the control subjects a significant negative correlation was shown between indices of hypoxia (CT90) and vagal modulation. This association between the severity of OSA in otherwise healthy subjects and modulation of autonomic activity in HRV is in keeping with other studies (Pan et al., 2016; Wang et al., 2008).

While autonomic nervous dysfunction has been extensively described among those with left heart failure (Danilowicz-Szymanowicz et al., 2016), less is known about autonomic modulation, in those with RV dysfunction (e.g. PH). Prior experiments in an animal model suggest the existence of a sympathetic reflex mechanism associated with pulmonary artery distention (Juratsch et al., 1977). Additionally, the increased pulmonary vascular resistance generates an increased RV afterload, resulting in RV hypertrophy, subsequent right atrial enlargement (Ciarka et al., 2010; McGowan et al., 2009), RV failure, consequent low systemic cardiac output, which in turn may activate compensatory sympathetic response but further loss of autonomic nervous system modulation, and result in concomitant myocytes injury in both ventricles (Vonk Noordegraaf et al., 2017).

Compared to control our PH subjects had impaired HRV as evidenced by significant reductions in both time (pNN50) and frequency (VLF, LF, HF) domain measures. Prior studies evaluating HRV in PAH patients were performed by assessment of daytime ECG or Holter monitor. These assessments did not take into consideration the potential influence of respiratory frequency (McGowan et al., 2009; Wensel et al., 2009; Yi et al., 2014). However, similar to the previous studies in PAH subjects...
Clinical characteristics of PH patients according to the presence of SA.

| Parameter | PH-SA | PH-NSA | p-value |
|-----------|-------|--------|---------|
| Age, years | 39 (12) | 52 (15) | 0.004 |
| Male sex, n (%) | 2 (12) | 8 (32) | 0.265 |
| Body mass index, kg/m² | 27 (8) | 32 (6) | 0.068 |
| WHO FC I/II/III, % | 12/37/50 | 12/52/36 | 0.743 |
| PH Risk Score 1/2/3, % | 31/65/0 | 40/56/4 | 0.576 |

**Abbreviations:** PH = pulmonary hypertension, SA = sleep apnea, WHO FC = World Health Organization functional class, PH Risk Stratification Score 1 = low, 2 = intermediate, 3 = high risk, CTEPH = chronic thromboembolic pulmonary hypertension, OA = obstructive sleep apnea, CSA = central sleep apnea, SaO₂ = oxygen saturation, CT90 = cumulative percentage time at SaO₂ < 90%, ODI = Oxygen desaturation index, WU = Wood unit.

Results are shown as Mean (SD) or n (%).

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Univariate analysis of heart rate variability predictors among pulmonary hypertension patients.

| Parameter | Mean difference in HF power ms² (%), 95% CI | p-value | Mean difference in LF/HF (%), 95% CI | p-value |
|-----------|---------------------------------------------|---------|-------------------------------------|---------|
| Age, per year | 0.001 (–0.002 to 0.005) | 0.491 | –0.001 (–0.008 to 0.006) | 0.793 |
| Male sex | –0.044 (–0.170 to 0.082) | 0.487 | 0.167 (–0.081 to 0.414) | 0.181 |
| Body mass index, kg/m² | –0.001 (–0.009 to 0.007) | 0.801 | 0.002 (–0.013 to 0.017) | 0.803 |
| Smoking | –0.071 (–0.424 to 0.281) | 0.684 | 0.576 (–0.104 to 1.256) | 0.095 |
| Hypertension | 0.008 (–0.130 to 0.145) | 0.909 | –0.026 (–0.300 to 0.249) | 0.851 |
| Epworth Sleepiness Scale, per unit | –0.1 (–0.024 to 0.065) | 0.194 | 0.022 (–0.008 to 0.052) | 0.139 |
| Apnea-hypopnea index, per event/h | –0.001 (–0.002 to 0.003) | 0.883 | 0.001 (–0.004 to 0.006) | 0.630 |
| Central index, per unit | –0.001 (–0.003 to 0.003) | 0.762 | 0.007 (0.002–0.012) | 0.014 |
| CT90, % | –0.001 (–0.001 to 0.001) | 0.956 | <0.001 (–0.003 to 0.003) | 0.895 |
| mPAP, per mmHg | –0.001 (–0.004 to 0.003) | 0.700 | 0.002 (–0.004 to 0.009) | 0.495 |
| PVR, per Wood unit | –0.011 (–0.023 to 0.001) | 0.059 | 0.017 (–0.016 to 0.050) | 0.293 |
| Cardiac index, per L/min/m² | 0.113 (–0.130 to 0.356) | 0.343 | 0.183 (–0.221 to 0.588) | 0.354 |
| DPG, per mmHg | –0.006 (–0.011 to 0.001) | 0.034† | 0.008 (0.002 to 0.018) | 0.113 |
| SvO₂, % | 0.002 (–0.006 to 0.001) | 0.592 | 0.001 (–0.014 to 0.017) | 0.850 |
| Right atrial pressure, mmHg | –0.009 (–0.025 to 0.008) | 0.271 | 0.008 (0.032 to 0.048) | 0.693 |
| Moderate to severe RV dysfunction | –0.156 (–0.260 to 0.005) | 0.005b | 0.121 (–0.093 to 0.335) | 0.261 |
| Pericardial effusion | –0.145 (–0.316 to 0.027) | 0.095 | 0.387 (0.083–0.691) | 0.014d |
| N-type natriuretic peptide <50 ng/L | 0.004 (–0.105 to 0.113) | 0.942 | –0.237 (–0.466–0.009) | 0.042† |

**Abbreviations:** HF = high frequency, LF/HF = low frequency/high frequency ratio, CT90 = cumulative percentage time at Oxygen saturation <90%, mPAP = mean pulmonary arterial pressure, PVR = pulmonary vascular resistance, DPG = diastolic pressure gradient, SvO₂ = mixed venous Oxygen saturation.

† Log10.

a Model R² = 0.125, Model R² adjusted = 0.099 (p = 0.034).
b Model R² = 0.196, Model R² adjusted = 0.175 (p = 0.005).
c Model R² = 0.146, Model R² adjusted = 0.124 (p = 0.014).
d Model R² = 0.165, Model R² adjusted = 0.140 (p = 0.014).
e Model R² = 0.130, Model R² adjusted = 0.101 (p = 0.042).
Technical differences in micropolyneuropathic assessment of sympathetic nervous activity is autonomic modulation through HRV. However, the performance of lead to more reliable ECG segments, but on the other hand may have exclusion of patients with excessive ectopic beats, which on one hand in the SA and NSA subgroups; the few subjects with severe OSA in PH; the frequency/high frequency ratio, CI due to the relative stability and predominantly metabolic regulation of cardiovascular and respiratory systems, during this sleep stage (Tobaldini et al., 2013). Finally, high coherence between respiration and HRV ensured that respiratory frequency was not inadvertently impacting HRV parameters (Notarius and Floras, 2001; Task Force of ESC/NASPE; Tobaldini et al., 2013).

There are multiple strengths of our study which include the relatively large sample size, the clearly defined group of PH subjects diagnosed by right heart catheterization, matched for potential confounders including age, sex, BMI and AHI to medication-free control subjects. Furthermore, HRV was confined to periods free of apnea and hypopneic events which can impinge upon HRV rhythmic oscillations (Tobaldini et al., 2013). By analyzing all data from a single sleep state we avoided potential effects of sleep stage on HRV. Additionally, the restriction of analysis to stage N2 avoided the possible influence of behavioral influences on HRV due to the relative stability and predominantly metabolic regulation of cardiovascular and respiratory systems, during this sleep stage (Tobaldini et al., 2013). Finally, high coherence between respiration and HRV ensured that respiratory frequency was not inadvertently impacting HRV parameters (Notarius and Floras, 2001; Task Force of ESC/NASPE; Tobaldini et al., 2013).

Potential limitations of our study include the small number of subjects in the SA and NSA subgroups; the few subjects with severe OSA in PH; the exclusion of patients with excessive ectopic beats, which on one hand lead to more reliable ECG segments, but on the other hand may have ruled out those with more severe PH; and the indirect measurement of autonomic modulation through HRV. However, the performance of micropolyneuropathic assessment of sympathetic nervous activity is technically difficult, and we wanted to use a feasible non-invasive marker that could be easily incorporated into clinical practice. Furthermore, neither the presence nor absence of a correlation can definitely establish causation or lack thereof.

5. Conclusions
In conclusion, our study confirmed previous findings of impaired HRV in PH compared to control subjects. We also demonstrated an impact of severe OSA on HRV in control subjects but not in PH. Therefore, we did not demonstrate greater autonomic dysregulation in those with both SA and PH. Further, larger studies should be conducted to confirm these findings and an assessment of the effect of treatment of SA in PH may identify a benefit in PH patients.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Declarations

Author contribution statement

Carolina Gonzaga Carvalho: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Richard Bresler: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ying Xuan Zhi: Analyzed and interpreted the data; Wrote the paper.

Hisham Alshaer, John T. Granton: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Clodagh M Ryan: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare the following conflict of interests: Carolina Gonzaga Carvalho was supported by a grant from the CNPq – National Council for Scientific and Technological Development, Brazil. John Granton’s institution has received funds to support research from Actelion Pharmaceuticals and Bayer. John Granton has served as a member of steering, data and safety monitoring, and adjudication committees for Bellerophon, Actelion, and United Therapeutics, respectively. These agencies had no role in the design, collection, analysis of data, or writing of the manuscript. Richard Bresler, Ying Xuan Zhi, Hisham Alshaer, Clodagh Ryan have nothing to disclose.

Additional information

No additional information is available for this paper.

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