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Autonomic nervous system activity in primary Raynaud's phenomenon: Heart rate variability, plasma catecholamines and [^{123}I]MIBG heart scintigraphy

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Abstract

Background and Aim: Primary Raynaud's phenomenon (pRP) is characterized by an exaggerated response to cold, resulting in the whitening typically of the fingers and toes. The patients are generally perceived as healthy individuals with a benign condition. However, the condition has been associated with increased cardiovascular mortality and changes in autonomic nervous system activity. This study aimed to investigate whether pRP is associated with pervasive changes in autonomic nervous activity. The hypothesis was that patients with pRP have increased sympathetic nervous activity.

Methods: The autonomic nervous activity of 22 patients with pRP was investigated by means of heart rate variability (HRV) and the plasma catecholamine response to head-up tilt and compared with 22 age- and gender-matched controls. In addition, the patients were examined with a [^{123}I]metaiodobenzylguanidine heart scintigraphy and compared with an external control group.

Results: The plasma norepinephrine response to head-up tilt was significantly lower in the patient group than in the control group. Similarly, the heart scintigraphy revealed a lower heart-to-mediastinum ratio in the patient group than in the control group. HRV analysis did not reveal significant differences between the groups.

Conclusion: The findings of the study showed that the autonomic nervous activity of patients with pRP was altered compared with the activity of healthy individuals. This was observed both during rest and after positional stress, but the findings did not uniformly concur with our initial hypothesis.

KEYWORDS

[^{123}I] metaiodobenzylguanidine, neurophysiology, parasympathetic nervous system, plasma epinephrine, plasma norepinephrine, sympathetic nervous system

1 | INTRODUCTION

Raynaud's phenomenon (RP) is characterized by the episodic whitening of the fingers triggered by cold, which can last from minutes to hours. RP can arise secondary to exposure to vibration, intake of certain medical drugs and the presence of various diseases such as connective tissue diseases (CTDs) (Herrick, 2012). However, for most patients with RP, no underlying cause can be identified. This condition is referred to as primary RP (pRP). pRP is typically perceived as an insignificant condition whose effects are entirely peripheral. However, Nietert et al. found that Caucasian patients with RP had a higher risk of experiencing angina as well as cardiovascular-related death compared with individuals without RP (Nietert et al., 2015), independent of underlying rheumatic disease or differences in age or gender. Furthermore, a systematic review by Garner et al. reported an association between pRP and cardiovascular disease (CVD), but not with typical risk factors for CVD such as diabetes, hypertension or hypercholesterolemia (Garner et al., 2015). Other studies found that patients with pRP demonstrated a higher prevalence of migraine and chest pain (O'Keeffe et al., 1992, 1993). These reports suggest that pRP is associated with symptoms that are more widespread than generally assumed.

Although the pathophysiology of RP is not completely understood, both autonomic and sensory nerve fibres are thought to be involved (Edwards et al., 1998; Herrick, 2012), which may explain the pervasiveness of symptoms in patients with pRP. A study investigating the autonomic nervous integrity of patients with pRP by analyzing heart rate variability (HRV) showed increased parasympathetic activation during positional stress in pRP patients compared with healthy individuals (Pancera et al., 1999). Furthermore, Klimiuk et al. assessed the cardiovascular response to Ewing's tests (Ewing & Clarke, 1982) and found that it triggered signs of early parasympathetic dysfunction (Klimiuk et al., 1988). Koszewicz et al. (2009) found a predominance of the low-frequency (LF) component during rest and decreased HRV during breathing in patients with pRP compared with healthy individuals, concluding a tendency toward elevated sympathetic activity in patients with pRP, which was also reported by Olsen et al. during positional stress (Olsen et al., 1987). Thus, previous findings of the literature have conveyed inconsistent conclusions about the autonomic nervous activity of patients with pRP. As decreased HRV have been reported to be an independent predictor of mortality (Malik et al., 1996; Thayer & Lane, 2007; Vinik & Ziegler, 2007), it seems important to further investigate the autonomic nervous system (ANS) activity of patients with pRP. If the patients have an imbalance of the ANS, this may provide a plausible link between this condition and the reported increased risk of CVD.

Assessment of autonomic nervous activity in patients with pRP should include investigations of overall HRV as well as the relative distribution of the parasympathetic and sympathetic pathways of the ANS. Thus, the present study included HRV analysis by time-domain and frequency-domain parameters, which mainly convey information on overall HRV and parasympathetic pathway; analysis of systolic blood pressure (sBP) overshoot in phase 4 of the Valsalva manoeuvre (VM), which provides an illustration of the integrity of the sympathetic component of the baroreflexes (Vogel et al., 2005); and the hormonal response to stress by measuring plasma catecholamine concentrations before and after a physiologically stressful stimulus. Given the inconsistent results of previous investigations of autonomic integrity in patients with pRP, the potential changes in autonomic nervous activity are likely to be subtle. Therefore, the study also included an examination that has been shown to be a sensitive marker of the sympathetic activity of the heart, the [^{123}I]metaiodobenzylguanidine ([^{123}I]MIBG) heart scintigraphy (Jacobson et al., 2010), which uses a norepinephrine (NE) analogue to assess cardiac sympathetic nervous activity during rest. Although the [^{123}I]MIBG heart scintigraphy has been investigated in numerous disorders, including heart failure, it has never been used to assess patients with pRP. A pathological [^{123}I]MIBG heart scintigraphy is able to predict an increased risk of cardiac events and heart failure progression (Gerson et al., 2011; Jacobson et al., 2010; Verberne et al., 2008), even in patients with preserved ejection fraction (Katoh et al., 2010). Thus, the heart scintigraphy was applied in the present study to reveal subtle changes in the cardiac sympathetic nervous activity in patients with pRP. The aim of the study was to investigate whether pRP is associated with pervasive changes in ANS activity, which might explain the reported higher risk of CVD and death. Based on the vasospastic nature of the condition as well as previous reports from the literature, we hypothesized that patients with pRP have increased sympathetic activity compared with healthy individuals.

2 | METHODS

2.1 | Patients with pRP

Twenty-two patients with pRP were recruited through advertisements in local newspapers. The diagnosis was clinically verified in accordance with the International Consensus Criteria for pRP developed by an international expert panel (Maverakis et al., 2014). Participants were diagnosed with RP by applying the three-step outline. Subjects were included only if they met the following diagnostic criteria for pRP: (1) physical examination without findings suggestive of secondary causes, (2) no history of existing CTD and (3) negative antinuclear antibodies (ANA). Furthermore, nailfold capillaries of all 10 fingers were inspected (using a USB digital microscope, ×25 to ×600 magnification) to document any abnormalities in nailfold capillary morphology that indicated an underlying CTD. Potential participants were excluded if they reported any secondary causes of RP, such as treatment with a beta-blocker or previous chemotherapy, exposure to vibration, symptoms of carpal tunnel syndrome, current smoking or pregnancy. The participants were questioned about chest pain and other heart-related symptoms, which were elaborated where relevant. In addition to ANA, the following blood analyses were performed on the included patients: haemoglobin, sodium, potassium, calcium (ionized), magnesium, thyroid-stimulating

hormone (TSH), vitamin D, vitamin B_{12}, folic acid and HbA1c. TSH, electrolytes and vitamins were analyzed to exclude competing causes of neuropathy, and HbA1c was analyzed to exclude diabetes.

The internal control group was also recruited through advertisements in the hospital and in local newspapers. Twenty-two age- and gender-matched participants were selected for the control group, which comprised healthy individuals without symptoms of RP or previous occupational exposure to vibration. Participants in the control group had no medical record of chronic illnesses or health issues affecting the blood vessels, especially heart or lung diseases or CTDs. They had no vitamin B_{12} deficiency or alcohol abuse and did not take prescription drugs, including drugs that could predispose them to RP. Except for ANA, the blood analyses outlined for the patient group were also examined in this group of healthy individuals, which will hereafter be referred to as the internal control group. This group participated only on the first day of examination and therefore did not undergo heart scintigraphy.

The heart scintigraphy results from an external control group (different from the internal group) were used as a reference. This control group, which will be referred to as the external control group, consisted of 14 healthy individuals examined in a previous study (von Scholten et al., 2016) that applied a similar protocol and equipment. This particular study is one of few studies investigating a healthy population applying a medium-energy general-purpose (MEGP) collimator, as is recommended in [^{123}I]MIBG imaging of the heart (Flotats et al., 2010; Inoue et al., 2003; Verberne et al., 2005). Image analysis of the external control group was done by an experienced observer.

2.2 | Tilt table testing, including VM, plasma catecholamines and HRV

On the days of examination, participants were asked to refrain from drinking coffee and tea, performing strenuous exercise and eating large meals before examination. Blood pressure was measured on both arms to exclude any side-to-side difference. Before examinations, a peripheral venous catheter (PVC) was placed in a cubital vein. The Task Force® Monitor (CNSystems Medizintechnik AG, Version 2.3.20.20, sampling frequency 1000 Hz) was applied to noninvasively measure and assess the beat-to-beat heart rate (HR) and blood pressure during HRV recordings. Before examination, four electrocardiogram electrodes were placed to record the six limb leads. The arm-cuff was placed on the upper arm on the same side of the PVC to measure the blood pressure for calibration, while the finger cuff was placed on the index and middle fingers of the opposite hand to measure the beat-to-beat HR and blood pressure. The participant then rested for 10 min in the supine position. For power spectral analysis, a 5-min recording was made with the participant resting while still in the supine position. To obtain a blood pressure signal strong enough to record, a heating pad was provided so that cold hands could be rewarmed before recording. The sBP, diastolic blood pressure (dBP) and HR were noted at baseline (at the time of the first blood sample) and after 15 min' head-up tilt (HUT) (second blood sample), respectively.

VM was performed after the first resting period. Upon a deep inspiration, the participant was asked to expire forcefully into a mouthpiece with a small leak to ensure an open glottis. The mouthpiece was attached to an electronic pressure gauge, and the participant was required to maintain expiration at a minimum pressure of 40 mmHg for 15 s. At least one attempt of appropriate length and quality was completed.

After completing the VM and before tilt-up, the participant rested in the supine position for 10 min, at which point the first blood sample was drawn. Subsequently, the participant was attached to the tilt table with straps and tilted to 60°. Thirty seconds after tilt-up, another 5-min recording was started to assess the HRV in response to tilt-up. After 15 min' HUT, the second blood sample was drawn. The investigation sequence is illustrated in Figure 1.

Plasma was initially stored at −20°C and subsequently at −80°C. Baseline and intervention samples from the respective participants were stored together continuously. Plasma epinephrine (p-E) and plasma norepinephrine (p-NE) levels were analyzed in both samples using liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Bergmann & Schmedes, 2020). Plasma samples were extracted using solid-phase extraction, and the eluate was evaporated and reconstituted before LC-MS/MS analysis. The analysis was calibrated using calibrators prepared in-house. LOQ was found to be 0.02 nmol/L for p-E and 0.20 nmol/L for p-NE with a coefficient of variation on five determinations of 9.6% and 8.4%, respectively.

The 5-min recordings were analyzed using a standardized analysis programme (Kubios HRV Premium) (Tarvainen et al., 2014),

FIGURE 1 The sequence of investigation for tilt table testing, recordings for heart rate variability, Valsalva manoeuvre (VM) and blood samples for plasma catecholamines

which provided detailed HRV analysis with time-domain and frequency-domain parameters, where the spectral analysis was estimated using Fast Fourier transformation. Participants with excessive extrasystoles (defined as >20 ectopic beats during the sampling period) or atrial fibrillation were excluded from further analysis. Ectopic beats were corrected using a threshold-based artefact correction algorithm (Lippman et al., 1993). Detrending was performed using smoothen priors with a lambda value of 500 (Tarvainen et al., 2002). Time and frequency-domain parameters were used to assess the HRV. The time-domain measures included the HR, the *standard deviation of RR intervals* (SDNN) and the *root mean square of* successive differences between normal heartbeats (RMSSD). The frequency-domain measures included the *total power*, the LF power, the *high-frequency power* (HF) and the *LF/HF rate*. LF and HF power were reported as absolute power (beats per minute [bpm]) and relative power (normalized units [n.u.]) (Malik et al., 1996).

2.3 | [^{123}I]MIBG heart scintigraphy

The [^{123}I]MIBG heart scintigraphy was carried out in the morning on a separate day. To protect the thyroid gland, patients were asked to ingest 400 mg potassium iodide upon arrival, and [^{123}I]MIBG was administered intravenously at least 30 min later; the average dosage was 302 MBq. Anterior planar images of the thorax were acquired 15 and 240 min after tracer injection. Before the scintigraphy, patients were asked to remove any clothing containing metal. Images were obtained using a Philips Brightview XCT gamma camera with a MEGP collimator and a 256 × 256 matrix and the software JETStream for Brightview XCT (Philips Medical Systems). Acquisition time was 900 s. A 20% energy window was centred on the 159-KeV photopeak of [^{123}I].

All images acquired from the patient group were assessed by one reader, and those from the control group by another reader. However, to be able to create regions of interest (ROIs) comparable to the ROIs from the external control group, image analysis was discussed with the observer who had assessed the images of the control group. ROIs were drawn over the heart and upper mediastinum, with the lung apices serving as the upper limit of the mediastinal ROI. Image analysis was done using the software Philips Intellispace, v6.0.5.12010. ROIs were drawn according to relevant guidelines and in accordance with the analysis applied to the images of the external control group (Flotats et al., 2010; von Scholten et al., 2016). The mean count within each ROI was reported from the early and late images, and an early and late heart-to-mediastinum ratio were obtained (HMR_{early} and HMR_{late}, respectively). The myocardial washout rate (WOR) was obtained by means of count-based calculations according to proposed guidelines (Flotats et al., 2010). A low HMR is a marker of altered cardiac sympathetic nervous activity; either due to chronically increased sympathetic activity as is seen in heart failure (Katoh et al., 2010) or decreased activity as in Parkinson's disease (Courbon et al., 2003). The WOR is an indicator of the sympathetic drive (Verberne et al., 2008).

All patient-related examinations were performed at the Department of Nuclear Medicine, Herlev Hospital, Copenhagen University Hospital, from February 2017 to March 2018. Plasma catecholamine analyses were performed at the Department of Biochemistry and Immunology, Vejle Hospital, University Hospital of Southern Denmark, Vejle, in April 2019.

The study was approved by the Research Ethics Committee of the Capital Region of Denmark (protocol H-16035842, approved on 5 October 2016). Written informed consent was obtained from each participant included in the study. All procedures involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

2.4 | Statistical analysis

Normal distribution of continuous variables was tested using the Q-Q plot and Shapiro–Wilk test. For non-normally-distributed variables, statistical comparison was done using the Wilcoxon rank-sum test and normally distributed continuous variables were compared using Student's *t* test. Categorical variables were compared using Fisher's exact test due to small subgroup numbers. A two-tailed $p < 0.05$ was interpreted as significant. All statistical analyses were done using R version 4.0.2.

For heart scintigraphy results, analysis of covariance (ANCOVA) was conducted to determine a significant difference between the patient group and the external control group on the HMRs, controlling for age, gender and body mass index (BMI). The explanatory variable *age* was chosen because HMR_{late} is known to decrease with age (Estorch et al., 1995). *Gender* and *BMI* were included due to the differences between patients and the external control group for these variables (see Table 1). HRV parameters of interest were selected from time-domain measures as well as frequency-domain measures according to guidelines on HRV standards of measurements (Malik et al., 1996). ANCOVA was applied to assess a significant difference between the patient group and the internal control group in HRV parameters after tilt-up and plasma catecholamine levels after 15 min' HUT, respectively, controlling for the respective baseline values and BMI. BMI was added as a covariate due to the significant difference between patient and control group for this variable. Average sBP 30 s before VM was subtracted from the peak sBP during Phase 4 of the VM to obtain the sBP overshoot after VM.

3 | RESULTS

3.1 | Clinical characteristics

The clinical characteristics of the patient and internal control group were similar except for BMI; although median BMI was within the normal reference range, it was significantly lower in the patient

TABLE 1 Clinical characteristics of the patient group with primary Raynaud's phenomenon (pRP), the internal control group and the external control group (von Scholten et al., 2016)

	pRP group ($n = 22$)	Internal control group ($n = 22$)	pRP versus Internal p value	External control group ($n = 14$)	pRP versus external p value
Age (years)	57.8 (49.2; 64.6)	59.0 (50.7; 67.7)	0.78	62.0 (56.3; 66.8)	0.22
Gender					
Female	19 (86.4)	19 (86.4)	1.0	6 (42.9)	0.01
Male	3 (13.6)	3 (13.6)		8 (57.1)	
Height (cm)	170 (165; 173)	166 (160; 171)	0.09	168 (164; 175)	0.72
Weight (kg)	66.3 (61.3; 72.0)	70.3 (62.9; 78.8)	0.25	72.5 (66.1; 78.5)	0.11
BMI (kg/m^2)	23.0 (21.0; 24.5)	25.0 (23.3; 26.5)	**0.02**	24.7 (23.3; 25.9)	0.07
Alcohol (units/week)	3 (1; 7)	3 (1; 7)	0.45		
Smoking status					
Never	11 (50.0)	13 (59.1)	0.54		
Current	0 (0.0)	1 (4.5)			
Former	11 (50.0)	8 (36.4)			
Smoking (pack-years)	0 (0; 5)	0 (0; 3)	1.0		
Menopause					
Yes	13 (59.1)	14 (63.6)	0.83		
No	4 (18.2)	5 (22.7)			
Unknown	5 (22.7)	3 (13.6)			
sBP (mmHg)	130 (18)	133 (15)	0.56		
dBP (mmHg)	82 (13)	81 (8)	0.83		

Note: Data are presented as *n* (%), mean (SD) or median (IQR). Bold value is significant *p* value.

Abbreviations: BMI, body mass index; dBP, diastolic blood pressure, HR, heart rate; sBP, systolic blood pressure.

group compared to the control group, where the mean value was 25.0 kg/m^2. None of the patients were treated with medication for RP. The blood analyses did not differ significantly between the patient group and the internal control group.

The patient group and the external control group differed only by gender, whereas the age, height, weight and BMI were similar between the groups. The clinical characteristics of the patient group and the internal as well as the external control group are presented in Table 1. Upon nailfold capillaroscopy examination, one patient revealed haemorrhages on some fingers. The nailfold capillaroscopy of another patient revealed suspected dilated capillaries on at least one finger. However, measurement of the capillary diameter was not possible due to the chosen capillaroscopic technique. The two patients did not fulfil the nine criteria required for the diagnosis of systemic sclerosis according to the American College of Rheumatology/European League Against Rheumatism (van den Hoogen et al., 2013). Consequently, both participants were still considered as patients with pRP and their results were included in the study.

3.2 | Plasma catecholamines

The *rest* as well as the *tilt* values of p-E and p-NE, respectively, were lower in the patient group than in the internal control group, albeit the difference was only significant for the *tilt* values. Further, the p-NE value after HUT increased significantly less in the patients compared with the controls. The plasma catecholamine results are presented in Table 2. The sBP, dBP and HR at baseline and after 15 min' HUT, respectively, are presented in Figure 2. The haemodynamic measurements were similar between the groups. The ANCOVA showed a significant difference between the groups on the p-NE ($p = 0.01$) concentration after 15 min' HUT, when adjusting for baseline p-NE level and BMI. No such difference between the groups was found for p-E ($p = 0.12$). The baseline levels for both p-E and p-NE were associated with the respective plasma concentrations after 15 min' tilt (both $p < 0.001$), while neither of the plasma catecholamines were associated with BMI.

TABLE 2 Results from the analysis of plasma epinephrine (p-E) and norepinephrine (p-NE) (nmol/L)

	pRP group ($n = 22$)	Internal control group ($n = 22$)	p value
Rest			
p-NE	1.18 (0.85)	1.36 (0.64)	0.44
p-E	0.02 (0.01; 0.05)	0.04 (0.02; 0.06)	0.19
Tilt			
p-NE	1.78 (1.09)	2.46 (0.97)	**0.03**
p-E	0.04 (0.02; 0.08)	0.08 (0.05; 0.13)	**0.03**
Rest to tilt			
p-NE	0.60 (0.68)	1.10 (0.79)	**0.03**
p-E	0.03 (0.00; 0.04)	0.04 (0.01; 0.08)	0.15

Note: *Rest* values were acquired after 10 min' rest in the supine position before tilting. *Tilt* values were the response to 15 min' head-up tilt to 60°. *Rest to Tilt* = the difference between *Rest* and *Tilt* values. Data are presented as mean (SD) or median (IQR). Bold values are significant p values.

Abbreviation: pRP, primary Raynaud's phenomenon.

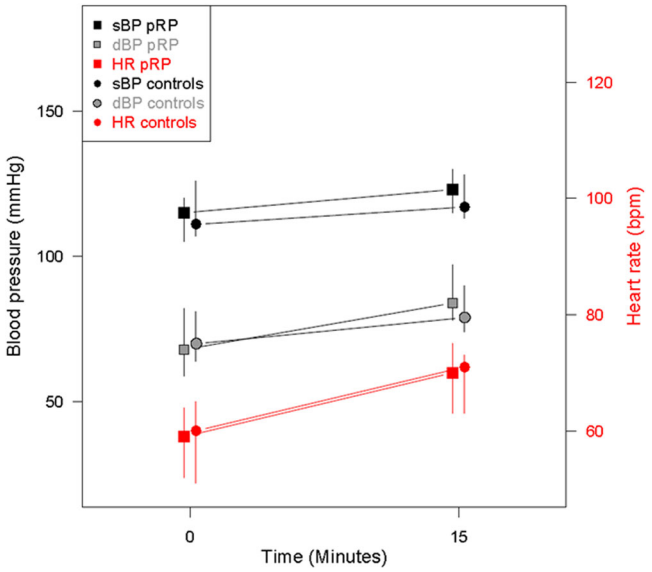

FIGURE 2 Blood pressure and heart rate (HR) measurements before and after 15 min' head-up tilt (HUT) for patients with primary Raynaud's phenomenon (pRP) and healthy controls. The points at baseline and after 15 min' HUT represent the median sBP (black), dBP (grey) and HR (red) for the patients (square) and the controls (circles) at the two-time points. The vertical lines represent the IQR. The *y* axis on the left relates to the blood pressure, while HR refers to the *y* axis on the right. dBP, diastolic blood pressure; sBP, systolic blood pressure

3.3 | [^{123}I]MIBG heart scintigraphy

The mean (SD) HMR_{late} was 2.40 (0.4) and 2.89 (0.4) for the patient group and the external control group, respectively. The difference was statistically significant ($p = 0.0004$).

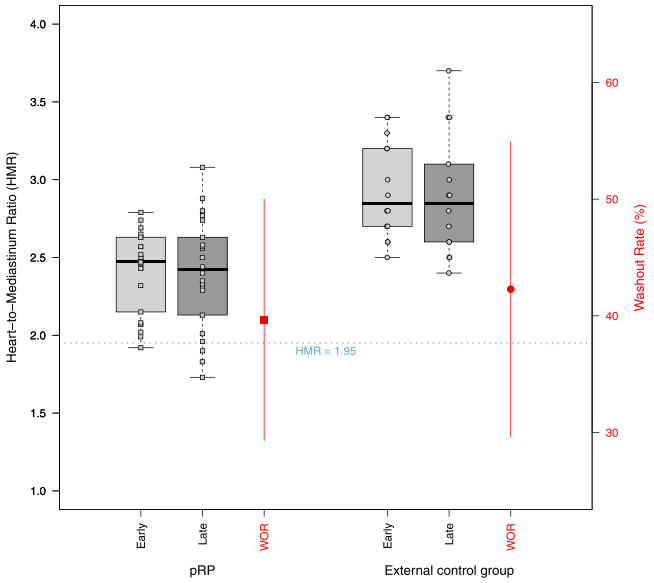

FIGURE 3 Boxplot illustrating the mean early and late heart-to-mediastinum ratio (HMR) as well as the washout rate for patients with primary Raynaud's phenomenon (pRP) (left) and the external control group (right) examined in a previous study (von Scholten et al., 2016). The boxes limit the interquartile range (IQR) and frame the median (horizontal bold black line). The whiskers limit the highest and lowest value within 1.5× IQR from the box. HMR values for each patient and healthy control are represented by small squares and circles, respectively. The mean WOR for patients and controls is represented by the large red square and circle, respectively. The vertical lines extending from the mean represent ± 1 SD. The blue dashed line marks the lower normal HMR_{late} limit of 1.95 as concluded by (Jacobson et al., 2010) and recalculated to fit the medium-energy general-purpose collimator according to (Nakajima et al., 2017; Verschure et al., 2018). HMR, heart-to-mediastinum; WOR, washout rate

In the patient group, mean (SD) HMR_{early} was 2.41 (0.3) and 2.94 (0.3) in the external control group. HMR_{early} also yielded a significant difference between the groups ($p = 5 \times 10^{-6}$). The mean (SD) WOR was 40% (10) in the patient group and 42% (13) in the control group. This difference was not significant ($p = 0.50$). Results are shown in Figure 3. The ANCOVA showed that there was a significant difference in HMR_{late} between the patients with pRP and the external control group ($p < 0.001$) when controlling for age, gender and BMI. Furthermore, HMR_{late} was inversely associated with age ($p = 0.02$) but not with gender or BMI. Model evaluations were carried out and the assumptions for ANCOVA were met. Use of Cook's distance plot revealed two outliers: one patient and one healthy participant. Both had the highest value of HMR_{late} in their respective group. Omission of the outliers increased the adjusted R^2 to .55 and the p value for the relation to age decreased to <0.001. Thus, their removal seemed to improve the model, but as the values for both outliers were genuine, they were preserved in the final model.

3.4 | HRV

None of the HRV parameters during rest or HUT were significantly different between the patient and the control group. Table 3 presents the HRV parameters during rest and upon tilt-up. Although SDNN was not significantly different between the groups during rest or HUT, the parameter remained notably stable in the patient group, while decreasing in the control group after tilt-up. Accordingly, the delta value from *rest* to *tilt* was borderline significantly different between the groups ($p = 0.06$). The ANCOVA showed no significant difference between the groups in HRV parameters after tilt-up when adjusting for HRV baseline values or BMI. The *tilt* values of three patients and one healthy individual were excluded from the analysis due to the presence of either many extrasystoles or technical issues. The median (IQR) sBP overshoot after VM was 27.2 (16.7; 51.9) mmHg in the patient group and 26.9 (19.1; 38.0) mmHg in the control group. This difference was not statistically significant ($p = 0.87$).

4 | DISCUSSION

The present study investigated the ANS in patients with pRP compared with age- and gender-matched healthy controls. The levels of p-E and p-NE were significantly lower in the patient group than in the control group after 15 min' HUT—as was the increase in p-NE from rest to tilt. In addition, HMR_{late} was significantly lower in patients with pRP compared to the external control group. The sBP overshoot after VM and HRV parameters at baseline and upon tilt were similar between groups.

TABLE 3 HRV parameters during rest and head-up tilt

Rest	pRP group ($n = 22$)	Internal control group ($n = 21$)	pRP versus internal p value
Time domain			
HR (bpm)	56.1 (6.9)	57.1 (7.7)	0.63
SDNN (ms)	29.5 (8.4)	31.7 (12.6)	0.51
RMSSD (ms)	32.5 (11.9)	33.8 (13.8)	0.75
Frequency domain			
Total power (ms^2)	834 (456; 1005)	994 (461; 1471)	0.53
LF power (ms^2)	312 (167; 520)	307 (208; 585)	0.62
HF power (ms^2)	314 (172; 560)	485 (166; 768)	0.71
LF power (n.u.)	44 (34; 60)	54 (33; 63)	0.62
HF power (n.u.)	56 (40; 66)	46 (37; 67)	0.62
LF/HF-ratio	0.79 (0.51; 1.5)	1.17 (0.49; 1.67)	0.62
Tilt	**pRP group ($n = 19$)**	**Internal control group ($n = 20$)**	**pRP versus internal p value**
Time domain			
HR (bpm)	65.8 (8.7)	66.0 (8.6)	0.94
SDNN (ms)	28.7 (11.0)	26.1 (8.8)	0.41
RMSSD (ms)	22.3 (9.1)	22.0 (9.4)	0.92
Frequency domain			
Total power (ms^2)	673 (234; 1025)	456 (302; 932)	0.78
LF power (ms^2)	361 (136; 644)	265 (146; 550)	0.60
HF power (ms^2)	89 (57; 236)	127 (45; 306)	0.62
LF power (n.u.)	73 (65; 82)	71 (59; 85)	0.60
HF power (n.u.)	27 (18; 35)	29 (15; 41)	0.60
LF/HF-ratio	2.7 (1.9; 4.5)	2.5 (1.5; 5.7)	0.60

Note: Data are presented as mean (SD) or median (IQR). The LF component is power in the low-frequency range (0.04–0.15 Hz), and the HF component is power in the high-frequency range (0.15–0.4 Hz).

Abbreviations: HR, heart rate; n.u., normalized units (LF or HF divided by [total power − very low frequency] × 100); pRP, primary Raynaud's phenomenon; RMSSD, square root of the mean of the sum of the squares of differences between adjacent NN intervals; SDNN, standard deviation of all NN intervals.

We demonstrated that the level of p-NE after 15 min' tilt increased significantly less in the patient group than in the control group, which is contrary to our initial hypothesis of increased sympathetic activity in patients with pRP. Previous studies of the plasma catecholamine level and response to a cold pressor test (Marasini et al., 1991; Surwit et al., 1982) found a similar response in their respective groups of patients with pRP, pointing to an impaired sympathetic pathway in response to stress. Moreover, Surwit et al. (1982) found a higher baseline level of plasma cortisol in patients with pRP compared with controls. The authors argued that the high level of plasma cortisol was responsible for the exaggerated vascular reactivity characterizing the condition, as glucocorticoids have been reported to increase vascular reactivity to p-NE (Surwit et al., 1982). Consequently, the low levels of p-NE were presumably due to a negative feedback mechanism. This potential feedback mechanism could explain the p-NE response to stress found in the current study as well as that of Surwit et al., which otherwise seems counterintuitive given the vasospastic nature of the disease. Contrary to these findings, Coppo et al. (2006), found a normal total p-NE spillover in patients, whereas their hand NE spillover was significantly increased. Although this finding refutes our hypothesis and supports the idea that pRP is caused by local adrenergic dysregulation, local dysregulation alone cannot explain the difference in the catecholamine response found in the present study. Hypothetically, inhibition of the plasma catecholamine response may occur in a central nervous system functional unit such as the central autonomic network (Thayer & Lane, 2007), which comprises a network of connected structural areas of the brain involved in autonomic regulation (Thayer & Lane, 2007). However, this does not explain the reduced HMR_{late} found in the current patient group.

The HRV data did not verify significantly different autonomic activity in the patient group compared with the controls. Although the parameter SDNN remained noticeably constant in the patient group while decreasing in the control group upon tilt-up, an increased vagal role during tilt was not confirmed by the parameters RMSSD or HF (n.u.) that showed a clear parasympathetic withdrawal after tilt-up similar to the control group. Pancera et al. (1999) actually did find increased vagal function upon tilt-up in patients with pRP, who had a significantly higher HF (n.u.) and lower LF/HF-ratio during HUT compared with the control group. As in the present study, HRV analysis revealed no differences between the patient and control group during rest (Pancera et al., 1999). This was refuted by Koszewicz et al. (2009), who found indications of an impaired parasympathetic pathway and elevated sympathetic activity during rest in patients with pRP. In addition, the findings by Koszewicz et al. indicated impaired parasympathetic activity during deep breathing, which was supported by Klimiuk et al. (1988). Contrarily, Manek et al. (2011) could not reveal altered autonomic activity in patients with pRP upon deep breathing or the VM. Olsen et al. (1987) found a tendency toward elevated sympathetic activity in patients with pRP by measuring the relative change in skin capillary blood flow. Thus, the results of previous autonomic nervous investigations in patients with pRP range from unaltered autonomic nervous activity to increased or impaired parasympathetic activity to increased sympathetic activity.

Similar to the HRV findings of the present study, assessment of the sBP overshoot after VM suggested intact sympathetic activity in the patient group as the results of both patient and control group resembled the results of healthy individuals previously studied (Vogel et al., 2005).

The [^{123}I]MIBG heart scintigraphy has proven to be a more sensitive marker of increased risk of cardiac events in heart failure patients than other applied measures, such as B-type natriuretic peptide and ejection fraction (Jacobson et al., 2010). The method makes it possible to visualize and quantify the sympathetic nerve activity of the heart. The patients of the current study had a significantly lower HMR_{late} than the control group. A late HMR of 1.60 is considered the lower normal limit, as ratios below this limit were found to be associated with an increased risk of arrhythmic events, cardiac death and all-cause mortality in heart failure according to the ADMIRE-HF study (Jacobson et al., 2010). The results of this multicentre study were based on the use of a low-energy/high-resolution collimator (LEHR), which has been shown to generate lower HMR values compared to the MEGP collimator used in the present study (Nakajima et al., 2017; Verschure et al., 2018). When applying the results from these two studies, investigating the significance of collimator choice, the HMR_{late} limit of 1.60 (LEHR) translates to an HMR_{late} limit of 1.95 when using a MEGP collimator. When applying the limit of 1.95 to the present data, three patients had an HMR_{late} below this limit (Figure 3). In contrast, none of the participants in the control group decreased below the limit. Although the HMR_{early} and the WOR (Figure 3) indicate that the difference between the groups in HMR_{late} may be due to differences in image analysis, the HMR_{late} results suggest that at least a small number of the patients with pRP are at increased risk of cardiac events and all-cause mortality, which could imply a number of clinical implications. However, this includes the assumption that the lower normal limit of HMR_{late} is similar for patients with and without known heart disease, as the limit concluded by Jacobson et al. was based on patients with heart failure, while none of the patients in the present study had a medical history of CVD. A low late HMR may be a sign of impaired cardiac adrenergic receptors as reported in Parkinson's disease (Borghammer et al., 2017) and diabetes (Freeman et al., 1997; Scott & Kench, 2004), but it may also reflect increased washout resulting from elevated sympathetic nervous activity as it is seen in heart failure (Jacobson et al., 2010). Whether the late HMR in three of the patients with pRP was decreased due to a hyperactivity of the sympathetic nervous system or an autonomic neuropathy could be suggested by the WOR, indicating the degree of the sympathetic drive (Verberne et al., 2008). However, the WOR did not differ significantly between the patient and the control group in this study, making further characterization of the autonomic activity difficult. The reduced HMR_{late} concurs with our hypothesis of increased sympathetic nervous activity in patients with pRP, although this was not confirmed by either HRV—especially, LF (n.u.) and LF/HF ratio—or the sBP after VM, which were both suggestive of a normal

cardiac autonomic control. Contrary to our hypothesis, p-NE showed a decreased response compared with the control group. The p-NE response cannot stand alone as a surrogate marker for autonomic dysfunction in the absence of a supportive clinical cardiovascular finding.

The study investigated the ANS from four different perspectives, exploring both parasympathetic and sympathetic pathways. The data set was largely complete. The MIBG data were the only proof of the presence of autonomic dysfunction of the heart in the present study. However, the extrapolated external control group limits the conclusion of the study. Also, the small sample size of the study is a limitation that may have affected the results of the WOR analysis. Small differences in protocols between the present study and the study describing the external control group (von Scholten et al., 2016) are considered to be of minor importance due to the use of the same collimator type (MEGP), which is highly influential on results (Inoue et al., 2003; Verberne et al., 2005), and the reporting in ratios. Interpretation of the HRV findings is limited by the fact that the measurements were not conducted under controlled breathing conditions. Vagal activity decreases with increasing breathing frequency, which means that respiration can affect the HF component as well as the LF/HF-ratio (Sanderson et al., 1996). The lack of controlled breathing in the present study could have blurred an actual difference in frequency-domain parameters between the groups.

The results of the current investigation indicate that autonomic nervous activity is altered in patients with pRP compared to healthy individuals. However, neither the present study nor previous studies have presented uniform conclusions as to whether the observed results favour the sympathetic or parasympathetic pathways or any at all.

The importance of the reported findings and their possible association with cardiovascular morbidity and death remain to be elucidated. Larger studies of the late HMR in patients with pRP should be conducted to confirm our findings, and these studies should preferably include a larger number of patients as well as age-, gender- and BMI-matched controls and an assessment of myocardial perfusion, which also could have influenced the results (von Scholten et al., 2016). Overall, investigations are needed to determine why patients with pRP, who are otherwise considered healthy individuals, are at increased cardiovascular risk. Until we know more about the potential consequences of the altered ANS activity, general practitioners and other relevant specialists might consider paying closer attention to cardiovascular symptoms in patients with pRP.

In conclusion, the study showed a significantly lower plasma catecholamine response to HUT and changes in the sympathetic activity of the heart in the resting condition in patients with pRP compared with healthy individuals. HRV and VM results were in agreement with a normal cardiac autonomic control. Although our findings support that the ANS is more pervasively affected by pRP than generally assumed, they do not uniformly concur with the hypothesis of increased sympathetic activity in patients with pRP. The importance of these findings and the possible association with previously found increased cardiovascular mortality must be subject to further investigations before conclusions can be drawn and recommendations can be made.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

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