

The transpulmonary ratio of endothelin I is elevated in patients with preserved left ventricular ejection fraction and combined pre- and post-capillary pulmonary hypertension

David F. Meoli¹, Yan Ru Su¹, Evan L. Brittain¹, Ivan M. Robbins², Anna R. Hemnes² and Ken Monahan¹

¹Division of Cardiovascular Medicine, Vanderbilt University Medical Center, Nashville, TN, USA; ²Division of Allergy, Pulmonary, and Critical Care Medicine, Vanderbilt University Medical Center, Nashville, TN, USA

Abstract

Pulmonary hypertension complicating left heart disease (PH-LHD) is associated with increased morbidity and mortality, especially in patients who develop combined pre- and post-capillary PH (Cpc-PH). Mechanisms underlying PH-LHD are incompletely understood, particularly for individuals with preserved left ventricular ejection fraction (LVEF). We hypothesized that transpulmonary concentrations of biomarkers representing signaling pathways with known effects on the pulmonary circulation could provide insight into the molecular etiology of PH-LHD in patients with preserved LVEF. Blood samples were collected from the pulmonary artery (PA) and wedge positions of outpatients with normal LVEF referred for right heart catheterization. Hemodynamic tracings were reviewed to classify patients as “no PH” (n = 23) or “PH-LHD” (n = 22). A biomarker’s transpulmonary ratio (TPR) was calculated as the quotient of wedge and PA concentrations. The TPR of endothelin I (ET-I) was elevated in Cpc-PH (n = 10) compared to no PH or isolated post-capillary PH (Ipc-PH, n = 12); cAMP and cGMP TPRs were not different among groups. Higher ET-I TPR in Cpc-PH was due to increased wedge ET-I concentration. Pulmonary vascular resistance (PVR) strongly correlated with wedge ET-I exclusively in Cpc-PH patients. In patients with normal LVEF and Cpc-PH, ET-I TPR is higher, due to elevated wedge ET-I, compared to those without PH or with Ipc-PH. Strong correlation between PVR and wedge ET-I, observed only in the Cpc-PH group, may suggest increased pulmonary vascular responsiveness to ET-I in these patients. These findings implicate elevated pulmonary ET-I as a marker of, and a potential contributor to, development of Cpc-PH in this population.

Keywords

biomarker, cardiopulmonary physiology and pathophysiology, endothelin, heart failure, hemodynamics

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Pulmonary hypertension due to left heart disease (PH-LHD) is defined as elevated mean pulmonary artery pressure (mPAP; ≥ 25 mmHg) in the setting of elevated left ventricular (LV) filling pressure (pulmonary artery wedge pressure [PAWP] > 15 mmHg).¹ PH-LHD occurs in the setting of heart failure with reduced or preserved left ventricular ejection fraction (LVEF) and/or left-sided valvular disease² and portends a worse prognosis compared to LHD with normal PA pressure.³ A subset of patients with PH-LHD develops increased pre-capillary pulmonary vascular resistance

(PVR) superimposed on elevated LV filling pressure, resulting in combined pre- and post-capillary PH (Cpc-PH).² Elucidating the pathophysiologic processes underlying this combined phenotype is important, as it may carry increased

Corresponding author:

Ken Monahan, Division of Cardiovascular Medicine, Vanderbilt University Medical Center, 1215 21st Avenue South – Medical Center East, 5th floor, Nashville, TN 37232, USA.

Email: ken.monahan@vanderbilt.edu



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morbidity and mortality compared to isolated post-capillary PH (Ipc-PH), yet has no targeted treatment with proven benefit.^{4–6}

In pulmonary arterial hypertension (PAH), dysregulation of the endothelin (ET), prostacyclin, and nitric oxide signaling pathways has a well-established pathophysiologic role; drugs that modulate these pathways are the mainstay of PAH treatment.⁴ In patients with heart failure with reduced LVEF (HF_{rEF}), there is evidence that impaired pulmonary release of cGMP, a secondary messenger downstream of nitric oxide and natriuretic peptides, plays a role in the development of pre-capillary PH.⁷ However, analogous mechanisms in patients with preserved LVEF are less well understood.

We hypothesized that measurement of the relative concentrations of biomarkers and mediators across the pulmonary circulation may provide insight into the molecular etiology of PH-LHD with preserved LVEF, including Cpc-PH. Enhanced understanding of the pathophysiologic processes underlying PH-LHD may enable development of novel therapies or personalized application of current medications and, ultimately, improve outcomes. We therefore conducted a study in patients with preserved LVEF comparing transpulmonary levels of putative biomarkers in patients with PH-LHD and no PH.

Methods

Participants

In this prospective, cross-sectional study, patients were eligible for enrollment if they were aged 18 years or older and had been referred for right heart catheterization (RHC) with or without left heart catheterization/coronary angiography as part of their usual clinical care. Exclusion criteria were LVEF \leq 40%, atrial fibrillation on the day of catheterization, significant anemia (hemoglobin $<$ 10 g/dL and hematocrit $<$ 30%), pregnancy, and treatment with PAH-specific medications or nitrates. The study was approved by the Vanderbilt University Institutional Review Board and all participants provided written informed consent.

Sample collection and processing

Swan-Ganz catheter placement in the proximal PA or wedge position was confirmed by pressure waveform analysis. A 5–10-cc blood sample was collected from each site with the minimal aspiration pressure necessary; samples were not sent for blood gas analysis. In patients who underwent fluid challenge, sample collection was repeated after normal saline administration (500 cc administered via peripheral IV over approximately 5 min) and acquisition of subsequent pressure measurements. Samples were immediately placed on ice and then hand-delivered to the Vanderbilt Core Laboratory for Cardiovascular Translational and Clinical Research. Plasma was separated

and stored in a -80°C freezer for later batched analysis as described below.

Hemodynamic classification

RHC pressure tracings (recorded at baseline, before fluid challenge, if performed) were reviewed independently by two authors (DFM and KM) for classification as precapillary PH (mPAP \geq 25 mmHg and pulmonary artery wedge pressure \leq 15 mmHg), PH-LHD (mean PA pressure \geq 25 mmHg and PAWP $>$ 15 mmHg) or no PH (mPAP $<$ 25 mmHg). PH-LHD was further subdivided into Cpc-PH (diastolic pressure gradient [DPG] \geq 7 mmHg and/or PVR $>$ 3 Wood units [WU]) and Ipc-PH (DPG $<$ 7 mmHg and PVR \leq 3 WU).⁴ All pressures were measured at end expiration.

Biomarker analysis

Patients were excluded from biomarker analysis if they had: precapillary PH (WHO group I, III, IV, or V); no PH, but co-existing acute cardiopulmonary disease discovered at or after catheterization (i.e. coronary disease and/or severe valvular disease); uninterpretable pressure tracings; or absence of wedge blood sample due to inability to obtain during catheterization. Plasma ET-1, cAMP, and cGMP concentrations were measured in the Vanderbilt Core Laboratory for Cardiovascular Translational and Clinical Research by ELISA (R&D Systems, Inc., Minneapolis, MN, USA; Cat# DET100, KGE002B, and KGE003, respectively) according to manufacturer's instructions. Each sample was run in duplicate. The ELISA plate was read on an Epoch microplate spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA) and data analysis was performed with Gen5 software (BioTek).

Statistical analysis

The transpulmonary ratio (TPR) of a biomarker was calculated as the quotient of its concentration in the wedge blood sample and its concentration in the PA sample. Statistical analysis was performed with Prism 7 (GraphPad Software, Inc., La Jolla, CA, USA). Box plots represent minimum, first quartile, median, third quartile, and maximum values. For continuous variables, normality of distribution was determined using the D'Agostino–Pearson test. For normally distributed variables, statistical significance was evaluated using two-tailed t-test with Welch's correction (for pairwise comparisons) or ANOVA followed by Tukey's test (for multiple comparisons). For non-normally distributed variables, statistical significance was evaluated by Wilcoxon–Mann–Whitney test (for pairwise comparisons) or Kruskal–Wallis test followed by Dunn's test (for multiple comparisons). The statistical significance of categorical variables was determined using a two-tailed Fisher's exact test. For scatter plots, Spearman correlation coefficients were

Table 1. Demographics and co-morbidities of participants with samples sent for biomarker analysis.

	No PH (n = 23)	PH-LHD (n = 22)	P value
Age (years)	61 ± 8	64 ± 8	0.19
Female (n (%))	12 (55)	14 (61)	0.77
BMI (kg/m ²)	27 ± 6	33 ± 8	0.015
LVEF	59 ± 5	61 ± 5	0.2
Hypertension (n (%))	8 (35)	18 (86)	<0.001
Diabetes (n (%))	7 (30)	10 (45)	0.37
Ischemic heart disease (n (%))	5 (22)	10 (45)	0.12
Chronic kidney disease (n (%))	11 (48)	8 (36)	0.55

The average BMI and prevalence of hypertension were higher in the group with PH-LHD than in the control group. For continuous variables, values represent mean ± SD.

PH, pulmonary hypertension; PH-LHD, pulmonary hypertension due to left heart disease; BMI, body mass index; LVEF, left ventricular ejection fraction.

calculated and statistical significance determined with two-tailed t-test. For all tests, a *P* value < 0.05 was considered statistically significant.

Results

The cohort consisted of 23 patients with no PH and 22 patients with PH-LHD. Patient demographic information and co-morbidities are shown in Table 1. LVEF was normal in both the no PH and PH-LHD groups. There was no significant difference in age or gender between the two groups. Consistent with previous reports,⁸ hypertension and obesity were more prevalent in the PH-LHD group than in the no PH group. RHC showed normal right atrial, pulmonary artery, and wedge pressures in the no PH group; as expected, all were elevated in the PH-LHD group (Fig. 1a).

To investigate potential molecular associations with PH-LHD, we calculated TPRs of mediators with known effects on the pulmonary vasculature (Fig. 1b). There was no statistically significant difference between the no PH and PH-LHD groups in the TPRs of ET-1, cAMP, or cGMP. However, the skewed distribution of ET-1 TPRs in the PH-LHD group suggested the presence of outliers with high TPR. Normality analysis and examination of individual patient TPRs revealed a non-Gaussian distribution in this group, suggestive of a discrete subset of patients with high ET-1 TPRs (Fig. 1c).

Postulating that patients with Cpc-PH have a distinct pathophysiology from those with Ipc-PH, we stratified the PH-LHD group based on DPG and PVR as recommended in recent guidelines.⁴ Clinical and hemodynamic characteristics of the two groups are shown in Table 2. There was no difference in heart rate, systolic or diastolic systemic blood pressure, right atrial pressure, PAWP, or cardiac index. The Cpc-PH group had higher mPAP and diastolic pulmonary artery pressure than the Ipc-PH group. As expected based

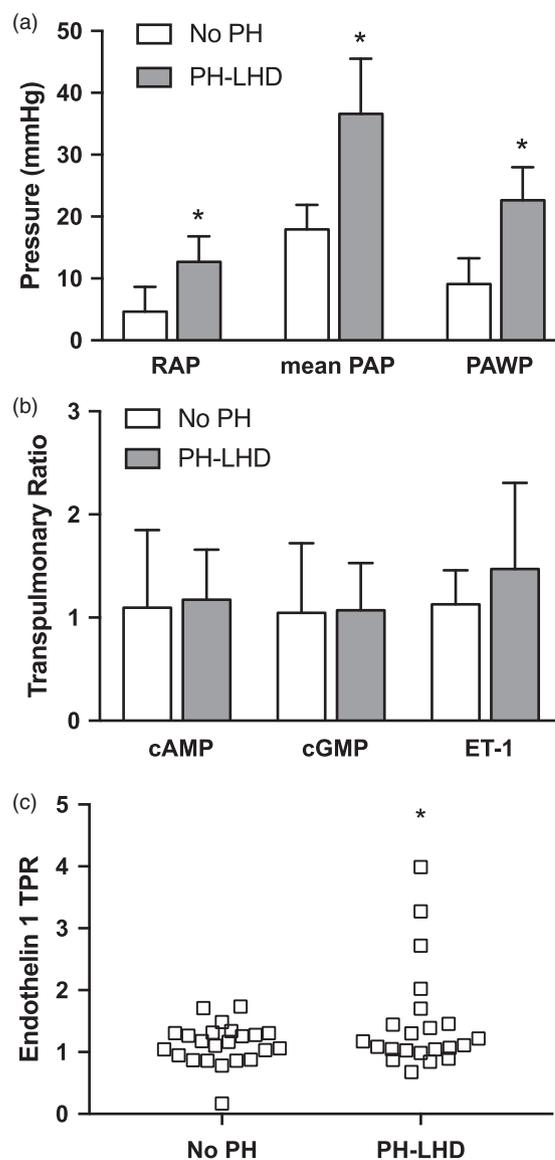


Fig. 1. A subset of patients with PH-LHD has elevated TPR of ET-1 compared to the rest of the group and individuals with no PH. (a) Hemodynamics from RHC showing higher right atrial pressure, mPAP, and wedge pressures in individuals with PH-LHD (*n* = 22) compared to those without PH (*n* = 23). **P* < 0.001 vs. no PH. (b) There was no difference in TPRs for any of the three biomarkers measured between the no PH and PH-LHD groups. (c) The distribution of TPR in the PH-LHD group is non-normally distributed due to outliers with high TPR. **P* < 0.001 for non-Gaussian distribution. RAP, right atrial pressure; mPAP, mean pulmonary artery pressure; PAWP, pulmonary artery wedge pressure; PH, pulmonary hypertension; PH-LHD, pulmonary hypertension due to left heart disease; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; ET-1, endothelin 1.

on the parameters used to define Cpc-PH, this group had higher PVR than the Ipc-PH group, and the latter included no patients with elevated DPG. A third measure of increased precapillary resistance, transpulmonary pressure gradient (TPG), was also higher in the Cpc-PH group.

Table 2. RHC hemodynamics in PH-LHD stratified by DPG and PVR.

	lpc-PH (n = 12)	Cpc-PH (n = 10)	P value
HR (bpm)	76 ± 21	66 ± 14	0.8
Systolic BP (mmHg)	129 ± 23	134 ± 21	0.84
Diastolic BP (mmHg)	70 ± 14	67 ± 13	0.92
RAP (mmHg)	11 ± 4	14 ± 4	0.12
mPAP (mmHg)	32 ± 5	42 ± 10	0.003
dPAP (mmHg)	22 ± 4	30 ± 6	0.003
PAWP (mmHg)	23 ± 5	23 ± 6	0.96
PVR (WU)	1.6 ± 0.4	4 ± 1.4	<0.001
PVR > 3 WU (n (%))	0 (0)	9 (90)	n/a
DPG ≥ 7 mmHg (n (%))	0 (0)	6 (60)	n/a
TPG (mmHg)	10 ± 3	19 ± 8	<0.001
CI (L/min/m ²)	3 ± 0.7	2.7 ± 0.7	0.35
Chronic HTN (n (%))	11 (85)	9 (90)	>0.99
Loop diuretic (n (%))	7 (58)	7 (70)	0.67
ACEI/ARB (n (%))	3 (25)	5 (50)	0.38

Diastolic pressure and mPAP were higher in the Cpc-PH group than the lpc-PH group, despite similar PAWP. Consistent with the definition of Cpc-PH used in this study, PVR and the prevalence of elevated DPG were higher in the Cpc-PH group. TPG, an additional measure of precapillary PH, was also significantly higher in Cpc-PH group. Values represent mean ± SD.

lpc-PH, isolated post-capillary pulmonary hypertension; Cpc-PH, combined pre- and post-capillary pulmonary hypertension; HR, heart rate; BP, blood pressure (systemic); bpm, beats per minute; RAP, right atrial pressure; mPAP, mean pulmonary artery pressure; dPAP, diastolic pulmonary artery pressure; PAWP, pulmonary artery wedge pressure; PVR, pulmonary vascular resistance; DPG, diastolic pressure gradient; TPG, transpulmonary pressure gradient; CI, cardiac index; HTN, hypertension; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

There was no difference between the two groups in clinical characteristics including prevalence of chronic hypertension, use of loop diuretics, or use of ACE inhibitors/angiotensin receptor blockers.

Patients with Cpc-PH had higher ET-1 TPR than those with no PH or with lpc-PH (Fig. 2a). There was no difference in the TPRs for cAMP or cGMP among the three groups (Fig. 2b). The ET-1 TPR was elevated because of higher wedge concentration rather than lower PA concentration (Fig. 2c), suggestive of either increased production or decreased clearance of ET-1 in the pulmonary circulation of patients with Cpc-PH. There was no difference in ET-1 TPR or wedge ET-1 between the no PH and lpc-PH groups. The finding of higher TPR and wedge ET-1 in Cpc-PH was durable across two alternative definitions of Cpc-PH: (1) TPG ≥ 12 mmHg; and (2) DPG ≥ 7 mmHg alone (Suppl. Fig. 1).

Next, we sought to determine whether elevated wedge ET-1 was related to LV filling pressure. While PAWP and wedge ET-1 correlated in the no PH group, there was no relationship between the two in either PH-LHD sub-group, suggesting that elevated wedge pressure alone does not drive ET-1 production or development of Cpc-PH (Fig. 3a).

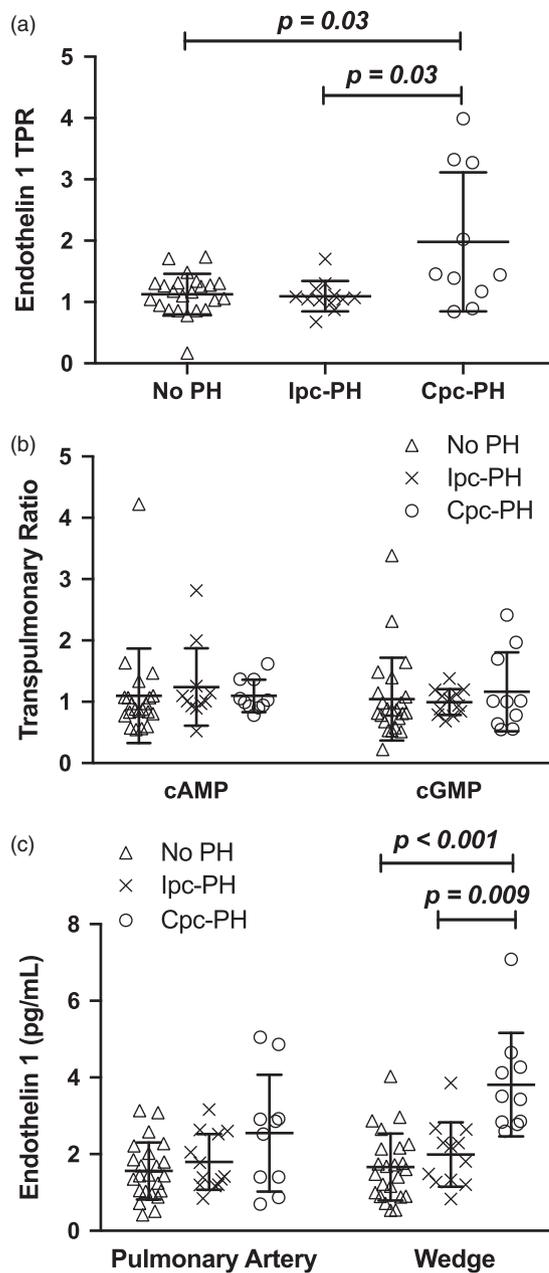


Fig. 2. The TPR of ET-1 is elevated in Cpc-PH due to high wedge ET-1. (a) ET-1 TPR was elevated in the Cpc-PH group (n = 10) compared to the lpc-PH (n = 12) and no PH (n = 23) groups. There was no difference in wedge ET-1 levels between the lpc-PH and no PH groups. (b) There was no difference in the TPRs of cAMP and cGMP among the three groups. (c) The elevated ET-1 TPR in Cpc-PH was due to higher wedge ET-1 rather than lower pulmonary artery ET-1. ET-1, endothelin I; PAWP, pulmonary artery wedge pressure; PH, pulmonary hypertension; lpc-PH, isolated post-capillary pulmonary hypertension; Cpc-PH, combined pre- and post-capillary pulmonary hypertension; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate.

To determine whether pulmonary ET-1 secretion could be stimulated by acute volume loading, we analyzed a subset of patients who had undergone fluid challenge during catheterization. In four individuals without PH and four with

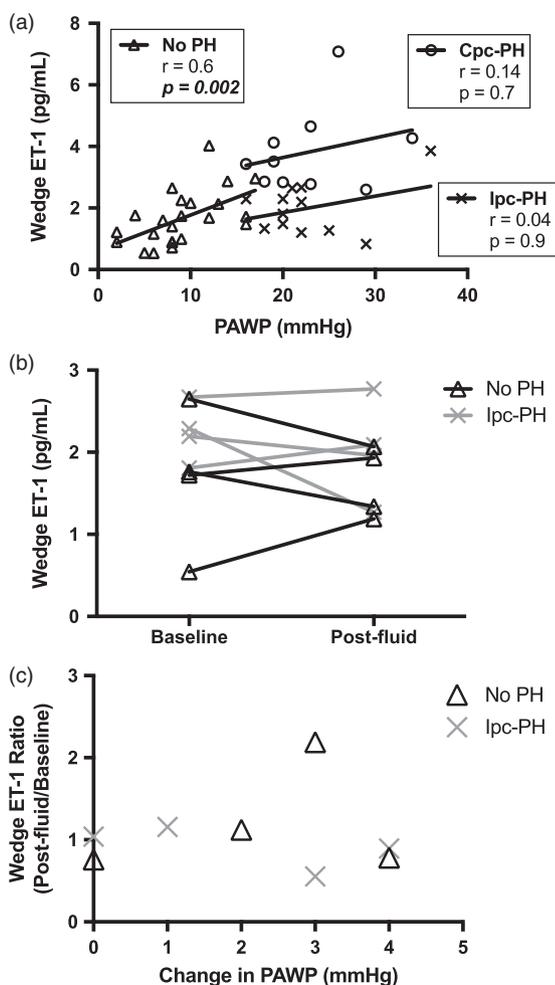


Fig. 3. Wedge ET-1 is neither correlated with PAWP in PH-LHD nor affected by acute fluid challenge in individuals with no PH or lpc-PH. (a) Scatter plot showing correlation between wedge ET-1 and PAWP in the no PH group, but not in either PH-LHD group. (b) There was no consistent change in wedge ET-1 after fluid challenge in four individuals with no PH and four with lpc-PH. (c) Scatter plot of the same participants depicted in (b) showing no relationship between the change in PAWP and the change in ET-1 in response to fluid challenge. ET-1, endothelin I; PH, pulmonary hypertension; PH-LHD, pulmonary hypertension due to left heart disease; PAWP, pulmonary artery wedge pressure; lpc-PH, isolated post-capillary pulmonary hypertension; Cpc-PH, combined pre-and post-capillary pulmonary hypertension.

lpc-PH there was no consistent difference in wedge ET-1 pre- and post-fluid bolus (Fig. 3a), regardless of the change in wedge pressure (Fig. 3b). These findings suggest that ET-1 secretion is not triggered by acute volume loading in these populations.

To investigate other potential determinants of ET-1 production, we compared wedge ET-1 concentration to systolic and diastolic systemic blood pressure, cardiac output, and cardiac index. As shown in Suppl. Fig. 2, the only significant correlation was between wedge ET-1 and systolic blood pressure in the Cpc-PH group. While it is possible that systolic hypertension promotes pulmonary ET-1 production in

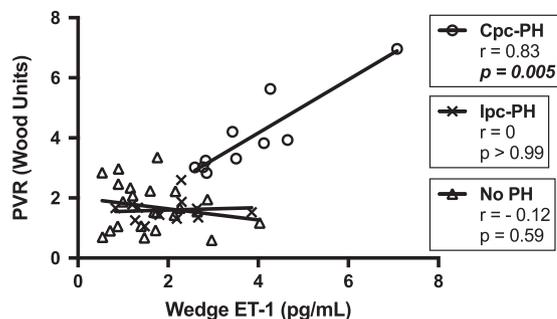


Fig. 4. Pulmonary ET-1 may contribute to increased PVR in patients with Cpc-PH. PVR was strongly correlated with wedge ET-1 in the Cpc-PH group. There was no correlation between PVR and wedge ET-1 in the lpc-PH and no PH groups. ET-1, endothelin I; Cpc-PH, combined pre- and post-capillary pulmonary hypertension; lpc-PH, isolated postcapillary pulmonary hypertension.

this group, it seems more biologically plausible that ET-1 is contributing to the determination of systolic blood pressure.

Finally, to assess whether elevated pulmonary ET-1 had a functional consequence, we compared PVR to the wedge ET-1 concentration in each study participant. In the Cpc-PH group, there was a strong, positive correlation, suggesting that ET-1 may contribute to elevated precapillary resistance in these patients (Fig. 4). There was no relationship between PVR and wedge ET-1 in the lpc-PH or no PH groups. As shown in Suppl. Fig. 3, there was no statistically significant correlation between PVR and ET-1 TPR, indicating that the absolute concentration of ET-1 to which the pulmonary circulation is exposed, rather than TPR, is likely the most physiologically relevant parameter.

Discussion

We have shown that in patients with preserved LVEF and Cpc-PH, wedge blood ET-1 concentration is elevated compared to patients with lpc-PH or without PH, potentially implicating ET-1 in the etiology of Cpc-PH. We have further shown that in patients with Cpc-PH, PVR strongly correlates with the ET-1 concentration in wedge blood and that wedge ET-1 concentration is not solely determined by LV filling pressures.

ET-1 has well-described functions in both systemic and pulmonary physiology, acting as a potent vasoconstrictor and smooth muscle cell mitogen.^{9–11} A role for ET-1 signaling in the pathophysiology of PAH is clearly established.^{12,13} Elevated blood levels of ET-1 and a decrease in PVR after acute administration of an ET receptor antagonist have been described in HFpEF.^{14–18} However, very little is known about the circulating concentration or pathophysiological effects of ET-1 in heart failure with preserved EF (HFpEF).¹⁹ In the one controlled clinical trial of ET receptor antagonism in HFpEF, sitaxsentan treatment resulted in increased treadmill time but was not associated with changes in echocardiographic or clinical outcomes.²⁰ Clinical trials

of ET receptor antagonists and other PAH medications in both HFrEF and HFpEF have been similarly neutral or even suggested detrimental effects.^{21–25}

Largely unaddressed in clinical trials, but increasingly appreciated, is the heterogeneous nature of PH-LHD. Although there is a growing body of data to suggest distinct pathophysiology in Cpc-PH patients,^{26,27} little is known about the specific molecular etiology of this newly-recognized phenotype. Measurement of transpulmonary biomarkers has the potential to help elucidate this molecular pathophysiology, refine HFpEF phenotypes, and identify treatments that may be effective in a given patient. Among the several studies that have evaluated transpulmonary ET levels, only one included a patient population potentially comparable to ours.²⁸ Of 27 patients included in that study, the five with elevated PAWP had no arteriovenous difference in ET immunoreactivity. Based on the available clinical information in that study as well as the early-generation ET assay used, it is challenging to compare directly that study to ours. A step-up in ET-1 concentration across the lungs, correlating with pulmonary pressures, has been demonstrated in PAH^{13,29} and HFrEF,^{30–32}; however those studies did not evaluate as a separate sub-group individuals that may have had Cpc-PH. Our study is unique among those done in patients with LHD in its focus on patients with preserved LVEF. In addition, our analysis of Cpc-PH and Ipc-PH as distinct phenotypes provides data that may implicate ET-1 in the pathogenesis of superimposed pre-capillary PH; this hypothesis could not have been generated in a study that grouped all PH-LHD patients together.

It is unknown whether development of superimposed pre-capillary PH is related to the magnitude or duration of elevation in left-sided filling pressures. The cross-sectional nature of our study does not allow careful examination of these determinants. However, the lack of difference in PAWP between the Cpc-PH and Ipc-PH groups may provide some evidence that neither development of Cpc-PH, nor elevation in wedge ET-1, is solely related to the magnitude of LV filling pressure elevation, consistent with prior findings.²⁷ Although data are available for only a small subset of the cohort, the lack of consistent change in ET-1 TPR after a fluid bolus in those with no PH or Ipc-PH raises the possibility that changes in transpulmonary ET levels cannot be induced rapidly with acute changes in volume status. Rather, our data suggest patient-intrinsic factors that result in increased ET-1 expression in response to elevated wedge pressure of some chronicity, coupled with increased pulmonary vasoreactivity to ET-1. Further study will be required to elucidate these mechanisms; possibilities include differences in pulmonary mechanosensing of increased post-capillary pressure, genetic or epigenetic variability in components of the ET-1 synthesis and post-translational processing pathways, and differences in absolute or relative expression of ET-A and ET-B receptors in the pulmonary circulation.³³

This study has several potential limitations. During sample collection, catheter positioning was evaluated only by waveform analysis; measuring oxygen saturation would

have allowed us to be more confident about the fidelity of sample acquisition, particularly from the wedge position. However, failure to achieve the wedge position would be expected to: (1) result in lower transpulmonary gradients in all patients; and (2) make identifying a difference between groups less likely. Importantly, this study does not allow us to definitively determine the anatomic source of ET-1 in the wedge samples. Given the relatively small volume of blood sampled in relation to the large capacity of the pulmonary circulation, it is likely we collected intrapulmonary, rather than left atrial, blood, perhaps even from the level of the resistance arteriole known to have a central role in the pathophysiology of PAH. The sample size was relatively small, especially the key sub-group of patients with Cpc-PH; confirmation of our findings in a larger population is needed. The clinical and diagnostic data needed to assess formally whether study patients had HFpEF were not uniformly available, although it is likely that many of the PH-LHD patients in this study qualify for that diagnosis.

Further, we evaluated only a focused panel of biomarkers chosen based on their known effects in the pulmonary circulation in PAH. However, the pathophysiology of PH-LHD is likely more complex than can be captured solely with the biomarkers evaluated in this study and an unbiased, larger-scale approach could lead to the identification of additional novel targets. As noted above, the study is also limited by its cross-sectional nature. Longitudinal data from serial RHCs would allow us to investigate the relative timing of PAWP elevation and rise in PAP. These sequential measurements would facilitate exploration of the hypothesis that elevated wedge pressure leads to elevated wedge ET-1 and subsequent elevation in PVR. A subset of patients in our cohort with Ipc-PH but elevated ET-1 TPR might represent the early stage of this progression. Ultimately, ET-1 TPR could be used to identify patients most likely to derive benefit in a clinical trial of ET receptor blockade.

In conclusion, this study highlights the potential of transpulmonary biomarkers to refine specific patient phenotypes in PH-LHD. Specifically, our findings suggest that Cpc-PH may occur, in part, due to exaggerated ET-1 production in response to elevated wedge pressure and increased responsiveness of the pulmonary vasculature to elevated ET-1. Therefore, this study lends support to the notion that Cpc-PH has a molecular pathophysiology distinct from Ipc-PH, potentially implicates ET-1 signaling in this pathophysiology, and may provide rationale for evaluation of ET receptor blockade in this selected population of patients.

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Conflict of interest

Ken Monahan received an investigator-initiated grant from Gilead Sciences for this study. Evan Brittain receives investigator-initiated funding from Gilead Sciences through the Gilead Sciences Research Scholars Program in Pulmonary Arterial Hypertension. Anna Hemnes serves as a consultant for United Therapeutics, Actelion Pharmaceuticals, Bayer, and GlaxoSmithKline.

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