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ORIGINAL ARTICLE

Inhaled nitric oxide testing in predicting prognosis in pulmonary hypertension due to left-sided heart diseases

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Abstract

Aims The pathophysiology of pulmonary hypertension (PH) due to left-sided heart disease (Group 2 PH) is distinct from that of other groups of PH, yet there are still no approved therapies that selectively target pulmonary circulation. The increase in pulmonary capillary pressure due to left-sided heart disease is a trigger event for physical and biological alterations of the pulmonary circulation, including the nitric oxide (NO)–soluble guanylate cyclase–cyclic guanosine monophosphate axis. This study investigated inhaled NO vasoreactivity tests for patients with Group 2 PH and hypothesized that these changes may have a prognostic impact.

Methods and results This was a single-centre, retrospective study with a median follow-up of 365 days. From January 2011 to December 2015, we studied 69 patients with Group 2 PH [age, 61.5 ± 13.0 (standard deviation) years; male:female, 49:20; left ventricular ejection fraction, 50.1 ± 20.4%; mean pulmonary arterial pressure, ≥25 mmHg; and pulmonary arterial wedge pressure (PAWP), >15 mmHg]. No adverse events were observed after NO inhalation. Thirty-four patients with Group 2 PH showed increased PAWP (ΔPAWP: 3.26 ± 2.22 mmHg), while the remaining 35 patients did not (ΔPAWP: −2.11 ± 2.29 mmHg). Multivariate analysis revealed that increased PAWP was the only significant predictor of all-cause death or hospitalization for heart failure (HF) after 1 year (hazard ratio 4.35; 95% confidence interval, 1.27–14.83; P = 0.019). The acute response of PAWP to NO differed between HF with preserved and reduced ejection fractions.

Conclusions Patients with Group 2 PH were tolerant of the inhaled NO test. NO-induced PAWP is a novel prognostic indicator.

Keywords Inhaled nitric oxide vasoreactivity tests; Pulmonary hypertension; Heart failure with preserved ejection fraction; Heart failure with reduced ejection fraction

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Introduction

The pathophysiology of pulmonary hypertension (PH) due to left-sided heart disease is distinct from that of other groups of PH. It is categorized as Group 2 PH by the World Health Organization and is associated with poor prognosis.[1] The increase in pulmonary arterial wedge pressure (PAWP) due to left-sided heart failure (HF) is a trigger of physical and biological alterations of the pulmonary circulation, including the nitric oxide (NO)–soluble guanylate cyclase (sGC)–cyclic guanosine monophosphate (cGMP) axis.[2] NO is an important regulator and mediator of numerous processes in the

biological systems. In cardiovascular function, NO relaxes smooth muscle by binding to the haem moiety of guanylate cyclase and increases intracellular levels of cGMP.[3] Tremendous process has been made in understanding the mechanisms of NO, leading to expanding clinical approach involved in the NO pathway. Examples include the use of isosorbide dinitrate for ischaemic heart diseases and systemic hypertension,[4] as well as sGC stimulators or phosphodiesterase inhibitors for PH.[5] The NO pathway has been explored as a primary target for developing new treatment strategies for cardiovascular diseases.

Recently, the VICTORIA trial, using an sGC stimulator, showed better clinical outcomes in patients with HF with reduced ejection fraction (HFrEF) and recent HF decompensation.[6] However, to date, most other clinical trials with sGC stimulators for patients with HF, particularly HF with preserved ejection fraction (HFpEF), have been disappointing,[7–9] likely due to their heterogeneous aetiologies, such as valvular disease, cardiomyopathies, ischaemic heart disease, or pulmonary arterial disease. These results suggest the need for the precise evaluation of pathophysiology and a novel investigation to distinguish the patients who may benefit from the therapies modulating the NO pathway.

The inhalation of NO is used for acute vasoreactivity testing in pulmonary arterial hypertension. A classic positive response is defined as a decrease of at least 10 mmHg in mean pulmonary arterial pressure (mPAP) to an absolute value of $<$40 mmHg with an increased or unchanged cardiac output (CO).[10] Guidelines recommend that acute vasoreactivity testing should be conducted to predict the effectiveness of calcium channel blockers.[1,11] However, most patients with PH do not meet the strict criteria, and the test is limited as an additional, optional test.

Mechanistically, the inhaled NO vasoreactivity test in catheterization should be useful in evaluating the function of the right and left ventricles in patients with HF, not only in patients with PH. Short-term inhalation of NO selectively reduces pulmonary arterial vascular resistance, and then inflow from the right ventricle to the left ventricle is increased, which may enable us to evaluate the reserve capacity of left ventricular (LV) function, such as the tolerance to exercise/haemodynamic stress or the responses of left ventricle to vasodilators. Therefore, the haemodynamic changes with the inhaled NO vasoreactivity test may provide additional information about cardiac function in patients with HF. However, there are no reports exploring the safety and clinical usefulness of the inhaled NO vasoreactivity test for patients with Group 2 PH. Thus, in this study, we investigated the haemodynamic changes associated with inhaled NO vasoreactivity tests in patients with Group 2 PH to elucidate their unmasked pathogenesis and hypothesized that these changes may have a prognostic impact.

Methods

Study design and participants

We retrospectively investigated 69 patients with PH due to LV disease, who underwent vasoreactivity testing with NO inhalation between January 2011 and December 2015. The diagnosis was established by echocardiography, computed tomography, spirometry, ventilation/perfusion lung scan, and right heart catheter examination, according to the 2015 European Society of Cardiology/European Respiratory Society guidelines,[10] including a resting mPAP measured through right cardiac catheterization $\geq$ 25 mmHg and PAWP $>$ 15 mmHg. Patients with significant valvular heart disease (moderate-to-severe stenosis and moderate-to-severe regurgitation) and heart transplant within 1 year were excluded. According to the above-mentioned guidelines,[10] we defined HFrEF as HF with left ventricular ejection fraction (LVEF) $<$ 50% and HFpEF as HF with LVEF $\geq$ 50%.

All patients were carefully treated with the latest HF treatment. Follow-up began on the day of the vasoreactivity test (right heart catheterization and NO inhalation) and was tracked through outpatient visits by cardiologists at Tohoku University Hospital or its satellite hospitals over a period of 1 year. All patient-level information was obtained via medical and administrative records to ensure that the HF episodes met the Framingham criteria. The study outcome was all-cause death or hospitalization due to HF. This study complied with the principles of the Declaration of Helsinki and was approved by the Medical Ethics Review Committee of Tohoku University Graduate School of Medicine. Also, as this is a retrospective study, we applied an opt-out method to obtain consent on this study from the patients by using the poster on the website approved by the Medical Ethics Review Committee (Approval No. 2021-1-684).

Right heart catheterization and nitric oxide inhalation

We performed a haemodynamic evaluation using right heart catheterization. A Swan-Ganz catheter (Edwards Life Science, Irvine, CA, USA) was inserted from the right, internal jugular vein and was placed in the pulmonary artery. Inhaled NO was consecutively performed as a part of a protocol for a diagnosis of PH or HF. Patients were excluded due to interstitial oedema on chest X-ray or physician's decision. Inhaled NO at 40 ppm for 10 min via a face mask was used to facilitate acute, pulmonary vasodilator testing.[12] Haemodynamic parameters measured before and during inhalation of NO were pulmonary arterial pressure, PAWP, right atrial pressure (RAP), heart rate (HR), CO, pulmonary vascular resistance (PVR), systemic vascular

resistance, mean aortic pressure (mAoP), and saturation of peripheral oxygen (SpO_2). All parameters were manually measured as mean of the waves and validated by different operators. CO was calculated using the thermodilution method, using the results of blood gas analysis and corrected for body surface area [cardiac index (CI)]. Echocardiography was performed 11 days (median: 0–89 days) before the NO vasoreactivity test at the Tohoku University Hospital. The following parameters were measured: ejection fraction, left ventricular end-diastolic diameter, left atrial diameter (LA), right atrial diameter, E/e', and trans-tricuspid pressure gradient.

Statistical analyses

All results are shown as the mean ± standard deviation. Comparisons of means between two groups with equal variances were performed using a two-tailed Student's *t*-test, while two groups with unequal variances were compared using the Mann–Whitney *U* test. Comparisons of mean responses associated with the two main effects of the different groups were performed using two-way analysis of variance (ANOVA) with interaction testing, followed by Tukey's honestly significant difference test for multiple comparisons. When comparing haemodynamic and other parameters between different groups of patients at rest and during inhaled NO, data were analysed using repeated measures, two-way ANOVA (if the number of patients in each group was the same) or a mixed-effects model analysis (if the number of patients in each group was different), as the data were taken over time from the same patient.

In order to examine the partial trends in the correlation between the NO inhalation-induced increase in PAWP and improvement in B-type natriuretic peptide (BNP) content, we applied 'piecewise linear regression models' more or less than delta 0 mmHg, as described previously.[13]

Univariate and multivariate analyses were performed to evaluate the association between the composite endpoint (death or hospitalization due to HF) and each haemodynamic change following the NO vasoreactivity test or basic characteristics.[14–16] Furthermore, we evaluated the independence of PAWP from PVR and BNP in the Cox model due to (i) the increase or decrease in PAWP, (ii) the decrease or increase in PVR in the vasoreactivity test, or (iii) the improvement in BNP level in the 1 year treatment as plausible candidates for prognostic factors in patients with Group 2 PH.

In survival analyses, we dichotomized haemodynamic changes following NO inhalation into increase or decrease (including 0) groups, regardless of how much they changed. We evaluated the association between the composite endpoint and haemodynamic changes following the NO vasoreactivity test or baseline characteristics. First, a univariate analysis of the patient characteristics or medication was performed (Supporting Information, *Table S1*). Next, we conducted Cox hazard analysis, including the following variables as potential confounders: age, sex, New York Heart Association (NYHA) functional class, worsening BNP during follow-up, E/e', increase in (or absence of) PAWP, PVR following NO test, and beta-blocker using backward selection (Supporting Information, *Table S2*).

A two-sided *P* value of <0.05 was considered statistically significant. Statistical significance was evaluated using GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA), R (Version 4.2.1; R Foundation for Statistical Computing, Vienna, Austria), or STATA 17 (College Station, TX, USA).

Results

Baseline patient characteristics

The baseline patient characteristics are shown in *Table 1*. The study population was characterized by a higher prevalence of males (71.0%). Regarding the cause of HF and cardiovascular risk, the prevalence of cardiomyopathy (58.0%) and hypertension (47.8%) was highest in these patients. Most of this population was not associated with connective tissue (2.89%) and pulmonary/deep venous thrombosis (0%). Patients were treated with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (over 70%), with beta-blockers (over 60%), and with mineralocorticoid receptor antagonists (approximately 50%). The echocardiography data showed mildly reduced systolic and diastolic performance, with mean LVEF of 50% and mean E/e' of 17. Spirometry showed relatively lower % vital capacity (78.0%). However, all patients did not receive any inhalant therapy. High age or the interval to spirometry after treatment for HF might have some influence on the results (*Table 2*). The catheterization data (*Table 2*) showed an elevated PAWP of 22 mmHg, mPAP of 33 mmHg, and a lower PVR of 212 dyne·s·cm^{-5}, suggesting that most of them at rest (baseline) were likely to be characterized by isolated post-capillary PH, rather than combined pre- and post-capillary PH.

Evaluation of haemodynamic and nitric oxide inhalation-induced alteration

After 10 min of inhaled NO, mAoP, HR, and SpO_2 were not altered (*Table 2*), and there were no adverse events, demonstrating its safety in patients with Group 2 PH.

Inhaled NO significantly decreased the PVR, mPAP, and RAP. However, none of the patients fulfilled the criteria of responders for the test, that is, a decrease of at least 10 mmHg

Table 1 Baseline characteristics of patients with Group 2 pulmonary hypertension (n = 69)

Age (years)	61.5 ± 13.0	Medications (n, %)	
Female (n, %)	20 (29.0)	Loop diuretics	50 (72.5)
BMI (kg/m^2)	24.2 ± 5.62	MRA	32 (46.4)
NYHA ≥ III (n, %)	32 (46.4)	ACE-I	30 (43.5)
eGFR (mL/min/1.73 m^2)	55.9 (39.9, 74.8)	ARB	20 (29.0)
BNP (pg/mL)	337.5 (111.2, 609.0)	Beta-blocker	46 (66.7)
HF aetiology (n, %)			
Moderate valvular heart disease	10 (14.5)	Echocardiography	
Ischaemic heart disease	9 (13.0)	LVEF (%)	50.1 ± 20.4
Hypertensive heart disease	10 (14.5)	LVDd (mm)	53.9 ± 12.0
Cardiomyopathy	40 (58.0)	LA (mm)	46.1 ± 8.9
		RA (mm)	42.4 ± 8.6
Previous history (n, %)		E/e'	17.2 ± 7.1
Hypertension	33 (47.8)	TR-PG (mmHg)	40.6 ± 15.0
Diabetes	14 (20.3)		
Dyslipidaemia	16 (23.2)	Spirometry	
Connective tissue disease	2 (2.89)	%VC	78.0 ± 14.9
Pulmonary/deep venous thrombosis	0 (0)	FEV1%	72.5 ± 13.8
		Inhalant therapy (n, %)	0 (0)

Continuous variables are expressed as mean ± standard deviation, except for eGFR and BNP, which are expressed as medians with inter-quartile ranges.
ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; BNP, brain natriuretic peptide; eGFR, estimated glomerular filtration rate; FEV1, forced expiratory volume in 1 s; HF, heart failure; LA, left atrial diameter; LVDd, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NYHA, New York Heart Association functional classification; RA, right atrial diameter; TR-PG, trans-tricuspid pressure gradient; VC, vital capacity.

Table 2 Haemodynamic changes at nitric oxide (NO) vasoreactivity test

	Rest	Inhaled NO	P value
mean AoP (mmHg)	89.3 ± 20.1	89.7 ± 19.7	0.776
HR (b.p.m.)	71.8 ± 15.4	71.5 ± 14.6	0.887
SpO_2 (%)	94.2 ± 6.5	94.3 ± 6.3	0.863
mean PAP (mmHg)	32.8 ± 6.9	31.0 ± 7.6	<0.0001
PVR (dyne·s·cm^{-5})	212.4 ± 126.0	148.4 ± 107.0	<0.0001
PAWP (mmHg)	22.6 ± 5.5	23.1 ± 6.4	0.193
RAP (mmHg)	9.8 ± 4.4	9.4 ± 4.5	<0.0001
CI (L/min/min^2)	2.5 ± 2.0	2.6 ± 1.8	0.107
SVR (dyne·s·cm^{-5})	1677.4 ± 678.8	1657.5 ± 705.8	0.310

Haemodynamics were measured following inhalation of 40 ppm NO for 10 min. Continuous variables are expressed as mean ± standard deviation. Comparison of parameters was performed using the Mann–Whitney U test.
AoP, aortic pressure; CI, cardiac index; HR, heart rate; PAP, pulmonary arterial pressure; PAWP, pulmonary arterial wedge pressure; PVR, pulmonary vascular resistance; RAP, right atrial pressure; SpO_2, saturation of peripheral oxygen; SVR, systemic vascular resistance.

in mPAP to an absolute value of <40 mmHg with an increased or unchanged CO. It is noteworthy that changes in PAWP following NO inhalation varied among patients (*Table 2*). In the comparison of mean values, the PAWP and CI were not altered during NO inhalation.

Comparison between patients with increased and decreased pulmonary arterial wedge pressure following nitric oxide vasoreactivity test

Thirty-four patients with Group 2 PH showed increased PAWP (ΔPAWP: 3.26 ± 2.22 mmHg), while the remaining 35 patients did not (ΔPAWP: −2.11 ± 2.29 mmHg). The patients showing increased PAWP at the time of the test were characterized as older, female, with a normal body mass index, compared with the decreased PAWP group. The increased PAWP group showed higher E/e', suggesting LV diastolic dysfunction (*Table 3*). At baseline haemodynamic, increased PAWP group showed relatively higher CI and lower mAoP, which were not significant (*Table 3*).

Patients with increased PAWP at the NO vasoreactivity test showed a trend towards an exacerbated BNP content at the 1 year follow-up, while the remaining 35 patients with decreased PAWP did not (increased group vs. decreased groups; R^2 = 0.347 vs. 0.00429, P < 0.001 vs. 0.7282, slope = 12.54 vs. 0.7988) (*Figure 1A*).

During the 1 year follow-up period, 6 patients with Group 2 PH died, and 14 patients were hospitalized due to HF. The Kaplan–Meier analysis of the composite endpoint (death and

Table 3 Characteristics of the patients showing an increase or decrease in pulmonary arterial wedge pressure (PAWP)

	Increased PAWP	Decreased PAWP	*P* value
Number	34	35	
Age (years)	65.5 ± 10.5	57.7 ± 14.1	0.022
Female (*n*, %)	10 (29.4)	10 (28.6)	>0.999
BMI (kg/m^2)	22.8 ± 3.5	25.5 ± 6.9	0.035
NYHA ≥ III (*n*, %)	20 (58.9)	12 (34.3)	0.055
eGFR (mL/min/1.73 m^2)	49.3 (40.7, 70.2)	62.8 (42.4, 75.1)	0.464
BNP (pg/mL)	292.1 (108.3, 554.8)	367.0 (117.6, 641.7)	0.539
Previous history (*n*, %)			
Hypertension	17 (50.0)	16 (45.7)	0.811
Diabetes	5 (14.7)	9 (25.7)	0.776
Dyslipidaemia	7 (20.6)	9 (25.7)	0.370
Echocardiography			
LVEF (%)	54.8 ± 18.1	45.6 ± 21.6	0.083
LVDd (mm)	51.4 ± 11.4	56.3 ± 12.1	0.023
LA (mm)	46.8 ± 8.4	45.4 ± 9.5	0.539
RA (mm)	41.6 ± 10.4	43.3 ± 5.8	0.301
E/e′	20.4 ± 7.9	14.1 ± 4.5	0.009
TR-PG (mmHg)	44.9 ± 14.7	36.7 ± 14.3	0.016
Haemodynamic			
mean AoP (mmHg)	84.9 ± 19.7	93.4 ± 19.8	0.0803
mean PAP (mmHg)	32.6 ± 6.7	32.9 ± 7.2	0.8427
PVR (dyne·s·cm^{-5})	224.2 ± 133.1	200.9 ± 119.5	0.6094
PAWP (mmHg)	21.9 ± 6.1	23.2 ± 4.9	0.0996
RAP (mmHg)	10.5 ± 4.7	9.1 ± 4.1	0.4708
CI (L/min/min^2)	2.9 ± 2.7	2.2 ± 0.6	0.0882
SVR (dyne·s·cm^{-5})	1740.2 ± 606.3	1612.7 ± 749.7	0.3695

Continuous variables are expressed as mean ± standard deviation, except for eGFR and BNP, which are expressed as medians with interquartile ranges. Comparison of parameters was performed using the Mann–Whitney *U* test.
AoP, aortic pressure; BMI, body mass index; BNP, brain natriuretic peptide; CI, cardiac index; eGFR, estimated glomerular filtration rate; LA, left atrial diameter; LVDd, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association functional classification; PAP, pulmonary arterial pressure; RA, right atrial diameter; RAP, right atrial pressure; SVR, systemic vascular resistance; TR-PG, trans-tricuspid pressure gradient.

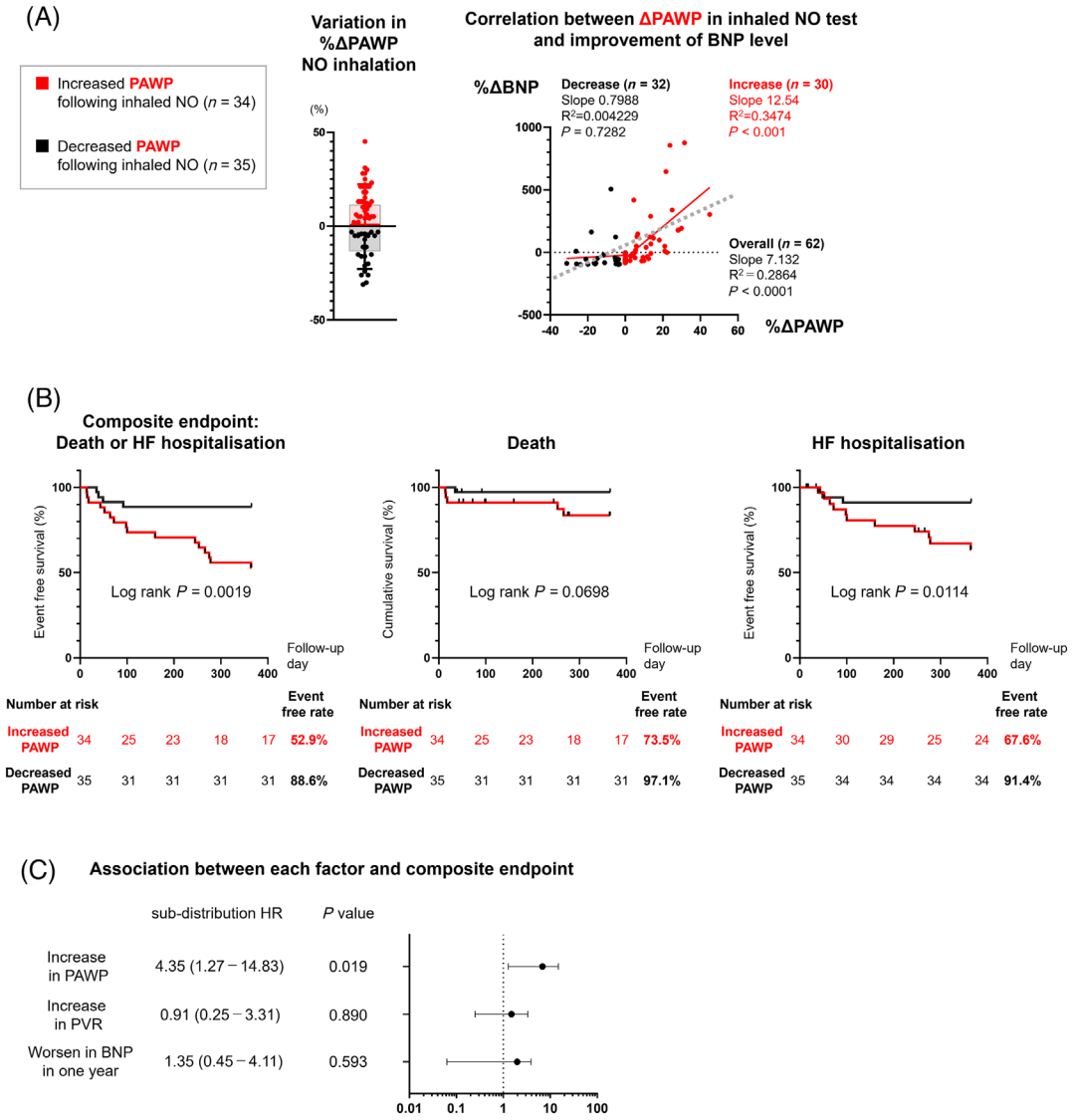
hospitalization due to HF) in patients with Group 2 PH demonstrated that event-free survival was substantially lower in the patients with increased PAWP following the NO vasoreactivity test, compared with the patients with decreased PAWP (*Figure 1B*). In the multivariate analysis of Model 1 including the variables age, sex, NYHA functional class, improvement rate of BNP, E/e′, increase or not in PAWP, PVR, and beta-blocker use as the potential confounders, increased PAWP following the NO vasoreactivity test was associated with the composite endpoint (hazard ratio 5.05; 95% confidence interval, 1.68–15.12; *P* = 0.004) (Supporting Information, *Table S2*). The correlated factor was unchanged even after adjusting for changes in PVR and BNP in Model 2 (Supporting Information, *Table S2*). Increased PAWP was the only variable that was selected as a parameter associated with composite events (hazard ratio 4.35; 95% confidence interval, 1.27–14.83; *P* = 0.019) (*Figure 1C*).

Subgroup analyses: heart failure with preserved and reduced ejection fractions

We then performed subgroup analysis to compare PH-HFpEF and PH-HFrEF. The baseline patient characteristics and haemodynamics are shown in *Tables 4* and *5*, respectively. The PH-HFpEF group was characterized by older age and a higher prevalence of females (*Table 4*). At baseline, PAWP was higher and LVEF was lower in patients with PH-HFrEF than in those with PH-HFpEF, whereas mPAP, PVR, and RAP were comparable in patients with PH-HFpEF and PH-HFrEF, indicating a similar severity of PH in both groups (*Tables 4* and *5*). Notably, following inhalation of NO, patients with PH-HFpEF showed an increase in PAWP, while a similar pattern was not observed in patients with PH-HFrEF (%change in PAWP: PH-HFpEF vs. PH-HFrEF: 9.1 ± 14.5% vs. −3.1 ± 15.0%, *P* = 0.0026) (*Table 5*), indicating less tolerance to NO in PH-HFpEF patients with Group 2 PH.

Consistent with the analyses of all patients with Group 2 PH, in both PH-HFpEF and PH-HFrEF patients, %ΔBNP level after 1 year was substantially correlated with %ΔPAWP in the NO vasoreactivity test (*Figure 2A*). Moreover, in the patients showing increased PAWP following NO vasoreactivity tests, the higher the PAWP, the higher the elevation in BNP at the 1 year follow-up in both PH-HFpEF and PH-HFrEF patients, compared with the patients showing no alteration or decrease in PAWP at the NO vasoreactivity test (*Figure 2A*).

Figure 1 Prognostic impact of increased pulmonary arterial wedge pressure (PAWP) following the nitric oxide (NO) inhalation test in patients with Group 2 pulmonary hypertension (PH). (A) Plasma brain natriuretic peptide (BNP) level in patients with Group 2 PH at the inhaled NO vasoreactivity test (n = 69) and at follow-up in the treatment of heart failure (HF) (overall: n = 62, increased PAWP group: n = 30, decreased PAWP group: n = 32). After consideration with piecewise linear regression model to evaluate the turning value of PAWP for partial slope, the point was set at 0 mmHg, which is clinically meaningful. (B) Kaplan–Meier curves for composite endpoint (death and HF hospitalization) in patients with Group 2 PH during follow-up. (C) Cox hazard analyses evaluated the correlation between composite endpoint (all-cause death and HF hospitalization) and changes in PAWP, pulmonary vascular resistance (PVR) following NO inhalation, or improvement in BNP after 1 year of treatment. In the analyses, we dichotomized haemodynamic changes following NO inhalation into increase or decrease (including 0) groups, regardless of how much they changed. %ΔPAWP = 100 × (PAWP following NO inhalation − PAWP at rest)/PAWP at rest. %ΔBNP = 100 × (BNP in 1 year − BNP at NO test)/BNP at NO test.



In the PH-HFpEF group, the patients with increased PAWP had higher E/e' (increased PAWP vs. decreased PAWP: 22.2 ± 8.5 vs. 14.5 ± 18.3, P = 0.029) than the patients with decreased PAWP, while the other parameters were comparable (*Table 6*). Similarly, the patients with PH-HFrEF with increased PAWP also showed an elevated trend of E/e' (increased PAWP vs. decreased PAWP: 17.8 ± 5.9 vs. 13.9 ± 3.8, P = 0.08).

During the 1 year follow-up period, three patients with PH-HFpEF and three patients with PH-HFrEF died. In addition, eight patients with HFpEF and six patients with PH-HFrEF were hospitalized due to HF. In both PH-HFpEF and PH-HFrEF, patients with increased PAWP showed a substantially higher incidence of all-cause death or hospitalization for HF than patients with decreased PAWP (*Figure 2B*).

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Table 4 Baseline characteristics of patients with pulmonary hypertension and heart failure with reduced and preserved ejection fractions (PH-HFrEF and PH-HFpEF, respectively)

	PH-HFpEF (n = 33)	PH-HFrEF (n = 36)	P value
Age (years)	65.6 ± 10.0	57.8 ± 14.3	0.017
Female (n, %)	13 (39.4)	7 (19.4)	0.110
BMI (kg/m^2)	23.2 ± 4.3	25.0 ± 6.6	0.318
NYHA ≥ III (n, %)	15 (45.5)	17 (47.2)	0.812
eGFR (mL/min/1.73 m^2)	56.2 (34.4, 78.9)	55.5 (46.7, 67.6)	0.927
BNP (pg/mL)	151.6 (78.7, 535.2)	376.5 (210.9, 630.9)	0.045
HF aetiology (n, %)			
Moderate valvular heart disease	5 (15.2)	5 (13.9)	>0.999
Ischaemic heart disease	5 (15.2)	4 (11.1)	0.728
Hypertensive heart disease	7 (21.2)	3 (8.3)	0.176
Cardiomyopathy	16 (48.5)	24 (66.7)	0.148
Previous history (n, %)			
Hypertension	19 (57.6)	14 (38.8)	0.151
Diabetes	6 (18.1)	8 (22.2)	0.769
Dyslipidaemia	6 (18.1)	10 (27.8)	0.402
Medications (n, %)			
Loop diuretics	22 (66.7)	28 (77.8)	0.419
MRA	13 (39.4)	19 (52.8)	0.33
ACE-I	12 (36.4)	18 (50.0)	0.336
ARB	10 (30.3)	10 (27.8)	>0.999
Beta-blocker	20 (60.6)	26 (72.2)	0.444
Echocardiography			
LVEF (%)	68.2 ± 8.8	33.5 ± 11.9	<0.0001
LVDd (mm)	46.2 ± 9.7	60.9 ± 9.3	<0.0001
LA (mm)	45.7 ± 9.7	46.5 ± 8.2	0.689
RA (mm)	41.9 ± 9.5	42.9 ± 7.6	0.289
E/e′	19.5 ± 8.7	15.4 ± 4.9	0.075
TR-PG (mmHg)	43.7 ± 17.3	38.0 ± 10.1	0.231

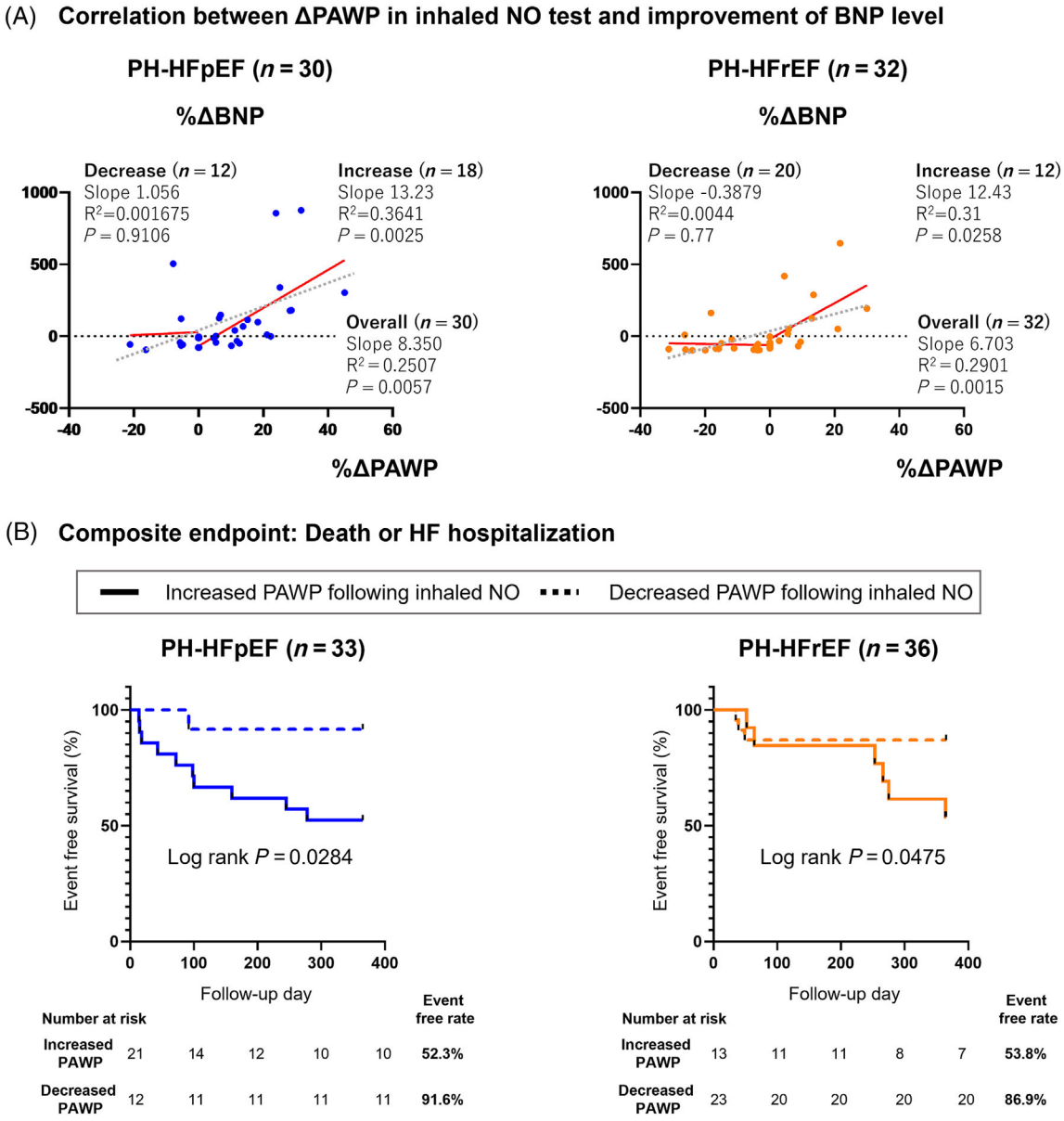
ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; BNP, brain natriuretic peptide; eGFR, estimated glomerular filtration rate; HF, heart failure; LA, left atrial diameter; LVDd, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NYHA, New York Heart Association functional classification; RA, right atrial diameter; TR-PG, trans-tricuspid pressure gradient.
Continuous variables are expressed as mean ± standard deviation, except for eGFR and BNP, which are expressed as medians with interquartile ranges. Comparison of parameters was performed using the Mann–Whitney U test.

Table 5 Haemodynamic change at nitric oxide (NO) vasoreactivity test in patients with pulmonary hypertension and heart failure with reduced and preserved ejection fractions (PH-HFrEF and PH-HFpEF, respectively)

	Rest	Inhaled NO	P value	Rest	Inhaled NO	P value	PH-HFpEF vs. PH-HFrEF Rest	PH-HFpEF vs. PH-HFrEF Inhaled NO
PH-HFpEF (n = 33)				PH-HFrEF (n = 36)				
mean AoP (mmHg)	88.7 ± 16.7	89.7 ± 17.5	0.972	89.8 ± 22.9	89.6 ± 21.8	0.999	0.996	0.999
HR (b.p.m.)	68.2 ± 15.5	68.3 ± 14.7	0.999	75.2 ± 14.9	74.5 ± 13.9	0.842	0.175	0.264
SpO_2 (%)	90.6 ± 17.9	90.6 ± 17.8	0.999	94.9 ± 5.4	95.0 ± 5.2	0.946	0.507	0.471
mean PAP (mmHg)	32.2 ± 6.6	31.2 ± 6.9	0.446	33.2 ± 7.2	30.7 ± 8.3	0.0002	0.945	0.981
PVR (dyne·s·cm^{-5})	227.5 ± 149.4	171.6 ± 134.8	0.001	198.6 ± 100.3	130.3 ± 77.5	0.0003	0.820	0.767
PAWP (mmHg)	20.2 ± 3.9	22.0 ± 5.2	0.013	24.7 ± 5.9	24.1 ± 7.3	0.657	0.010	0.459
RAP (mmHg)	10.5 ± 3.8	10.4 ± 3.3	0.967	9.2 ± 4.9	8.5 ± 5.2	0.026	0.539	0.201
CI (L/min/min^2)	3.1 ± 2.7	3.0 ± 2.5	0.864	2.1 ± 0.49	2.1 ± 0.49	0.281	0.116	0.194
SVR (dyne·s·cm^{-5})	1559.7 ± 649.7	1559.9 ± 682.2	0.881	1785.3 ± 695.9	1744.3 ± 724.5	0.907	0.543	0.521

AoP, aortic pressure; CI, cardiac index; HR, heart rate; PAP, pulmonary arterial pressure; PAWP, pulmonary arterial wedge pressure; PVR, pulmonary vascular resistance; RAP, right atrial pressure; SpO_2, peripheral oxygen saturation; SVR, systemic vascular resistance.
Continuous variables are expressed as mean ± standard deviation. The data were analysed using a mixed-effects model analysis, as the data were taken over time from the same patient.

Figure 2 Inhaled nitric oxide (NO)-induced increase in pulmonary arterial wedge pressure (PAWP) predicts poor clinical course in patients with pulmonary hypertension (PH) and heart failure with reduced and preserved ejection fractions (PH-HFrEF and PH-HFpEF, respectively). (A) Correlation between change rates in plasma brain natriuretic peptide (BNP) level after 1 year and PAWP following NO inhalation in patients with PH-HFpEF (n = 30) and PH-HFrEF (n = 32). After considering the piecewise linear regression model to evaluate the turning value of PAWP for the partial slope, the point was set at 0 mmHg, which is clinically meaningful. (B) Kaplan–Meier curves for the composite endpoint (death and hospitalization due to HF) in patients with PH-HFpEF (n = 33) and PH-HFrEF (n = 36). Three patients with PH-HFpEF and three patients with PH-HFrEF died. Eight patients with PH-HFpEF and six patients with PH-HFrEF were hospitalized for HF during the 1 year follow-up period. %ΔPAWP = 100 × (PAWP following NO inhalation − PAWP at rest)/PAWP at rest. %ΔBNP = 100 × (BNP in 1 year − BNP at NO test)/BNP at NO test.



Discussion

The major findings of this study regarding Group 2 PH were as follows: (i) The inhaled NO vasoreactivity test did not reduce mean blood pressure or cause any adverse events; (ii) increased PAWP was a significant predictor of all-cause death or hospitalization for HF; and (iii) the acute response of PAWP to the NO inhalation test was different between the PH-HFpEF and PH-HFrEF groups.

Table 6 Characteristics of patients with pulmonary hypertension and heart failure with reduced and preserved ejection fractions (PH-HFrEF and PH-HFpEF, respectively), showing an increase or decrease in pulmonary arterial wedge pressure (PAWP)

	Increased PAWP	Decreased PAWP	*P* value
Patients with PH-HFpEF (number)	21	12	
Age (years)	66.8 ± 9.9	63.5 ± 10.5	0.489
Female (*n*, %)	7 (35.0)	6 (50.0)	0.465
BMI (kg/m^2)	22.7 ± 3.9	24.0 ± 4.6	0.227
NYHA ≥ III (*n*, %)	10 (47.6)	4 (33.3)	0.152
eGFR (mL/min/1.73 m^2)	44.6 (34.4, 84.1)	64.2 (35.8, 76.3)	0.943
BNP (pg/mL)	189.7 (81.9, 599.4)	117.6 (73.7, 349.9)	0.369
Previous history (*n*, %)			
Hypertension	13 (61.9)	6 (50.0)	0.716
Diabetes	3 (14.3)	3 (25.0)	0.643
Dyslipidaemia	3 (14.3)	3 (25.0)	0.643
Echocardiography			
LVEF (%)	66.9 ± 7.9	70.3 ± 9.7	0.208
LVDd (mm)	46.8 ± 8.6	45.0 ± 10.9	0.915
LA (mm)	46.5 ± 8.7	43.9 ± 10.7	0.359
RA (mm)	41.7 ± 10.2	42.4 ± 7.5	0.822
E/e′	22.2 ± 8.5	14.5 ± 5.7	0.029
TR-PG (mmHg)	45.2 ± 16.0	41.1 ± 18.3	0.389
Patients with PH-HFrEF (number)	13	23	
Age (years)	63.5 ± 11.6	54.6 ± 14.9	0.842
Female (*n*, %)	3 (23.1)	4 (17.4)	>0.999
BMI (kg/m^2)	22.9 ± 2.8	26.2 ± 7.6	0.149
NYHA ≥ III (*n*, %)	9 (69.2)	8 (34.8)	0.134
eGFR (mL/min/1.73 m^2)	52.9 (45.0, 59.8)	58.6 (47.5, 71.2)	0.819
BNP (pg/mL)	315.3 (216.3, 382.1)	544.8 (238.3, 835.3)	0.249
Previous history (*n*, %)			
Hypertension	4 (30.8)	10 (43.5)	0.756
Diabetes	2 (15.4)	6 (26.1)	0.734
Dyslipidaemia	4 (30.8)	6 (26.1)	>0.999
Echocardiography			
LVEF (%)	35.0 ± 10.5	32.7 ± 12.8	0.739
LVDd (mm)	59.3 ± 11.3	61.7 ± 8.2	0.385
LA (mm)	47.2 ± 7.5	46.1 ± 8.8	0.562
RA (mm)	41.4 ± 10.8	43.9 ± 3.9	0.704
E/e′	17.8 ± 5.9	13.9 ± 3.8	0.088
TR-PG (mmHg)	41.2 ± 7.8	36.4 ± 10.9	0.137

BMI, body mass index; BNP, brain natriuretic peptide; eGFR, estimated glomerular filtration rate; LA, left atrial diameter; LVDd, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association functional classification; RA, right atrial diameter; TR-PG, trans-tricuspid pressure gradient.
Continuous variables are expressed as mean ± standard deviation, except for eGFR and BNP, which are expressed as medians with interquartile ranges. Comparison of parameters was performed using the Mann–Whitney *U* test.

The safety of nitric oxide inhalation vasoreactivity test in patients with Group 2 pulmonary hypertension

The European Society of Cardiology/European Respiratory Society guideline has not recommended acute vasoreactivity tests for patients with Groups 2–5 PH, assumingly due to the absence of evidence regarding its safety.[1,10] Possible adverse effects include rebound PH or pulmonary oedema in patients with Group 2 PH.[11]

Mechanistically, under normal or high oxygen concentrations, NO tends to convert to NO_2 or Hb-NO,[17] resulting in specific effects in the pulmonary arteries and minimal systemic effects. Moreover, inhaled NO has a short half-life (3 min) compared with other drugs for vasoreactivity tests, such as epoprostenol or sildenafil,[18] indicating relatively minimal stress for the patient. Despite the retrospective design, the present study of patients with Group 2 PH provided detailed data of the cardiopulmonary response to the inhaled NO test and demonstrated that there was no significant alteration in blood pressure, HR, SpO_2, or any other adverse event at the time of the test (*Table 2*). Further studies are warranted to determine whether this method is applicable to other types of PH.

Prognostic impact of increased pulmonary arterial wedge pressure in inhaled nitric oxide vasoreactivity test

There have been some reports about prognostic parameters in Group 2 PH, such as PVR, LA stiffness, and BNP

level, based on baseline data.[15,19,20] In recent years, increasing efforts have also been made to establish the reliable assessment of provocation testing in order to obtain additional diagnostic data. Originally, the NO vasoreactivity test was recommended for patients with Group 1 PH to evaluate pulmonary arterial function or to predict responsiveness to calcium blockers.[11] In the current study, the multivariable analysis demonstrated that increased PAWP following NO inhalation was a novel and significantly correlated factor of prognosis in patients with Group 2 PH (*Figure 1B,C*).

An abnormal response to the acute vasoreactivity test of NO inhalation could be related to (i) progression of disease in which some degree of vasoconstriction is impaired due to vascular remodelling, (ii) medical treatment with pulmonary vasodilators that may limit the remaining vascular dilatory action, and (iii) altered reserve capacity of cardiac function, which may become evident following the increased inflow from the right ventricle to the left ventricle.

In the present study, 86% of patients with Group 2 PH showed a decrease in PVR in the NO vasoreactivity test, indicating preserved responses of pulmonary arteries, and no patients were treated with medication that predominantly exerted pulmonary dilatory action. Indeed, alterations in PVR were not associated with prognosis. These findings may be related in part to the patient characterization of isolated post-capillary PH.

Therefore, we focused on alterations in the haemodynamics of the left side of the heart following NO inhalation. NO inhalation shifts the pressure from the right ventricle to the left ventricle via selective pulmonary arterial dilation, in which patients with potential or manifest LV dysfunction would be intolerant to the change, resulting in increased LV filling pressure, while preserved left ventricle can compensate (Supporting Information, *Figure S1*). Similarly, previous studies have demonstrated that an abnormal increase in PAWP after fluid loading or exercise stress is indicative of reduced functional capacity, associated with diastolic compliance or valvular heart disease.[2] Therefore, while the mechanisms underlying these stress tests may differ significantly, LV filling pressure following the inhaled NO test could potentially reflect the reserve capacity of the left ventricle. In fact, this study provides clear evidence of prognostic significance of increased PAWP in patients with Group 2 PH. The NO inhalation test has a clinical advantage in evaluating underlying functional impairment, particularly diastolic-related properties in both PH-HFpEF and PH-HFrEF. Previous studies have reported that patients with severe LV dysfunction, such as candidates for LV transplantation, show increased PAWP following the inhaled NO test, which is related to the clinical course after surgery.[21–23]

Clinical implication: the possibility of acute haemodynamic changes with nitric oxide inhalation to explore the application of nitric oxide signalling treatment

The assessment of NO-inhaled vasoreactivity can provide pivotal information, not only as a prognostic tool but also as a diagnostic tool for the application of NO-signalling treatment. The present study demonstrated that, following NO inhalation, patients with PH-HFpEF showed an increase in PAWP, while a similar pattern was not observed in patients with PH-HFrEF (*Table 5*). These findings may be related in part to the recent diverse results of clinical trials with sGC stimulators for HFrEF and HFpEF. The phase 3 VICTORIA trial found that vericiguat, an sGC stimulator, was effective in patients with HFrEF and recent HF decompensation. In a recent phase 2b study of the VITALITY-HFpEF, vericiguat did not improve the physical limitation score of the Kansas City Cardiomyopathy Questionnaire. These inconsistent outcomes suggest that treatment with NO–sGC–cGMP signalling may require patient selection in patients with Group 2 PH. This is important for patients with Group 2 PH because 49.2% of the enrolled patients in the present study showed an increase in PAWP with the NO vasoreactivity test. Therefore, the NO vasoreactivity test is expected to be like a 'Merk'Mal', which makes the administration of NO–sGC–cGMP treatment safer and more effective in clinical practice (Supporting Information, *Figure S1*).

Study limitations

The present study has several limitations. First, this was a single-centre, retrospective study with a relatively small number of patients, and additional subgroup analyses were difficult. Second, it should be noted that the follow-up period in our study was relatively short, spanning only 1 year, despite the well-known poor prognosis of Group 2 PH. Previous studies have demonstrated mortality rates of approximately 20% at 1 year and 40% at 3 years for this patient population.[15,24] Additionally, higher PVR values exceeding 3 Wood units have been associated with increased mortality rates of 30% at 1 year and 60% at 5 years.[15] Longer term follow-up would provide a more comprehensive understanding of the clinical course, including the response to medications. Third, the treatment strategy for HF depended on the physician's decision; the use of guideline-recommended therapies, including beta-blockers or mineralocorticoid receptor antagonist treatment, seemed not to be adequate.[1,25] Moreover, the present patients did not receive the current progressed treatments for HF, such as sodium glucose cotransporter 2-inhibitors or angiotensin receptor-neprilysin inhibitor, during the

follow-up period.[26,27] It is possible that these treatments may change the correlation between alterations following inhaled NO tests or clinical outcomes. Fourth, there is currently insufficient evidence regarding the mechanism by which the acute vasoreactivity test can predict future outcomes and assess the reserve capacity of cardiac function in patients with Group 2 PH. Combination assessment with other stress tests, such as exercise or fluid loading, may be beneficial in subjectively evaluating the significance or determining a cut-off value for the changes after NO inhalation. Further research is necessary to investigate this matter.

Conclusions

Patients with Group 2 PH are likely to tolerate the inhaled NO vasoreactivity test. Increased PAWP after NO inhalation indicates reserve capacity of cardiac function and is a novel and significantly correlated factor of prognosis in patients with Group 2 PH.

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Conflict of interest

Y.S. has received grant support from Takeda Pharmaceutical, Abbott, and Boston Scientific and lecture fees from Daiichi Sankyo and Bristol Myers Squibb.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1: Univariable analysis of patient characteristics or medication.
Table S2: Prognostic significance of PAWP in patients with Group 2 pulmonary hypertension (PH).
Figure S1: The haemodynamic changes following inhaled NO vasoreactivity test indicate reserve capacity and provide additional information of clinical course and the tolerance to NO-sGC-cGMP treatment of the patients with Group 2 PH. PAWP, pulmonary arterial wedge pressure; PVR, pulmonary vascular resistance; sGC, soluble guanylate cyclase; cGMP, cyclic guanosine monophosphate; PDE5, phosphodiesterase type 5.

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