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Original Article

Prognostic value of follow-up vasoreactivity test in pulmonary arterial hypertension☆

Satoshi Ishii (MD, PhD) [a], Masaru Hatano (MD, PhD, FJCC) [a,b,*], Hisataka Maki (MD, PhD) [a,c], Shun Minatsuki (MD, PhD) [a], Akihito Saito (MD, PhD) [a], Hiroki Yagi (MD, PhD) [a], Mai Shimbo (MD, PhD) [a,d], Katsura Soma (MD, PhD, FJCC) [a], Genri Numata (MD, PhD) [a], Takayuki Fujiwara (MD, PhD) [a,d], Norifumi Takeda (MD, PhD, FJCC) [a], Issei Komuro (MD, PhD, FJCC) [a]

[a] Department of Cardiovascular Medicine, The University of Tokyo Hospital, Tokyo, Japan
[b] Department of Advanced Medical Center for Heart Failure, The University of Tokyo Hospital, Tokyo, Japan
[c] Department of Cardiovascular Medicine, Jichi Medical University Saitama Medical Center, Saitama, Japan
[d] Department of Computational Diagnostic Radiology and Preventive Medicine, The University of Tokyo Hospital, Tokyo, Japan

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ABSTRACT

Background: Acute vasoreactivity test with inhaled nitric oxide (NO) is performed during diagnostic right heart catheterization (RHC) to identify patients with pulmonary arterial hypertension (PAH) who respond to calcium channel blockers. Our purpose was to investigate the prognostic importance of follow-up vasoreactivity test after treatment.
Methods: We retrospectively analyzed 36 PAH patients (mean age, 47 years; 61 % treatment-naïve), who underwent diagnostic and follow-up RHC and vasoreactivity tests at our center. The primary outcome was all-cause mortality.
Results: The median time between baseline and follow-up RHC was 9.7 months. Absolute change in mean pulmonary arterial pressure (ΔmPAP) during NO challenge was less pronounced after treatment, but there was great variability among patients. Overall cohort was dichotomized into two groups: preserved vasoreactivity (ΔmPAP ≤ −1 mmHg) and less vasoreactivity (ΔmPAP ≥0 mmHg) at follow-up RHC. Less vasoreactivity group had higher usage rate of endothelin receptor antagonists and parenteral prostacyclin analogues. During a median observation period of 6.3 years after follow-up RHC, 7 patients died, of which 6 showed less vasoreactivity at follow-up. Absolute ΔmPAP ≥0 at follow-up RHC was associated with all-cause mortality in univariable Cox regression analysis (hazard ratio, 8.728; 95 % confidence interval, 1.045–72.887; $p = 0.045$), whereas other hemodynamic parameters were not. Absolute ΔmPAP ≥0 at follow-up RHC was associated with all-cause mortality in multivariable Cox analysis adjusted for age and known PAH prognostic factors (HR, 12.814; 95 % CI, 1.088–150.891; $p = 0.043$). Kaplan-Meier survival analysis revealed a significantly worse survival of less vasoreactivity group compared to preserved vasoreactivity group (log-rank test, $p = 0.016$).
Conclusions: Follow-up vasoreactivity test after treatment could contribute to the detection of high-risk subgroups who might need careful monitoring and referral for lung transplantation.

Introduction

Pulmonary arterial hypertension (PAH) is a disease characterized by increased pulmonary arterial pressure and vascular resistance due to vasoconstriction and remodeling of the pulmonary arterioles, eventually leading to severe right heart and respiratory failure, and death [1–3]. Invasive hemodynamic assessment by right heart catheterization (RHC) is required for the definitive diagnosis and is useful for the judgement of treatment effectiveness during follow up [4]. Acute vasoreactivity test is performed during the diagnostic RHC to identify a subset of PAH patients who may exhibit a favorable long-term response to high-dose calcium channel blockers [4–6]. Inhaled nitric oxide (NO) is preferred for vasoreactivity testing, and intravenous epoprostenol, intravenous adenosine, and inhaled iloprost are also acceptable alternatives [4]. A positive acute vasodilator response is currently defined as a drop in mean pulmonary arterial pressure (mPAP) by ≥10 mmHg leading to mPAP ≤40 mmHg with increased or unchanged cardiac output; however, the positive acute response during the first diagnostic RHC is found in only about 10 % of idiopathic PAH (IPAH) patients [4].

Given that NO is the most potent endogenous vasodilator and the bioactivity could be altered in various pathophysiological conditions

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* Corresponding author at: Department of Cardiovascular Medicine, The University of Tokyo Hospital, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.
E-mail address: hatanoma@pg8.so-net.ne.jp (M. Hatano).

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[7,8], the pulmonary vascular response to inhaled NO may change over time with disease progression and/or in the treatment condition. Recently, Tooba et al. showed the pulmonary vascular reactivity decreased over time in PAH cohorts including non-idiopathic etiology [9]; however, there was great variability among patients and the prognostic importance of acute vasoreactivity test with inhaled NO achieved after PAH-specific treatment has not been established. Therefore, we sought to retrospectively investigate the prognostic value of follow-up vasoreactivity test after treatment by analyzing 36 PAH patients, who underwent diagnostic and follow-up RHC and vasoreactivity tests at our referral center.

Materials and methods

Study patients and data collection

We conducted a retrospective cohort study using the records of consecutive 39 PAH patients, who underwent at least two vasoreactivity tests with inhaled NO (>2 months apart) at the University of Tokyo Hospital between March 2010 and November 2020 (Fig. 1). Of 91 PAH patients who underwent vasoreactivity test with inhaled NO during the period, 52 patients with single vasoreactivity testing were excluded. All patients were diagnosed as PAH with mPAP ≥25 mmHg and pulmonary arterial wedge pressure ≤ 15 mmHg [4]. As previous reports [9,10], we included patients who were diagnosed with PAH and already received single or combination therapy with calcium channel blockers, parenteral or oral prostacyclin analogues, endothelin receptor antagonists, phosphodiesterase type V inhibitors, or immunosuppressive therapy for at least two weeks before referral to our center. Among 39 patients, we excluded 3 patients with uncorrected congenital heart disease, who received corrective surgery during the observation period, and the remaining 36 patients were included in this study. In current guidelines, acute vasoreactivity testing is only recommended in idiopathic and heritable PAH (I/HPAH) in terms of detecting calcium channel blocker responders [4]. However, because our objective is to elucidate the change and prognostic value of pulmonary vascular response to inhaled NO after PAH treatment rather than to investigate calcium channel blocker responsiveness, we included PAH with etiologies other than I/HPAH, as previous reports [9,10]. The present study was performed according to the ethical guidelines of the University of Tokyo (approval by the Ethical Committee of the University of Tokyo: No. 2650) and in accordance with the Declaration of Helsinki. Due to the nature of the retrospective study, written informed consent was waived.

Right heart catheterization and acute vasoreactivity test with inhaled NO

Hemodynamic parameters were measured in the cardiac catheterization laboratory during RHC. Cardiac output was measured by the thermodilution method. Baseline RHC and vasoreactivity testing were scheduled at the referral to our hospital, and the follow-up timing to re-evaluate the vasodilative reserve in pulmonary circulation after initiation or intensification of therapy was at the attending physicians' discretion. Oral vasodilators for PAH were interrupted 12 h before RHC. After the routine RHC measurement, patients were administered with NO delivered using a respiratory synchronizer (Sansoserver, TEIJIN LIMITED, Tokyo, Japan) at a concentration of 20 ppm continuously for 10 min through a tight-fitting facial mask, and subsequently hemodynamic parameters after NO test were recorded.

Follow-up and outcome

After the second RHC, patients were followed up through July 31, 2021. The primary outcome was all-cause mortality.

Statistical analysis

All statistical analyses were performed using SPSS statistics 19 (SPSS Inc., Chicago, IL, USA). All data are expressed as mean ± standard deviation or median (interquartile range) unless otherwise specified. Continuous and categorical variables between groups were compared using unpaired *t*-test and Fisher's exact-test, respectively. Differences between baseline and follow-up RHC and changes in parameters during NO challenge were compared with paired samples t-test. Continuous variables among multiple groups were compared using one-way ANOVA. Patients were dichotomized into two groups at the median value of absolute difference in mPAP between before and after NO challenge at follow-up RHC (ΔmPAP): preserved vasoreactivity group (ΔmPAP ≤ −1 mmHg) and less vasoreactivity group (ΔmPAP ≥0 mmHg). Univariable Cox proportional hazards analysis was used to assess factors associated with all-cause mortality. In multivariable Cox proportional hazards analysis, known prognostic factors in PAH were included [4]. Kaplan-Meier analysis was performed to assess the differences in survival among patients according to the dichotomous classification of variables, and the log-rank test was used to compare the distribution of survival. For all analyses, p-value <0.05 was considered statistically significant.

Results

Patient characteristics and hemodynamic parameters at baseline and follow-up RHC

Patient characteristics and hemodynamic parameters at baseline and follow-up RHC are shown in Table 1. The 36 patients included 23 women (63.4 %), I/HPAH in 11 (30.6 %), PAH related to congenital heart disease (CHD-PAH) in 6 (16.7 %), and PAH related to connective tissue disease (CTD-PAH) in 15 (41.6 %) including 3 patients with

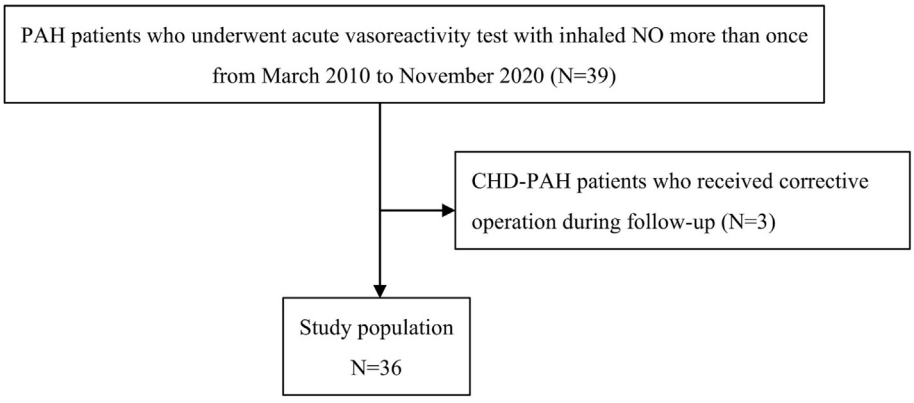


Fig. 1. Flowchart of the patients included in the study.
CHD-PAH, congenital heart disease associated pulmonary arterial hypertension; NO, nitric oxide; PAH, pulmonary arterial hypertension.

Table 1
Patient characteristics at baseline and follow-up RHC.

Variable	Overall cohort ($n = 36$)		P value
	Baseline RHC	Follow-up RHC	
	Mean ± SD, n (%)	Mean ± SD, n (%)	
Age (years)	46.9 ± 14.8	47.9 ± 14.6	
Sex (female)	23 (63.9 %)		
Types of PAH, n (%)			
I/HPAH	11 (30.6 %)		
CHD-PAH	6 (16.7 %)		
CTD-PAH	15 (41.6 %)		
SSc	3 (8.3 %)		
Non-SSc	12 (33.3 %)		
PoPH	3 (8.3 %)		
Drug-induced	1 (2.8 %)		
Medication management, n (%)			
Ca channel blockers	4 (11.1 %)	8 (22.2 %)	0.172
Parenteral prostacyclin analogues	2 (5.6 %)	5 (13.9 %)	0.214
Oral prostacyclin analogues	11 (30.6 %)	17 (47.2 %)	0.113
ERA	11 (30.6 %)	28 (77.8 %)	<0.001
PDE-V inhibitors	11 (30.6 %)	24 (66.7 %)	0.002
sGC stimulator	0	3 (8.3 %)	0.120
Immunosuppressive therapy	8 (22.2 %)	9 (25.0 %)	0.500
Medication number, n (%)			<0.001
0 or 1	22 (61.1 %)	6 (16.7 %)	
2 or more	14 (38.9 %)	30 (83.3 %)	
Hemodynamic parameters			
HR (bpm)	73.5 ± 12.2	70.3 ± 11.6	0.092
mRAP (mmHg)	6.3 ± 3.1	5.7 ± 2.5	0.286
mPAP (mmHg)	45.1 ± 12.6	35.9 ± 11.0	<0.001
mPAWP (mmHg)	8.4 ± 3.3	9.1 ± 3.0	0.221
Cardiac index (L/min/m^2)	2.69 ± 0.64	3.01 ± 0.70	0.004
PVR (dynes.sec.cm^{-5})	756.2 ± 478.7	464.4 ± 230.7	0.001

bpm, beat per minutes; CTD-PAH, connective tissue disease-associated pulmonary arterial hypertension; CHD-PAH, congenital heart disease-associated pulmonary arterial hypertension; ERA, endothelin receptor antagonist; HR, heart rate; I/HPAH, idiopathic/heritable pulmonary arterial hypertension; mPAP, mean pulmonary arterial pressure; mPCWP, mean pulmonary arterial wedge pressure; mRAP, mean right atrial pressure; PAH, pulmonary arterial hypertension; PDE-V, phosphodiesterase type V; PoPH, portopulmonary hypertension; PVR, pulmonary vascular resistance; RHC, right heart catheterization; sGC, soluble guanylate cyclase; SSc, systemic sclerosis.

systemic sclerosis. At baseline RHC, the mean age was 46.9 ± 14.8 years old, and 22 (61.1 %) were treatment-naïve, whereas 14 (38.9 %) had already received medical treatment for PAH. Hemodynamic parameters were compatible with pre-capillary pulmonary hypertension [mPAP, 45.1 ± 12.6 mmHg; mean pulmonary arterial wedge pressure, 8.4 ± 3.3 mmHg; pulmonary vascular resistance (PVR), 756.2 ± 478.7 dynes. Sec.cm^{-5}; cardiac index (CI), 2.69 ± 0.64 L/min/m^2].

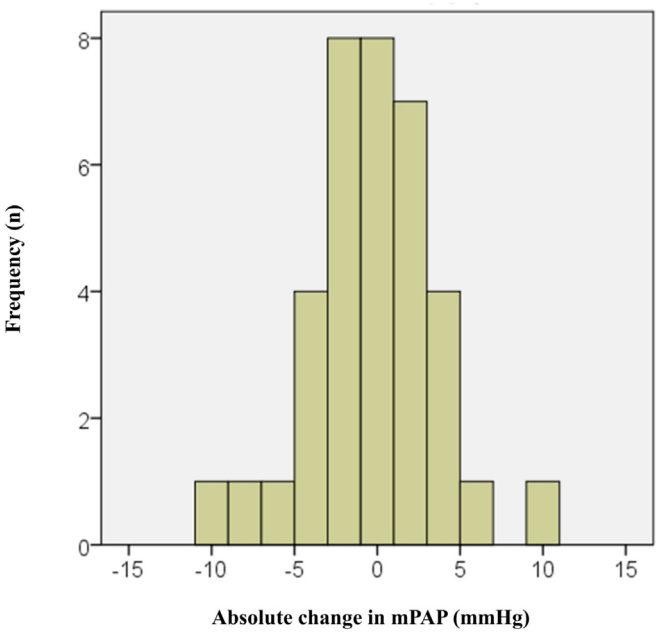


Fig. 2. Histogram on the absolute change in mPAP during inhaled NO challenge at follow-up RHC.
mPAP, mean pulmonary arterial pressure; NO, nitric oxide; RHC, right heart catheterization.

The median time between baseline and follow-up RHC was 9.7 (interquartile range, 5.6–16.6) months. At follow-up RHC after initiation or intensification of therapy, all patients received at least one PAH-specific medication, and the hemodynamic parameters showed significant improvements in mPAP, PVR, and CI (mPAP, 35.9 ± 11.0 mmHg; mean pulmonary arterial wedge pressure, 9.1 ± 3.0 mmHg; PVR, 464.4 ± 230.7 dynes. Sec.cm^{-5}; CI, 3.01 ± 0.70 L/min/m^2).

Hemodynamic changes during NO challenge at baseline and follow-up RHC

Table 2 shows changes in hemodynamic parameters during inhaled NO challenge at baseline and follow-up RHC. At baseline RHC, inhaled

Table 2
Changes in hemodynamic parameters during inhaled NO challenge at baseline and follow-up RHC.

Variable	Before NO challenge	During NO challenge	*p*-Value
	Mean ± SD	Mean ± SD	
Baseline RHC			
mPAP (mmHg)	45.1 ± 12.6	41.9 ± 13.4	0.006
mPAWP (mmHg)	8.4 ± 3.3	9.4 ± 3.9	0.007
Cardiac index (L/min/m^2)	2.69 ± 0.64	2.71 ± 0.69	0.667
PVR (dynes.sec.cm^{-5})	756.2 ± 478.7	675.5 ± 501.8	0.004
SpO_2 (%)	94.5 ± 2.9	93.2 ± 3.5	0.002
Follow-up RHC			
mPAP (mmHg)	35.9 ± 11.0	35.1 ± 12.0	0.215
mPAWP (mmHg)	9.1 ± 3.0	10.1 ± 3.9	0.011
Cardiac index (L/min/m^2)	3.01 ± 0.70	3.05 ± 0.66	0.400
PVR (dynes.sec.cm^{-5})	464.4 ± 230.7	414.8 ± 221.5	0.004
SpO_2 (%)	95.0 ± 2.8	93.6 ± 3.6	<0.001
Variable	Baseline RHC	Follow-up RHC	*p*-Value
	Mean ± SD	Mean ± SD	
Absolute ΔmPAP (mmHg)	−3.2 ± 6.6	−0.81 ± 3.8	0.009
Percentage ΔPVR (%)	−12.1 ± 19.9	−9.5 ± 19.0	0.402

mPAP, mean pulmonary arterial pressure; mPCWP, mean pulmonary arterial wedge pressure; NO, nitric oxide; PVR, pulmonary vascular resistance; RHC, right heart catheterization; Δ, change during NO challenge; SpO_2, oxygen saturation of peripheral artery.

NO challenge significantly decreased mPAP and PVR without a decrease in CI in overall cohorts, and 3 patients (8.3 %) fulfilled the positive acute pulmonary vasoreactivity criteria defined as a drop in mPAP by ≥10 mmHg leading to mPAP ≤40 mmHg with increased or unchanged cardiac output. Inhaled NO challenge at follow-up RHC significantly further improved PVR without a decline in CI, whereas mPAP did not change in overall cohorts; percentage changes in PVR (ΔPVR) during NO challenge at baseline and follow-up RHC did not show significant difference (decrease by −12.1 ± 19.9 % vs. -9.5 ± 19.0 %; $p = 0.402$), but absolute ΔmPAP at follow-up NO challenge was less pronounced than at baseline (absolute ΔmPAP, −3.2 ± 6.6 mmHg vs. -0.81 ± 3.8 mmHg; $p = 0.009$). Only one patient (2.8 %) showed positive acute vasoreactivity at follow-up RHC; however, the histogram on absolute ΔmPAP showed the wide distribution including both ΔmPAP-positive and ΔmPAP-negative patients (Fig. 2), suggesting that patients might be further stratified according to their absolute ΔmPA*P* values after PAH treatment.

There was no significant difference in the mean value of absolute ΔmPAP at follow-up NO challenge between PAH subgroups (IPAH/HPAPH, 0.0 ± 3.3 mmHg; CHD-PAH, −0.7 ± 3.5 mmHg; CTD-PAH, −2.1 ± 4.5 mmHg; portopulmonary hypertension, 1.3 ± 1.5 mmHg; $p = 0.482$).

Table 3
Patient characteristics stratified by vasoreactivity to inhaled NO at follow-up RHC.

	Preserved vasoreactivity	Less vasoreactivity	*p*-Value
Patient number, n	20	16	
Interval between two RHCs (months)	12.6 ± 9.2	10.9 ± 7.2	0.538
Observation period after follow-up RHC (years)	6.2 ± 2.1	5.3 ± 2.7	0.290
Age (years)	46.6 ± 14.2	49.5 ± 15.4	0.554
Sex (female), n (%)	14 (70 %)	9 (39.1 %)	0.493
PAH etiology, n (%)			0.034
I/HPAH	3 (15 %)	8 (50 %)	
PAH with other etiology	17 (85 %)	8 (50 %)	
Medication management, n (%)			
Ca channel blockers	4 (20 %)	4 (50 %)	1.000
Parenteral prostacyclin analogues	0	5 (31.3 %)	0.012
Oral prostacyclin analogues	9 (45 %)	8 (50 %)	1.000
ERA	12 (60 %)	16 (100 %)	0.005
PDE-V inhibitors	12 (60 %)	12 (75 %)	0.481
sGC stimulator	1 (5 %)	2 (12.5 %)	0.574
Immunosuppressive therapy	8 (40 %)	1 (6.3 %)	0.026
Medication number, n (%)			0.024
0 or 1	6 (30 %)	0	
2 or more	14 (70 %)	16 (100 %)	
Hemodynamic parameters			
Baseline RHC			
Before NO challenge			
mPAP (mmHg)	42.9 ± 14.0	47.8 ± 10.2	0.250
mPAWP (mmHg)	7.5 ± 2.9	9.4 ± 3.5	0.078
Cardiac Index (L/min/m^2)	2.82 ± 0.58	2.53 ± 0.70	0.189
PVR (dynes.sec.cm^{-5})	674.2 ± 313.1	858.7 ± 624.7	0.256
SpO_2 (%)	94.9 ± 3.2	94.1 ± 2.4	0.385
During NO challenge			
mPAP (mmHg)	37.6 ± 13.5	47.4 ± 11.5	0.027
mPAWP (mmHg)	8.8 ± 3.0	10.3 ± 4.7	0.238
Cardiac index (L/min/m^2)	2.83 ± 0.68	2.69 ± 0.60	0.551
PVR (dynes.sec.cm^{-5})	555.3 ± 304.1	825.9 ± 653.4	0.109
SpO_2 (%)	93.6 ± 2.4	92.6 ± 4.6	0.435
Change during NO challenge			
Absolute ΔmPAP (mmHg)	−5.4 ± 7.0	−0.4 ± 4.9	0.023
Percentage ΔPVR (%)	−17.6 ± 19.2	−5.2 ± 19.1	0.063
Absolute ΔSpO_2 (%)	−1.2 ± 2.0	−1.2 ± 3.3	0.966
Follow-up RHC			
Before NO challenge			
mPAP (mmHg)	34.3 ± 10.1	37.9 ± 12.1	0.333
mPAWP (mmHg)	9.4 ± 3.5	8.9 ± 2.4	0.644
Cardiac index (L/min/m^2)	3.01 ± 0.60	3.01 ± 0.83	0.982
PVR (dynes.sec.cm^{-5})	438.1 ± 205.5	497.3 ± 261.8	0.453
SpO_2 (%)	95.3 ± 2.4	94.6 ± 3.3	0.501
During NO challenge			
mPAP (mmHg)	30.9 ± 9.5	40.4 ± 12.8	0.015
mPAWP (mmHg)	10.1 ± 4.5	10.2 ± 3.0	0.948
Cardiac index (L/min/m^2)	2.99 ± 0.57	3.13 ± 0.78	0.554
PVR (dynes.sec.cm^{-5})	352.2 ± 158.1	492.9 ± 266.6	0.057
SpO_2 (%)	94.2 ± 2.9	92.8 ± 4.2	0.234
Change during NO challenge			
Absolute ΔmPAP (mmHg)	−3.5 ± 2.5	2.5 ± 2.3	<0.001
Percentage ΔPVR (%)	−16.2 ± 17.4	−1.1 ± 18.1	0.016
Absolute ΔSpO_2 (%)	−1.1 ± 1.9	−1.8 ± 1.8	0.279

ERA, endothelin receptor antagonist; I/HPAH, idiopathic/heritable pulmonary arterial hypertension; mPAP, mean pulmonary arterial pressure; mPCWP, mean pulmonary arterial wedge pressure; NO, nitric oxide; PAH, pulmonary arterial hypertension; PDE-V, phosphodiesterase type V; PoPH, portopulmonary hypertension; PVR, pulmonary vascular resistance; RHC, right heart catheterization; sGC, soluble guanylate cyclase; SpO_2, oxygen saturation of peripheral artery; Δ, change during NO challenge.

Table 4
Univariate Cox proportional analysis of factors associated with all-cause mortality.

Parameters	Hazard ratio (95 % CI)	*p*-Value
Age (years)	1.066 (1.033–1.133)	0.041
Sex (female)	2.729 (0.610–12.214)	0.189
Baseline		
mRAP (mmHg)	0.950 (0.738–1.222)	0.688
mPAP (mmHg)	1.026 (0.971–1.084)	0.366
Cardiac index (L/min/m^2)	1.444 (0.453–4.604)	0.535
PVR (dynes.sec.cm^{-5})	1.000 (0.998–1.001)	0.928
Absolute ΔmPAP (mmHg)	1.010 (0.898–1.135)	0.873
Percentage ΔPVR (%)	0.989 (0.954–1.026)	0.556
Follow-up		
mRAP (mmHg)	0.920 (0.673–1.257)	0.600
mPAP (mmHg)	1.034 (0.970–1.101)	0.306
Cardiac index (L/min/m^2)	2.632 (0.820–8.444)	0.104
PVR (dynes.sec.cm^{-5})	1.001 (0.998–1.004)	0.658
Absolute ΔmPAP (mmHg)	1.125(0.928–1.364)	0.232
Percentage ΔPVR (%)	0.995 (0.954–1.038)	0.833
Absolute ΔmPAP ≥0	8.728 (1.045–72.887)	0.045
Percentage ΔPVR ≥-5.5 %	2.318 (0.449–11.957)	0.315

mPAP, mean pulmonary arterial pressure; mPCWP, mean pulmonary capillary wedge pressure; mRAP, mean right atrial pressure; NO, nitric oxide; PVR, pulmonary vascular resistance; Δ, change during NO challenge.

Prognostic significance of repeating vasoreactivity test after therapeutic intervention

Patients were dichotomized into two groups at the median value of absolute change in ΔmPAP at follow-up RHC: preserved vasoreactivity (ΔmPAP ≤ −1 mmHg, $n = 20$) and less vasoreactivity (ΔmPAP ≥0 mmHg, $n = 16$) (absolute ΔmPAP, −3.5 ± 2.5 mmHg vs. 2.5 ± 2.3 mmHg; $p < 0.001$) (Table 3). There were no statistically significant differences between groups in age, sex, and time interval between two RHCs. Preserved vasoreactivity group included more patients taking immunosuppressive treatments for CTD-PAH, while less vasoreactivity group included more I/HPAH patients with higher usage rate of endothelin receptor antagonists and parenteral prostacyclin analogues. At baseline and follow-up RHCs, hemodynamic parameters before NO challenge showed no significant differences between two groups; however, preserved vasoreactivity group presented a significantly larger drop in absolute ΔmPAP also during baseline NO challenge compared to less vasoreactivity group (absolute ΔmPAP, −5.4 ± 7.0 mmHg vs. -0.4 ± 4.9 mmHg; $p = 0.023$).

During a median observation time of 6.3 (interquartile range, 5.0–7.0) years after follow-up RHC, 7 patients died: 1 patient in preserved vasoreactivity group and 6 patients in less vasoreactivity group. Of 7 patients, 4 patients died from heart failure, 2 from malignancy, 1 from liver failure. In univariable Cox regression analysis, sex, hemodynamic parameters at baseline and follow-up RHCs, absolute ΔmPAP or percentage ΔPVR as a continuous variable, follow-up percentage ΔPVR as a dichotomous variable by median (−5.5 %), showed no association with the poor prognosis (Table 4). On the contrary, age [hazard ratio (HR), 1.066; 95 % confidence interval (CI), 1.033–1.133; $p = 0.041$]

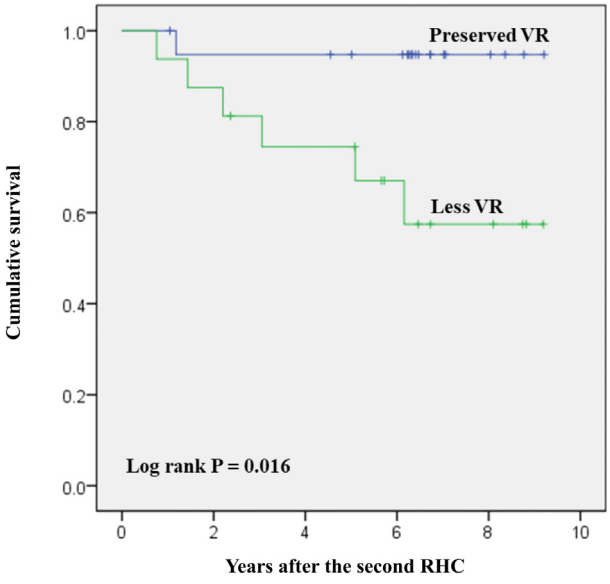
Table 5
Multivariate Cox proportional analysis of factors associated with all-cause mortality.

Parameters	Hazard ratio (95 % CI)	*p*-Value
Age (years)	1.113 (1.007–1.231)	0.036
mRAP at follow-up (mmHg)	0.707 (0.454–1.100)	0.124
Cardiac index (L/min/m^2) at follow-up	4.751 (0.969–23.299)	0.055
Absolute ΔmPAP ≥0 at follow-up	12.814 (1.088–150.891)	0.043

mPAP, mean pulmonary arterial pressure; mRAP, mean right atrial pressure; NO, nitric oxide; Δ, change during NO challenge.

and absolute ΔmPAP ≥0 at follow-up RHC (HR, 8.728; 95 % CI, 1.045–72.887; $p = 0.045$) were significantly associated with increased all-cause mortality. Absolute ΔmPAP ≥0 at follow-up RHC was significantly associated with all-cause mortality in multivariable Cox analysis adjusted for age and known PAH prognostic factors including mean right atrial pressure and CI at follow-up RHC (HR, 12.814; 95 % CI, 1.088–150.891; $p = 0.043$) (Table 5). Absolute ΔmPAP ≥0 at follow-up RHC was significantly associated with all-cause mortality also in the population without calcium channel blocker responders identified at baseline RHC (**Online** Table 1).

Kaplan-Meier survival analysis for all-cause mortality after follow-up RHC showed that less vasoreactivity group had a significantly worse survival compared to preserved vasoreactivity group (log-rank test, $p = 0.016$) (Fig. 3). In detail, 1 out of 20 patients (5 %) in preserved vasoreactivity group died during an estimated mean survival time of 8.8 years (95 % CI, 8.0–9.6), and the 3-year and 5-year survival rates were 94.7 %. On the other hand, 6 of 16 patients (38 %) in less vasoreactivity group died during a mean survival time of 6.7 years (95 % CI, 5.1–8.3) and survival rates at 3 and 5 years were 81.3 % and 74.5 %, respectively. Kaplan-Meier survival analysis in the population without calcium channel blocker responders also indicated that less vasoreactivity group had a significantly worse survival compared to preserved vasoreactivity group (log-rank test, $p = 0.024$) (**Online** Fig. 1).



Number at risk	T_0	1 y	2 y	3 y	4 y	5 y	6 y	7 y	8 y
Preserved VR	20	20	18	18	18	17	16	6	4
Less VR	16	15	14	12	11	11	7	4	4

Fig. 3. Kaplan-Meier curve comparing all-cause mortality after the second RHC in overall cohort.
RHC, right heart catheterization; VR, vasoreactivity; y, years.

Discussion

Numerous studies have evaluated the prognostic value of clinical variables at the time of diagnosis of PAH and during the disease course, and current guidelines recommend a multidimensional approach including invasive hemodynamic assessment at the time of follow-up after initiation or intensification of therapy as well [4,11,12]. Acute vasoreactivity test with inhaled NO is currently recommended exclusively to identify calcium channel blocker responders as part of an initial assessment of PAH patients, and several studies demonstrated that the responsiveness was associated with survival outcomes in PAH patients [13,14]. However, the prognostic importance of follow-up acute vasoreactivity test with inhaled NO achieved after PAH-specific treatment has not been established. In the present retrospective study of 36 PAH patients undergoing diagnostic and follow-up RHC and vasoreactivity tests at our referral center, we demonstrated that the pulmonary vascular reactivity decreased over time; however, the remaining vascular dilatory reserve at the follow-up test after treatment could predict the subsequent all-cause mortality, whereas regularly measured hemodynamic parameters obtained simultaneously did not.

Recently, Tooba, et al. reported results of the second vasoreactivity testing with inhaled NO for 54 PAH patients, and showed the pulmonary vascular reactivity decreased over time; however, there was great variability among patients [9]. Consistent with this study, the positive vasoreactivity rate in the present study decreased from 8.3 % at baseline RHC to 2.8 % at follow-up RHC, and 44.4 % patients (less vasoreactivity group) had ΔmPAP ≥0 mmHg during the follow-up NO challenge. Since hemodynamic parameters such as mPAP, PVR, and CI improved in most patients at follow-up RHC after initiation or intensification of therapy, the successful PAH treatment which reduced their remaining vascular dilatory reserve in pulmonary circulation, might be responsible for the less vasoreactivity at follow-up. The disease progression in which vasoconstrictor responses are lost due to vascular remodeling might also explain our observation. Furthermore, there is an alternative possibility that the redox status of soluble guanylate cyclase (sGC), stimulated by NO under physiological conditions, might be altered by oxidative stress, which contributes to the pathogenesis of PAH, and vasodilation via NO-sGC signaling might be compromised [15,16]. Tooba's study and ours demonstrated that acute vasoreactivity to inhaled NO in PAH patients could change over time, and the criteria for vasoreactivity at diagnostic RHC could not be simply applicable at follow-up RHC.

We divided our cohort into two groups: preserved vasoreactivity group (ΔmPAP ≤ −1 mmHg) and less vasoreactivity group (ΔmPAP ≥0 mmHg) based on the result of the follow-up NO challenge, and compared clinical features and prognosis. There were no significant differences in hemodynamic parameters before NO challenge at follow-up RHC between groups; however, less vasoreactivity group had higher usage rates of endothelin receptor antagonists and parenteral prostacyclin analogue, suggesting that less vasoreactivity group presented with more advanced PAH and less remaining vascular dilatory reserve.

Less vasoreactivity group unexpectedly showed a paradoxical increase in mPAP during follow-up NO challenge and had a poorer survival compared with preserved vasoreactivity group. In previous reports, 23–29 % of PAH patients demonstrated a paradoxical mPAP elevation during NO challenge with unknown mechanisms and clinical implications [17–19]. Increased muscular remodeling of small veins as well as fixed arterial remodeling, might exist in advanced PAH lungs and be associated with heterogeneity in response to medical therapies and prognosis [20,21]. Alternatively, inhaled NO might quickly react with excessive endothelial superoxide radicals, and elicit adverse effects by yielding a toxic vasoconstrictor peroxynitrite, which is reported to be increased in patients with advanced pulmonary hypertension [16,22–24]. Although the underlying mechanism is unknown, in the present

study, we demonstrated that there existed distinct subpopulations of PAH patients with different vasoreactivity to inhaled NO at follow-up RHC, which could provide prognostic information: patients with preserved vasoreactivity at follow-up time may present benign and slowly progressive phenotype under PAH therapy, whereas those with less vasoreactivity may run out of remaining vascular dilatory reserve, and might have to be carefully monitored and referred for early lung transplantation. Given that sGC stimulator can stimulate only the reduced form of sGC and vascular relaxation via NO-sGC signaling can be impaired in the presence of oxidized form of sGC, vasoreactivity test to inhaled NO might also identify responder to sGC stimulator. In this regard, future studies are warranted [15].

This study had several limitations. First, it was a single center retrospective study with a small sample size in Japan. The small sample size could make it difficult to detect the difference in vasoreactivity among PAH subgroups. The small sample size and low number of events could also hinder the multivariable analysis from including all potential confounding variables or detecting statistically significant variables. In addition, prognostic factors associated with right ventricular failure or exercise intolerance, such as brain natriuretic peptide, the index of right ventricular function, or six-minute walking test were not available in our study [4]. Second, our cohort included both treatment-naïve cases and patients who already received treatment at baseline RHC with various PAH etiologies as previous reports [9,10]. This heterogeneity in our cohort might explain the reason why prognostic factors previously known such as right atrial pressure and CI at baseline RHC were not associated with the all-cause mortality in this study [4]. Third, there might be a survival bias in selecting patients who underwent repeat vasoreactivity tests. Our cohort had a higher 5-year survival rate (85.7 %) than previously reported in registry data [25]. Fourth, given that there are no definite criteria for vasoreactivity at follow-up test and our objective is to elucidate prognostic value of vasoreactivity after treatment rather than to investigate calcium channel blocker responsiveness, we adopted a new definition about vasoreactivity at follow-up assessment. The cut-off value of vasoreactivity during follow-up NO challenge may change depending on hospital types (i.e. tertiary or referral center) or patient populations. Fifth, the optimal timing of follow-up RHC in PAH patients is still uncertain [4], and it was at the attending physicians' discretion in our study. Intervals between vasoreactivity tests in our study might be relatively short given that pulmonary vascular remodeling in human PAH occurs over months and years [26]. Further validation of the optimal timing of follow-up vasoreactivity test is required in future research. Although there are limitations as described above, our study demonstrated for the first time that vasoreactivity to inhaled NO at follow-up RHC could give more prognostic information than regularly measured hemodynamic parameters. Further prospective studies with large sample sizes and multiple institutions, which adjust for known prognostic factors, allow subgroup analysis according to PAH etiology, cause of CTD-PAH or treatment status, and investigate the relationship between vasoreactivity and tissue remodeling, are warranted to confirm our findings and explore the underlying mechanisms.

Conclusions

In conclusion, acute vasoreactivity with inhaled NO in PAH patients decreased over time; however, there existed distinct subpopulations with different vasoreactivity at follow-up test, and less vasoreactivity group showed higher risk of all-cause mortality. Follow-up acute vasoreactivity test achieved after PAH-specific treatment could contribute to the detection of such high-risk subgroups who might have to be carefully monitored and referred for early lung transplantation.

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Ethical approval

the Ethical Committee of the University of Tokyo (No. 2650).

CRediT authorship contribution statement

SI, MH, HM, SM, AS, HY, MS, KS, GN, and TF contributed to the conception and design or analysis and interpretation of data, NT and IK contributed to drafting of the manuscript or revising it critically for important intellectual content, and MH contributed final approval of the manuscript submitted.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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