*Pulmonary Circulation*

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RESEARCH ARTICLE OPEN ACCESS

# A Prospective Analysis of Vasoreactivity and Mortality in WHO Group 3 Pulmonary Hypertension

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**Received:** 28 June 2024 | **Revised:** 10 January 2025 | **Accepted:** 2 April 2025

**Funding:** The authors received no specific funding for this work.

**Keywords:** inhaled nitric oxide | lung disease | mean pulmonary artery pressure | pulmonary vascular resistance

## ABSTRACT

Prognostic markers of Group 3 pulmonary hypertension (PH) remain largely unknown. In this study, we evaluate clinical data to provide a comprehensive profile of patients with Group 3 PH and evaluate the potential use of vasoreactivity testing as a prognostic tool within this population. We hypothesized that patients with a stronger vasoconstrictive component of their pulmonary vascular disease would have a more favorable prognosis. Patients were given inhaled nitric oxide during their right heart catheterization to determine if they met the European Respiratory Society guidelines for having a positive vasoreactivity test as defined for patients with Group 1 pulmonary arterial hypertension (PAH). While vasoreactivity response is proven to predict survival in subgroups of PAH, there was no significant relationship between change in mean pulmonary artery pressure (mPAP) during acute vasodilator challenge and survival within our cohort. On the contrary, patients with larger decreases in pulmonary vascular resistance (PVR) during the acute vasodilator challenge were at a significantly higher risk of mortality. The data suggests that the change in PVR during acute vasodilator challenge may be a better indicator of survival in patients with WHO Group 3 PH than the change in mPAP.

## 1 | Introduction

Pulmonary hypertension (PH), a complex and debilitating condition, is characterized by elevated pressures within the pulmonary circulation, leading to right heart failure and substantial morbidity and mortality [1]. Among its diverse classifications, Group 3 PH is associated with underlying lung disorders, such as chronic obstructive pulmonary disease (COPD), emphysema, interstitial lung disease (ILD), combined pulmonary fibrosis and emphysema (CPFE), and chronic hypoxia. This represents a challenging subset that has not yet been comprehensively researched [2]. In addition, Group 3 PH treatment options are limited, with only inhaled treprostinil approved by the FDA for the ILD subgroup of this classification. While this treatment has yielded improvements in exercise capacity, it has minimal effectiveness in improving the quantity or quality of life and reducing disease burden for patients, further underscoring the critical need for novel therapeutic strategies and prognostic tools in this patient population [3]. To advance diagnostics and therapeutics in this group, there must be a delineation of the pathophysiological mechanisms underlying this disease.

Vasoreactivity testing is utilized to evaluate pulmonary vascular responsiveness to vasodilators and gain insights into potential responsiveness to vasodilator therapy and prognosis of Group 1 pulmonary arterial hypertension (PAH). The European Respiratory Society (ERS) has provided guidelines for conducting

vasoreactivity testing during right heart catheterization, advocating for the use of short-acting selective pulmonary vasodilators such as inhaled nitric oxide [4]. According to these guidelines, a positive vasoreactivity test is defined as a reduction in mean pulmonary artery pressure (mPAP) by $\geq 10$ mmHg to reach an absolute value $\leq 40$ mmHg, without a drop in cardiac output [5]. A positive response indicates a vasoreactive component and has been associated with a favorable prognosis as well as a greater likelihood of a favorable long-term response to high-dose calcium channel blockers (CCBs) in certain subgroups of PAH [4]. Pulmonary vasoreactivity testing is currently recommended only in patients with idiopathic pulmonary arterial hypertension (IPAH), heritable pulmonary arterial hypertension (HPAH), and drug- and toxin-induced pulmonary arterial hypertension (DPAH) [6]. Studies have shown that the number of acute vasoresponders in other forms of PAH is extremely low [7]. Further studies have suggested that only about half of vasoreactive IPAH patients maintain long-term response to CCBs [4]. However, due to its critical role in identifying suitable treatment options, vasoreactivity testing remains a standard practice in IPAH, HPAH, and DPAH.

Despite the prognostic value of vasoreactivity testing in certain sub-groups of PAH, its application in Group 3 PH has not been systemically explored. In this study, we used a single center PH registry to evaluate acute vasoreactivity testing in Group 3 PH, including its potential utility as a prognostic tool. Additionally, by comparing responders and non-responders, we sought to improve the understanding of this disease. We hypothesized that patients who met the ERS guidelines for a positive vasoreactivity test would have better outcomes as assessed by time to death after diagnosis.

## 2 | Methods

Data were collected prospectively from the Tufts Medical Center Pulmonary Hypertension Center database, under a study approved by the Tufts Medical Center Institutional Review Board (IRB# 00004908) titled "A Prospective study of Vasoreactivity and Mortality in WHO Group 3 Pulmonary Hypertension." As a non-interventional study, it was not entered into clinicaltrials.gov. All patients in our database were screened for possible eligibility in this study.

### 2.1 | Patients

Thirty-six adult patients ( $\geq 18$ years) with suspicion of Group 3 PH based on disproportionate dyspnea and/or exercise limitation and echocardiographic or computerized tomography (CT) findings and undergoing a right heart catheterization (RHC) with iNO challenge between March 2006 and July 2023 were enrolled in this study. All patients were followed up until January 17, 2024. Blood was collected around the time of their RHC or during a follow-up after the RHC. Demographics and clinical characteristics were recorded from medical records at the time of enrollment. The sample size for this study was not predetermined through statistical power calculations due to the exploratory nature of the research. Instead, it was based on the availability of patients who met the inclusion and exclusion criteria at our site over the study period.

### 2.2 | Inclusion and Exclusion Criteria

Patients were included in the study if they had significant chronic lung disease and PH as defined by a resting (mPAP) $> 20$ mmHg, pulmonary capillary wedge pressure (PCWP) $\leq 15$ mmHg, and pulmonary vascular resistance (PVR) $> 2$ Wood units (WU). The chronic lung disease was evaluated by two pulmonary specialists, who relied on evidence from clinical notes, a CT scan of the chest, and a pulmonary function test (PFT). Hemodynamics, radiologic imaging, PFTs, laboratory results, and clinical assessments were used to exclude patients meeting criteria for Group 1 PAH, or Groups 2, 4, and 5 PH according to the WSPH criteria of 2018 [8]. This study was limited to incident patients and only the baseline/diagnostic RHC was used for evaluation.

### 2.3 | Hemodynamic Measurements

A right heart catheterization was performed on each subject, guided by ultrasound and fluoroscopy. Measurements of right atrial pressure, mPAP, PCWP, and cardiac output (CO) via thermodilution were recorded both while the patient was breathing ambient air or their baseline supplemental oxygen and subsequently during the administration of 20 PPM of inhaled nitric oxide (iNO) (Ikaria, Hampton, N.J., USA) using a snug-fitting mask, for 10 min. All parameters were measured at the end of expiration.

During iNO challenge, supplemental oxygen (28% $FiO_2$) was provided for patients not requiring oxygen. For patients already receiving supplemental oxygen during the initial hemodynamic measurements, the same concentration of oxygen was continued during subsequent assessments. This technique is widely utilized as a hemodynamic test for patients with PAH. However, it has yet to be utilized in patients with Group 3 PH.

### 2.4 | Variables

The variables collected included demographics, clinical characteristics, brain natriuretic peptide (BNP), 6-min walk distance (6MWD), pulmonary function tests (PFTs), pulmonary hemodynamics, echocardiographic parameters of right atrium size, right ventricular size and function, left atrial size, left ventricle ejection fraction, and survival. Measurements were recorded within 90 days of the right heart catheterization. The survival period was defined as the time between the baseline RHC and either death or the study end date of January 17, 2024.

### 2.5 | Statistical Analysis

Data were collected from the Epic electronic medical record system. Continuous variables are presented as median [IQR].

Categorical variables are presented as frequencies and percentages.

We employed a two-stage statistical analysis to identify significant predictors of mortality. Initially, univariate Cox proportional hazards models were applied to each potential predictor to assess its individual association with mortality. Variables demonstrating a *p*-value of less than 0.10 in these univariate analyses as well as variables considered relevant by clinical expertise including age, sex, and mPAP, were selected for further evaluation. Subsequently, we constructed a multivariate Cox proportional hazards model incorporating these selected variables. A backward stepwise elimination process was implemented to systematically remove variables if their association with the outcome, adjusted for the presence of other variables in the model, resulted in a *p*-value greater than 0.10.

We employed Kaplan–Meier survival analysis to investigate the impact of change in PVR and change in mPAP during iNO challenge as well as baseline PVR and baseline mPAP on survival outcomes within our data set. Two distinct groups were then created based on the median of change in PVR and mPAP or the median baseline PVR and baseline mPAP. A log-rank test was used to statistically compare the survival distributions between the two groups.

We conducted a linear regression to explore the relationship between baseline PVR and the reduction in PVR during iNO challenge. Visual representations of the regression lines were created to show the relationships between the variables.

The Python code used for the Kaplan–Meier curves, Cox proportional hazard models, linear regression, and other statistical analysis for this study can be found using the following link: https://github.com/dstrick17/Vasoreactivity-Testing-in-Group-3-Pulmonary-Hypertension.git.

## 3 | Results

### 3.1 | Study Population

Thirty-six patients diagnosed with Group 3 PH, 17 with COPD, 12 with ILD, 6 with CPFE, and 1 with developmental restrictive lung disease (hypoplastic left lung) were enrolled between March 2006 and July 2023. One patient was lost to follow up and was not included in survival analysis.

The clinical, demographic, hemodynamic, and echocardiographic characteristics are presented in Table 1. The median age of patients in this study was 71.94 [IQR: 64.45–76.98], and half were female. Most patients (34) were treatment naïve, while the remaining two patients were on phosphodiesterase type 5 inhibitors. The average 6MWD was reduced, BNP was elevated, and all patients were symptomatic, in New York Health Association (NYHA) functional Class II or III. Left ventricular ejection fraction (LVEF) was measured in 12 subjects with a median of 60.00% [IQR: 55.00–62.50]. Echocardiogram evaluations showed abnormal right ventricle function, right ventricular size, and atrial sizes in most patients.

**TABLE 1** | Characteristics of Group 3 PH patients in this cohort.

| **Demographics and clinical characteristics of Group 3 PH patients** | |
|---|---|
| **Parameter** | ***N*, median [IQR]** |
| Age at diagnosis, years | 71.94 [64.45–76.98] |
| Female, *N* (%) | 18 (50%) |
| Etiology, *N* (%) | |
| COPD | 17, (47.2%) |
| ILD | 12, (33.3%) |
| CPFE | 6, (16.7%) |
| Developmental restrictive lung disease (hypoplastic left lung) | 1, (2.8%) |
| Pre-RHC medications | None: 34, Sildenafil: 2 |
| 6 min-walk test | 18, 237.50 [192.00–338.00] |
| NYHA functional class | 24, 5: II, 19: III |
| BNP (pg/mL) | 120.00 [59.50–487.75] |
| **Echocardiogram parameters** | |
| LVEF (%) | 12, 60.00 [55.00–62.50] |
| Right ventricular size | 18, 5: normal, 13: enlarged |
| Right ventricular function | 17, 5: normal, 12: reduced |
| Left atrium size | 19, 15: normal, 4: enlarged |
| Right atrium size | 19, 6: normal, 13: enlarged |

Abbreviations: BNP, brain natriuretic peptide; COPD, chronic obstructive pulmonary disease; CPFE, combined pulmonary fibrosis and emphysema; ILD, interstitial lung disease; IQR, interquartile range; LVEF (%), left ventricular ejection fraction; RHC, right heart catheterization.

PFTs showed abnormal lung function patterns (Table 2). To analyze pulmonary function, the patients were divided into four groups: total population, obstructive disease, restrictive disease, and mixed disease. Notably, the diffusing capacity for carbon monoxide (DLCO) was severely reduced.

### 3.2 | Hemodynamics and Acute Vasoresponsiveness in Group 3 PH

MPAP and PVR were elevated, while PCWP, CO, and CI were normal at baseline (Table 3). During the administration of iNO, the median decrease in PVR was 1.13 WU [IQR: 0.53–1.93], while the median decrease in mPAP was 5.00 mmHg [IQR: 2.75–7.00] in the entire cohort. In addition to the improvements in pulmonary hemodynamics, there was a slight decrease in CO and CI during the administration of iNO. A paired t-test comparing hemodynamics during iNO challenge to baseline hemodynamics revealed significant reductions in both mPAP and PVR. There were three subjects who met the acute vasoreactivity criteria by the ERS guidelines. Hemodynamic and

**TABLE 2** | Pulmonary function testing in patients with Group 3 PH separated by disease group.

| Parameter | Total *N*, median [IQR] | Obstructive *N*, median [IQR] | Restrictive *N*, median [IQR] | Combined *N*, median [IQR] |
|---|---|---|---|---|
| FVC (L) | 18, 2.3 [1.64–2.91] | 11, 2.68 [1.96–3.02] | 4, 2.3 [1.87–2.43] | 3, 1.63 [1.46–1.65] |
| FVC (%) | 17, 68.0 [60.0–72.0] | 10, 66.5 [62.5–92.25] | 4, 68.5 [58.5–69.75] | 3, 51.0 [48.0–64.0] |
| FeV1 (L) | 18, 1.23 [0.94–1.93] | 11, 0.99 [0.92–1.62] | 4, 1.98 [1.57–2.12] | 3, 1.31 [1.15–1.47] |
| FeV1 (%) | 17, 59.0 [32.0–76.0] | 10, 45.5 [31.0–68.0] | 4, 75.0 [57.5–84.5] | 3, 63.0 [55.0–70.5] |
| FeV1/FVC | 18, 63.5 [46.25–83.25] | 11, 53.0 [37.0–59.5] | 4, 85.0 [81.25–85.75] | 3, 78.0 [77.0–89.0] |
| TLC (L) | 10, 4.13 [3.39–6.0] | 6, 5.58 [4.06–8.48] | 2, 3.87 [3.6–4.14] | 2, 3.43 [3.35–3.52] |
| TLC (%) | 10, 81 [72.0–113.25] | 6, 109.5 [92.25–125.25] | 2, 67.0 [65.5–68.5] | 2, 72.5 [72.25–72.75] |
| DLCO (mL/min/mmHg) | 10, 6.55 [5.0–8.07] | 6, 7.6 [6.48–9.48] | 2, 4.25 [3.98–4.53] | 2, 5.75 [5.22–6.28] |
| DLCO (%) | 10, 33.5 [29.0–36.25] | 6, 35.5 [34.0–37.0] | 2, 17.5 [16.75–18.25] | 2, 30.0 [29.0–31.0] |

Abbreviations: DLCO, diffusing capacity for carbon monoxide; $FEV_1$, forced expiratory volume in one second; FVC, functional vital capacity; IQR, interquartile range; TLC, total lung capacity.

**TABLE 3** | Right heart catheterization with acute vasodilator challenge in patients with Group 3 PH.

| Hemodynamic parameter Term | Baseline *N*, median [IQR] | During iNO challenge *N*, median [IQR] | Change in parameter during iNO challenge *N*, median [IQR] |
|---|---|---|---|
| RAP (mmHg) | 36, 6.00 [3.00–9.00] | | |
| RVSP (mmHg) | 35, 56.00 [41.00–67.00] | | |
| RVDP (mmHg) | 35, 5.00 [1.00–7.50] | | |
| mPAP (mmHg) | 36, 36.00 [29.00–42.50] | 36, 31.00 [23.75–36.50] | 36, −5.00 [−2.75 to −7.00] |
| PCWP (mmHg) | 36, 9.00 [7.75–12.00] | 36, 10.00 [8.00–13.00] | 36, 1.00 [−2.00 to 2.00] |
| CO by TD (L/min) | 36, 4.28 [3.37–4.81] | 36, 4.25 [3.30–4.80] | 36, −0.07 [−0.47 to −0.28] |
| CI by TD ($L/min/m^2$) | 36, 2.29 [1.92–2.64] | 36, 2.21 [1.92–2.61] | 36, −0.04 [−0.24 to 0.17] |
| PVR (Wood Units) | 36, 6.25 [4.33–8.51] | 36, 4.71 [3.27–7.51] | 36, −1.13 [−1.93 to −0.53] |

Abbreviations: CI by TD, Cardiac Index by thermodilution; CO by TD, cardiac output by thermodilution; IQR, interquartile range; mPAP, mean pulmonary artery pressure; mRAP, mean right atrial pressure; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; RVDP, right ventricular diastolic pressure; RVSP, right ventricular systolic pressure.

pulmonary function data on these three patients are presented in Supporting Information: S2, S9.

### 3.3 | Survival in Group 3 PH

The median survival time for all subjects in this study was 31.7 months after diagnosis. Of the three vasoreactive patients, all experienced mortalities before the study endpoint, with survival times of 1.9, 46.6, and 53.5 months after diagnosis. The median survival time of non-vasoreactive patients was 25.4 months.

Kaplan–Meier curves comparing subjects with a reduction in mPAP of greater than 5 mmHg during iNO challenge to those with a milder response showed no significant difference in survival times (Figure 1D). Interestingly, subjects with a reduction in PVR of more than 1.2 WU during iNO challenge were at a statistically significant increased risk of mortality compared to those with a milder response, with median survival times of 23.3 months and 47.8 months respectively (Figure 1C). Subjects in the large reduction of PVR group had an average baseline PVR of 6.9 wood units, while the average baseline PVR of subjects in the small reduction of PVR group was only 4.9 WU. A Mann–Whitney *U* test showed a statistically significant difference in the distributions of PVR values between groups ($p = 0.01$). PCWP increased by an average of 1.72 mmHg in the group with a greater absolute reduction in PVR, whereas it decreased by an average of 0.28 mmHg in the group with a lesser absolute reduction in PVR. This difference was statistically significant ($p = 0.03$). Additionally, mPAP dropped by an average of 2.45 mmHg more in the group with a greater absolute reduction in PVR than the group with a smaller absolute reduction in PVR. Notably, cardiac output increased by an average of 0.13 L/min in the greater absolute reduction in PVR group, compared to a decrease of 0.38 L/min in the smaller absolute reduction in PVR group during the iNO challenge. Our data indicate that changes in mPAP, CO, and PCWP all contributed to the drop in PVR in the group with the greater decrease during iNO challenge.

There was no significant correlation between a reduction in mPAP during iNO challenge with survival (Figure 2D and Table 4). There was also no significant correlation between the change in PCWP and survival (Table 4). Subjects with a greater

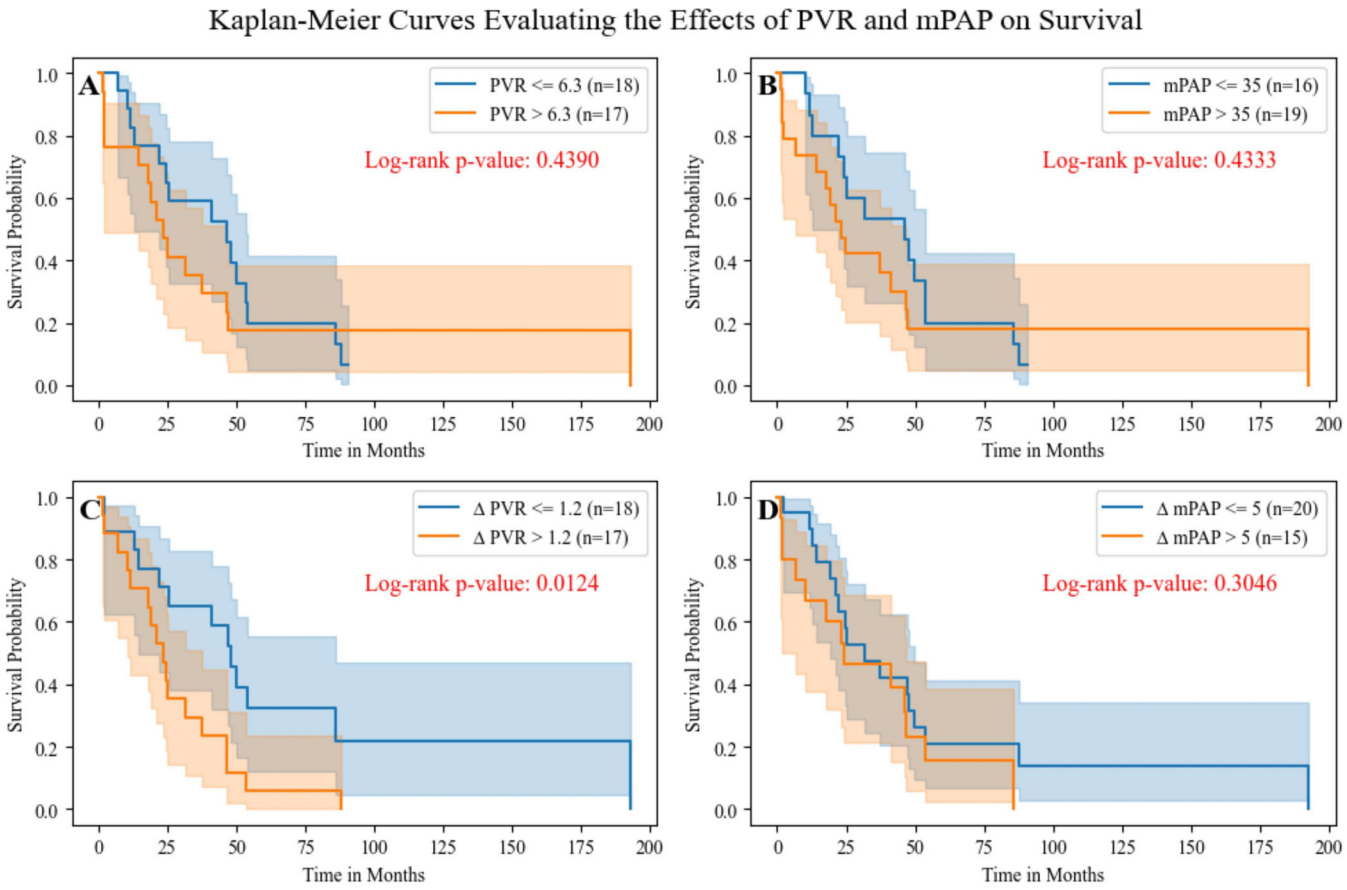


**FIGURE 1** | Kaplan–Meier estimates survival of two groups of patients in this cohort illustrating the estimated survival probabilities over time (in months). (A) Divides the subjects into two groups based on median baseline PVR of 6.3 wood units. (B) Divides the subjects into two groups based on median baseline mPAP of 35 mmHG. (C) Divides the subjects into two groups based on median reduction in PVR during iNO challenge of 1.2 Wood units. (D) Divides the subjects into two groups based on median reduction in mPAP during iNO challenge of 5 mmHG. Only (C) showed a statistical significance in the survival between the two groups demonstrating that subjects with a greater reduction in PVR during iNO challenge were at an increased risk of mortality than subjects with a lower reduction in PVR. Of note, the average baseline PVR of subjects in the reduction of PVR by more than the median was 6.9 wood units, while the average baseline PVR of subjects in the reduction of PVR by the median or less was only 4.9 wood units. mPAP, mean pulmonary artery pressure; PVR, pulmonary vascular resistance.

absolute decrease in PVR during iNO challenge were at a greater risk of mortality (Figure 2C and Table 4). In Supporting Information: Figure 1, Kaplan–Meier estimates assessed the subjects based on the median percentage change in PVR and median percentage change in mPAP. Neither graph showed a statistically significant difference in greater percent decrease in PVR or mPAP compared to lower percent decrease in PVR and mPAP group. However, there appears to be a weak relationship suggesting that patients who experienced a greater percent reduction in PVR are at a greater risk of mortality with a *p*-value of 0.07. There was a positive relationship between baseline PVR and reduction in PVR during iNO challenge, with a coefficient of 0.43 (Figure 3).

Univariate analysis utilizing Cox Proportional Hazard modeling elucidated that a higher baseline CO and CI were associated with a lower risk of mortality, while a higher baseline PVR, BNP, and a reduction of PVR during iNO challenge were associated with an increased risk of mortality (Table 4). Vasoreactivity testing revealed no significant survival difference between patients stratified by median change in mPAP. However, significant correlation was observed between reductions in PVR and increased mortality risk, emphasizing the prognostic value of vasoreactivity testing in Group 3 PH. Multivariate analysis further suggested that a reduction in PVR during iNO challenge and baseline BNP were associated with an increase in mortality risk with hazard ratios of 1.36, and 1.07, respectively (Table 4).

Lastly, although the sample size was small—there was no significant difference in mortality between the three subjects who met the ERS guidelines for having a positive vasoreactivity test for Group 1 PAH and the other 32 subjects, with a *p*-value of 0.77. All data on the three vasoreactive patients can be found in Supporting Information: Tables 1–5.

## 4 | Discussion

In this study, we describe a selected cohort of Group 3 PH patients with various subtypes evaluated with vasoreactivity testing. Vasoreactivity testing with iNO was well tolerated in this cohort of patients. During the RHC, administration of iNO was found to reduce mPAP and PVR in the study population. However, only a small subset of patients, specifically three out

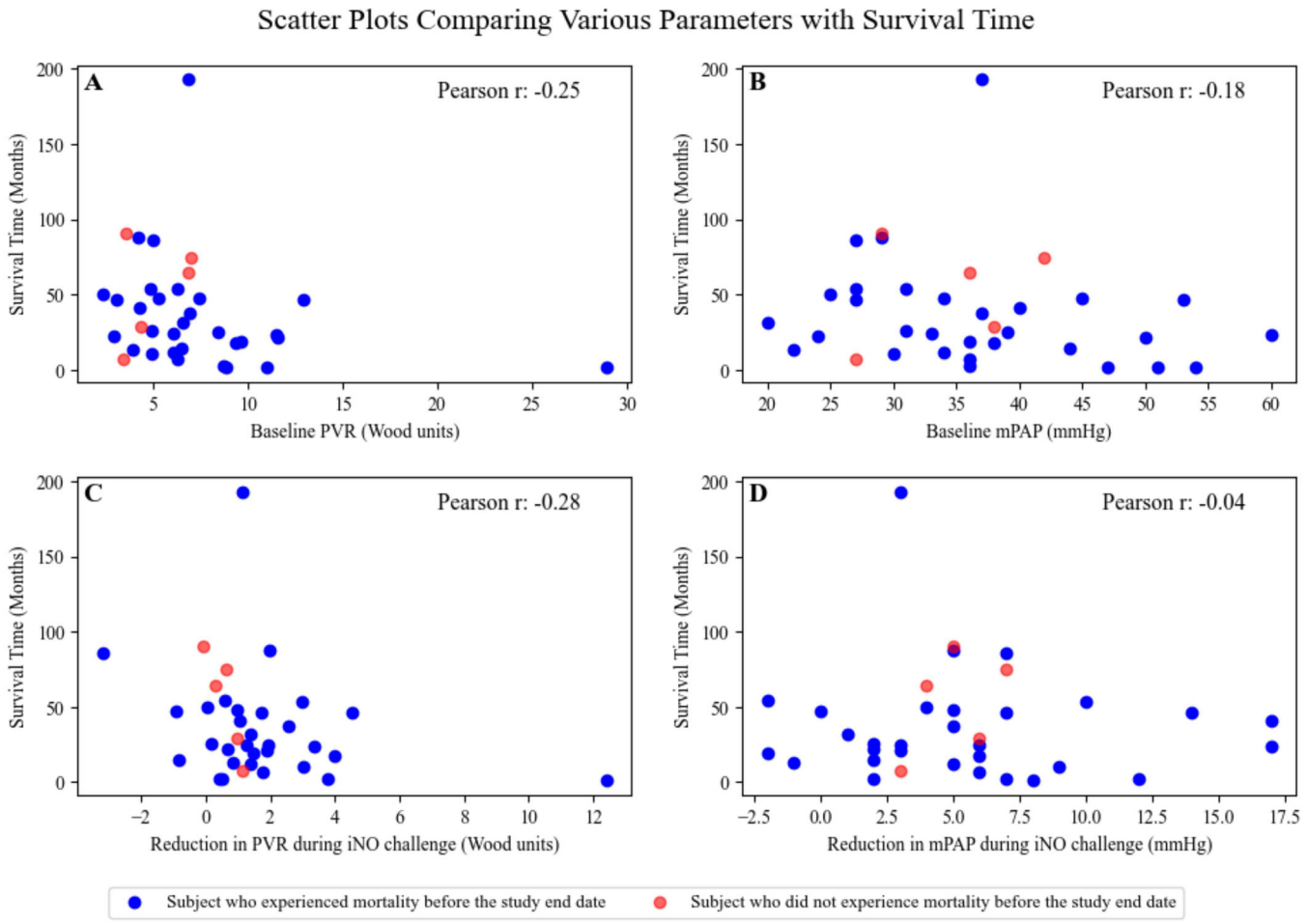


**FIGURE 2** | This scatter plot provides a visual representation of the distribution of survival times relative to mPAP and PVR. (A) Displays the correlation between Baseline PVR and survival for each subject. (B) Displays the correlation between Baseline mPAP and survival for each subject. (C) Displays the correlation between the reduction in PVR during iNO challenge and survival for each subject. (D) Displays the correlation between the reduction in mPAP during iNO challenge and survival for each subject. The pearson correlation coefficient is presented on each graph. mPAP, mean pulmonary artery pressure; PVR, pulmonary vascular resistance.

of the 36 individuals (8.3%), demonstrated positive vasoreactivity during the RHC, as per the accepted criteria of the intervention in PAH. According to the 2022 ERS guidelines, less than 10% of patients with IPAH, HPAH, or DPAH respond to acute vasodilator testing [6].

While vasoreactivity testing is very well documented and only recommended in sub-groups of PAH, existing literature has called for more research on its use for other types of PH [9–11]. Studies in patients with PH due to left heart disease associated with heart failure with preserved ejection fraction (Group 2 PH) concluded that acute vasodilator testing did not predict outcomes but needs to be further investigated [9]. Other studies have shown that a decrease in mPAP > 10.4% during iNO is a predictor of long-term survival and freedom from lung transplantation in adult patients with chronic thromboembolic pulmonary hypertension (Group 4 PH) who are undergoing pulmonary endarterectomy [10]. Vasoreactivity testing has also been used as a prognostic method for patients with rare forms of Group 5 PH [11].

It is unclear if vasoreactivity testing in Group 3 PH has an important prognostic value. The practice remains underexplored, even as clinicians are striving to learn more about the disease, as morbidity and mortality rates remain unacceptably high. While further research is requisite to delineate treatment strategies based on vasoreactivity testing, initiating patient phenotyping will be a pivotal step toward ultimately enhancing care. Our findings provide evidence that a small proportion of Group 3 PH patients may exhibit a positive vasoreactivity test according to the accepted guidelines for patients with Group 1 PAH.

We hypothesized that patients who met the ERS guidelines for a positive vasoreactivity test in PAH would have more favorable outcomes. Since only three subjects met these guidelines, we decided not to compare this small sample size to the rest of the population. Instead, we looked for a relationship between the reduction in mPAP and PVR during iNO challenge and survival. Our adjusted hypothesis predicted greater reductions in mPAP during iNO challenge would have more favorable outcomes, however, we did not find any significant association between these parameters. As expected, an elevated baseline PVR and BNP were associated with an increased risk of mortality, and elevated CO and CI were associated with a decreased risk of mortality. Surprisingly, while looking through other parameters, we observed that a

**TABLE 4** | Univariate and multivariate adjusted risk factors associated with mortality in patients with Group 3 pulmonary hypertension undergoing vasoreactivity testing ($n = 35$).

| **Initial univariate analysis** | | |
|---|---|---|
| **Variable** | **Hazard ratio (95% CI)** | ***p*-value** |
| Age at Cath per 10-year increase | 1.33 (0.88–1.99) | 0.18 |
| Sex_Male | 1.40 (0.67–2.92) | 0.37 |
| Vasoreactive_Yes | 1.20 (0.36–4.02) | 0.77 |
| Baseline RAP per 1 mmHG increase | 1.04 (0.93–1.16) | 0.47 |
| Baseline mPAP per 1 mmHG increase | 1.03 (0.99–1.08) | 0.11 |
| Baseline PCWP per 1 mmHG increase | 1.00 (0.90–1.11) | 0.97 |
| Baseline CO per 1 L/min increase | 0.64 (0.41–1.01) | 0.06 |
| Baseline CI per 1 L/min/$m^2$ increase | 0.36 (0.14–0.91) | 0.03 |
| Baseline PVR per 1 Wood Unit increase | 1.20 (1.07–1.33) | 0.001 |
| Δ mPAP per 1 mmHG decrease during iNO challenge | 1.02 (0.93–1.10) | 0.72 |
| Δ PCWP per 1 mmHG decrease during iNO challenge | 0.98 (0.87–1.11) | 0.75 |
| Δ CO per 1 L/min decrease during iNO challenge | 0.75 (0.41–1.39) | 0.36 |
| Δ CI per 1 L/min/$m^2$ decrease during iNO challenge | 0.60 (0.20–1.74) | 0.34 |
| Δ PVR per 1 wood unit decrease during iNO challenge | 1.35 (1.10–1.67) | 0.004 |
| % Δ mPAP per 1% decrease during iNO challenge | 0.99 (0.96–1.03) | 0.77 |
| % Δ PVR per 1% decrease during iNO challenge | 1.01 (0.99–1.03) | 0.19 |
| BNP level per 50 pg/mL increase | 1.06 (1.01–1.12) | 0.02 |
| **Final multivariate summary** | | |
| **Covariate** | **Hazard ratio (95% CI)** | ***p*-value** |
| Age per 10-year increase | 1.63 (0.99–2.68) | 0.05 |
| Baseline CI per 1 L/min/$m^2$ increase | 0.46 (0.19–1.09) | 0.08 |
| Δ PVR per 1 Wood Unit decrease during iNO challenge | 1.36 (1.11–1.66) | 0.003 |
| BNP level per 50 pg/mL increase | 1.07 (1.01–1.13) | 0.03 |

Abbreviations: BNP, brain natriuretic peptide; CI, Cardiac Index; CI, confidence interval; CO, cardiac output; IQR, interquartile range; mPAP, mean pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; RAP, right atrial pressure.

greater drop in PVR during iNO challenge was associated with an increased risk of mortality. A plausible conclusion is that patients had greater reductions in PVR due to higher baseline PVRs, which allows for a greater drop. PCWP rose more in the group with a greater reduction in PVR, raising the possibility that the iNO challenge revealed a component of left-sided heart dysfunction which could have contributed to greater subsequent mortality. Our multivariate regression incorporating both baseline PVR and reduction in PVR during iNO challenge showed that the reduction in PVR during iNO was more predictive than baseline PVR regarding mortality. Given these findings, the current ERS guidelines for vasoreactivity testing in Group 1 PAH may not be applicable to Group 3 PH. Further research is warranted to investigate the use of reduction in PVR during iNO as the primary indicator of vasoreactivity in patients with WHO Group 3 PH.

While the statistical methods used in this study were comprehensive, they carry some important limitations. One of the primary limitations of this study is the small sample size, with only 36 patients enrolled over 17 years. This reflects the low prevalence of WHO Group 3 PH among patients undergoing right heart catheterization at our center and the fact that vasoreactivity testing is not commonly performed in this population. This is particularly relevant to our observation that a greater reduction in PVR in response to iNO is associated with a greater risk of mortality. Lacking a larger sample size, we are reluctant to draw too strong an inference until it can be replicated. Additionally, the data were collected from a single site which may limit the generalizability of the results. Much of the data recorded predates the implementation of comprehensive electronic medical records which limited the authors' ability to specify ILD classifications when phenotyping patients even though this difference may be important in analyzing this data. Future studies with access to detailed electronic records could provide better insights into the impact that specific ILD subtypes can have on survival outcomes in Group 3 PH. The initial variable selection based on clinical expertise and univariate analysis may have subjectivity and potential bias. The backward elimination process may have excluded key predictors to the *p*-value limitation. The Kaplan–Meier analysis and the log-rank test do not consider confounding factors. Future studies should include multiple research centers to improve generalizability, employ more robust variable selection with a larger sample size, and explore other statistical models to ensure the reproducibility of the findings.

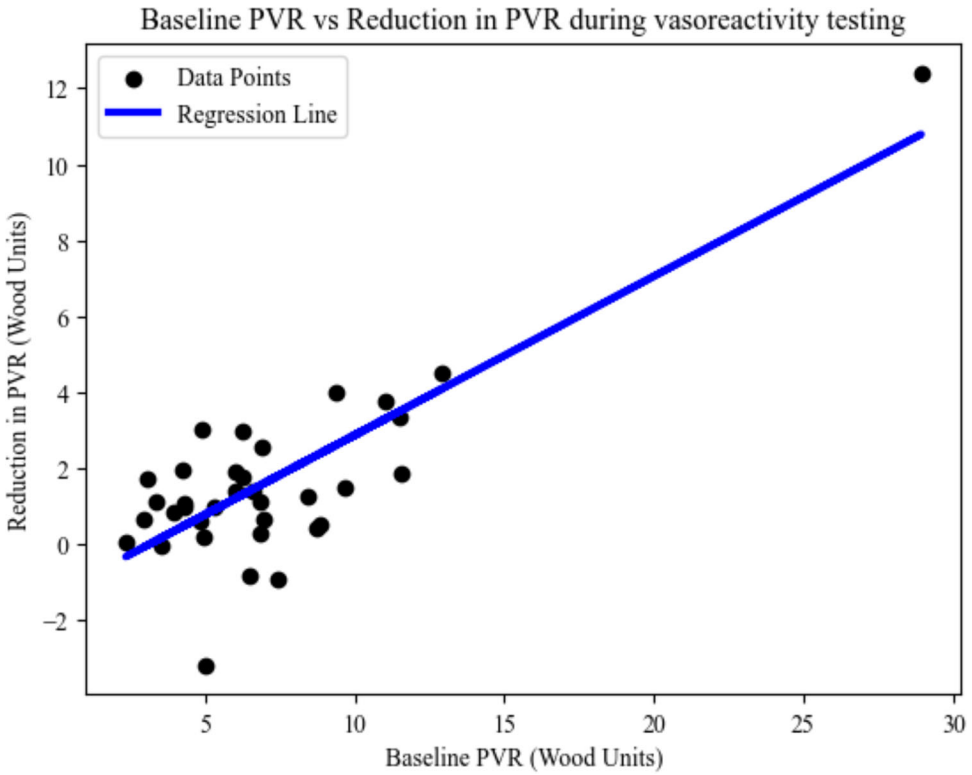


**FIGURE 3** | Linear regression of baseline PVR in wood units (PVR) compared to reduction in PVR in wood units during inhaled nitric oxide challenge (Δ PVR) with a coefficient of 0.43 and an R-squared value of 0.66. PVR, pulmonary vascular resistance.

### Author Contributions

Daniel Strick collected the data, conducted the statistical analysis, and drafted the manuscript. Meredith Kaplan assisted with data collection as well as drafting the manuscript. Dr. Carl Tanba, Dr. David Condon, Dr. Ioana Preston, and Dr. Harrison Farber helped conceive and design the study and revised the manuscript critically for important intellectual content.

### Acknowledgments

We thank Rod Warburton for his advice on statistical methods and technical assistance in data collection. The authors received no specific funding for this work.

### Ethics Statement

The study was approved by the Tufts Medical Center Institutional Review Board (IRB# 00004908).

### Conflicts of Interest

Daniel J. Strick has received travel support from Actelion. Carl Tanba, MD declares no conflicting interests. Meredith Kaplan declares no conflicting interests. Nicholas S. Hill, MD receives research grants from Fisher Paykel and is a consultant for Liquidia, Aerovate, Merck, and United Therapeutics. Harrison W. Farber, MD is a consultant for Actelion, Altavant, Acceleron, Aerovate, United Therapeutics, and Arami. David Condon, MD declares no conflicting interests. Ioana R. Preston, MD receives research grants from United Therapeutics, Liquidia, **Merck** and **Janssen**, is a consultant for Respira, **Merck, Gossamer, Liquidia, United Therapeutics**, and **Janssen**.

### Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

### Guarantor

Dr. Nicholas Hill accepts full responsibility for the work and/or the conduct of the study. He is the principal investigator for the study Titled "A Prospective Study of Vasoreactivity and Mortality in WHO Group 3 Pulmonary Hypertension" which has been approved by the Tufts Medical Center Institutional Review Board (IRB# 00004908).

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**Supporting Information**

Additional supporting information can be found online in the Supporting Information section.