

# Non-Invasive Assessment of Airway Markers in the Diagnosis of Pulmonary Hypertension

Geoffrey Warwick<sup>1</sup>, Anu Krishnan<sup>1</sup>, Sharron Chow<sup>2</sup>, Eugene Kotlyar<sup>3</sup>, Paul S. Thomas<sup>2</sup>, Deborah H. Yates<sup>1</sup>

<sup>1</sup>Department of Thoracic Medicine, St. Vincent's Hospital, Sydney; <sup>2</sup>Inflammatory Diseases Research Unit, University of New South Wales, Sydney; <sup>3</sup>Department of Cardiology, St. Vincent's Hospital, Sydney

## Background

Despite the development of new treatments, the clinical diagnosis of pulmonary hypertension (PH) is difficult. A simple, non-invasive screening test would enable early diagnosis of PH, so that treatment may be offered when most effective.

Biomarkers, such as natriuretic peptides and endothelin, have been used for assessing, screening, prognosis, and evaluating response to treatment in PH patients. Certain biomarkers have been shown to correlate with measures of exercise capacity and haemodynamic and echocardiographic parameters<sup>1,2,3</sup>.

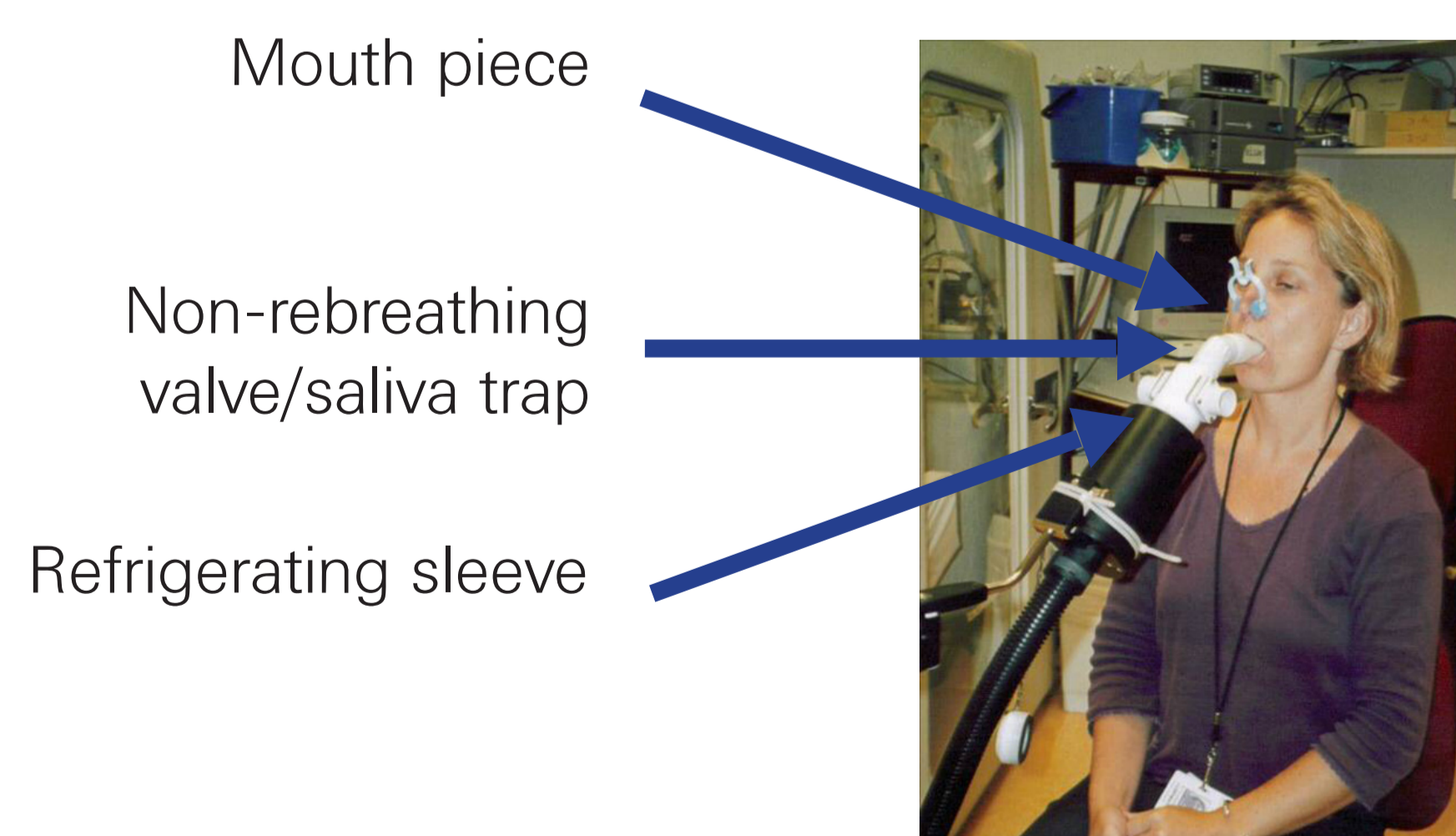
Exhaled nitric oxide (eNO) and exhaled breath condensate (EBC) have recently been recognised as non-invasive methods for assessing the lungs. These methods are quick, safe, easy to perform and can be performed in patients for whom other tests might not be suitable. eNO is low in PH patients and this is supported by laboratory studies where reduced NO is found in pulmonary arteries from patients with PH. Endothelin-1 (ET-1) is a potent vasoconstrictor peptide produced by the vessels in the lungs. It is increased in patients with PH<sup>1</sup> and thus a target for drug therapy. ET-1 has been measured in EBC, but there have been no studies to date evaluating EBC in PH.

This study was designed to evaluate the utility of EBC and eNO in diagnosing PH compared with conventional tests.

## Methods

- Patients were recruited from the respiratory and PH out-patient clinics at St. Vincent's Hospital in Sydney.
- In patients with PH, pulmonary pressures were estimated by right heart catheter, as defined by a mean pulmonary artery pressure > 25 mmHg at rest or > 30 mmHg with exercise.
- eNO measurements were performed on-line by chemiluminescence, and EBC was collected using a refrigerating exhaled breath circuit.
- Samples were tested for pH, 8-isoprostane (8-iso), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), nitrates/nitrites (NOx), 6-keto-prostaglandin F<sub>1α</sub> (PGF<sub>1α</sub>), ET-1 and N-terminal pro B-type natriuretic peptide (NT-proBNP).
- Data were analysed with one-way ANOVA and Bonferroni's multiple comparison test using SPSS for MS Windows, version 12.0. Non-parametric data were log-transformed prior to analysis.
- Correlation of markers between EBC and blood was performed using Pearson's correlation coefficient test. p values < 0.05 were considered significant.

Figure 1. Exhaled breath condensate collection method



The subject breathes tidally via a two-way non-rebreathing valve, which also serves as a saliva trap, into a refrigerated collecting tube. Between 1 and 2 mL are collected in 10 min and aliquoted into 200 μL tubes for immediate storage at -70°C.

## Results

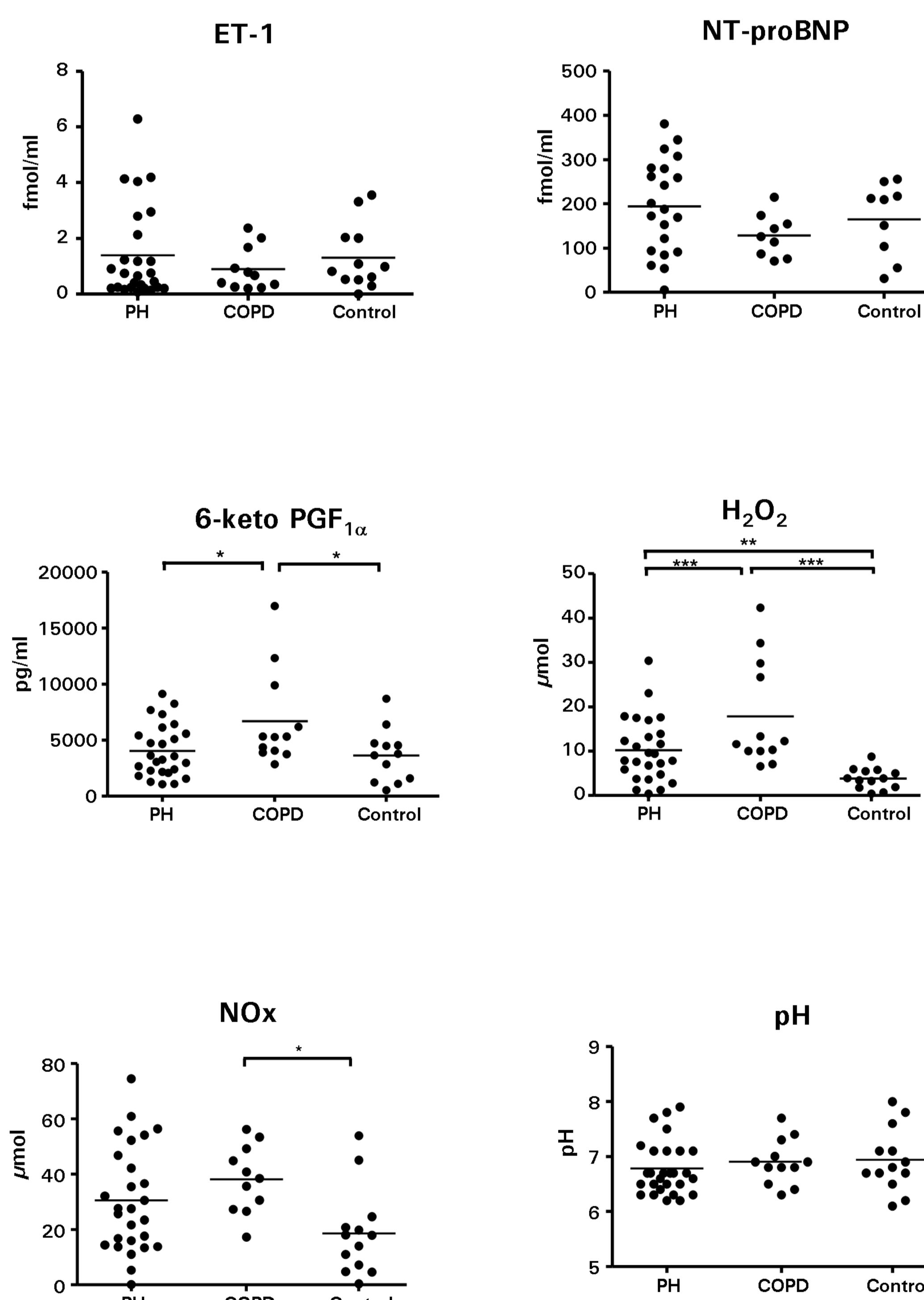
Table 1. Patient characteristics

Parameter	PH (n = 28)	COPD (n = 12)	Control (n = 13)
Mean age (years)	50	70	33
Male/female (n)	9/19	8/4	8/5
Mean FEV1 % predicted (L)	86.1	60.2	101
Mean FVC % predicted (L)	90.5	90.3	105
Mean FEV1/FVC	79	58	80
Mean PASP (mmHg)	85.5	-	-
Mean mPAP (mmHg)	55.96	-	-
Current smokers (%)	0	50	31
Ex-smokers (%)	32	50	23
Inhaled steroid (%)	0	50	0
Oral steroid (%)	0	17	0
Endothelin receptor antagonist (%)	96	0	0
PDE-5 inhibitor (%)	36	0	0
Prostanoid (%)	4	0	0

COPD = chronic obstructive pulmonary disease; FEV1 = forced expiratory volume in 1 s; FVC = forced vital capacity; PASP = pulmonary artery systolic pressure; mPAP = mean pulmonary arterial pressure; PH = pulmonary hypertension; PDE-5 = phosphodiesterase-5

Twenty eight patients with pulmonary hypertension (PH) were enrolled in the study (Table 1). Ten of these had idiopathic PH, 6 had PH secondary to congenital heart disease, 6 had connective tissue disease and 6 had chronic thromboembolic PH. A positive control group of 12 patients with chronic obstructive pulmonary disease (COPD) were studied, as were 13 healthy controls with no cardio-respiratory disease. All of the PH patients were receiving treatment: 27 were receiving an endothelin receptor antagonist and one was on an inhaled prostanoid. Ten patients were receiving additional phosphodiesterase-5 inhibitor.

Figure 2. Comparison of biomarkers from exhaled breath condensates of PH patients, COPD patients and controls



\*p<0.05; \*\*p<0.01; \*\*\*p<0.001  
6-keto PGF<sub>1α</sub> = 6-keto-prostaglandin F<sub>1α</sub>; COPD = chronic obstructive pulmonary disease; ET-1 = endothelin-1; H<sub>2</sub>O<sub>2</sub> = hydrogen peroxide; NOx = nitrogen oxides; NT-proBNP = N-terminal pro B-type BNP; PH = pulmonary hypertension

Table 2. Mean levels of biomarkers in exhaled breath condensates

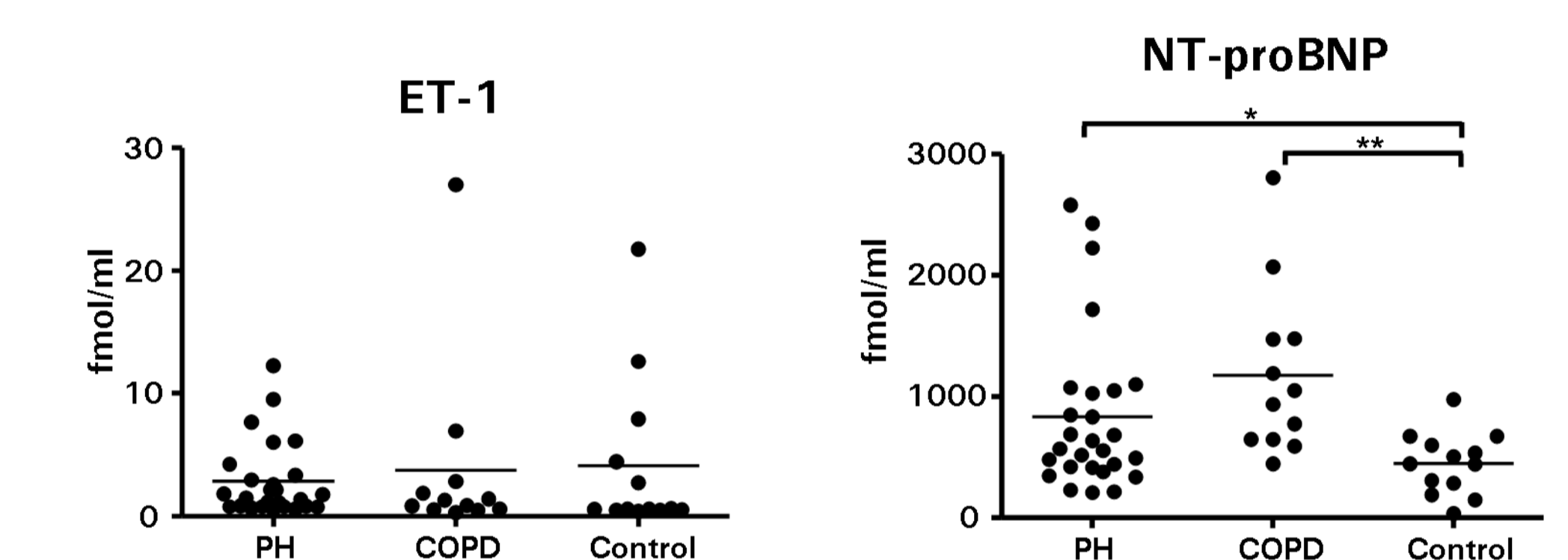
Parameter	PH	COPD	Control
NT-proBNP (fmol/mL)	155 (27)	135 (19)	151 (35)
ET-1 (fmol/mL)	1.51 (0.52)	0.94 (0.31)	1.22 (0.46)
H <sub>2</sub> O <sub>2</sub> (μM)	12.69 (2.22)	16.19 (3.87)	3.45 (0.67)
6-keto PGF <sub>1α</sub> (pg/mL)	4852 (565)	8267 (1906)	5179 (676)
NOx (μM)	33.85 (5.24)	32.47 (3.96)	21.71 (7.60)
8-isoprostane (pg/mL)	10151 (5294)	12868 (10105)	14578 (9010)
pH	6.76 (0.11)	6.94 (0.17)	6.91 (0.16)
Protein (μg/mL)	42.82 (6.42)	53.48 (8.11)	41.97 (7.96)

Data are presented as means (standard errors)  
6-keto PGF<sub>1α</sub> = 6-keto-prostaglandin F<sub>1α</sub>; COPD = chronic obstructive pulmonary disease; ET-1 = endothelin-1; H<sub>2</sub>O<sub>2</sub> = hydrogen peroxide; NOx = nitrogen oxides; NT-proBNP = N-terminal pro B-type BNP; PH = pulmonary hypertension

NT-proBNP was detectable in the EBCs of patients from all groups (Figure 2 and Table 2). There was no statistically significant difference in the levels of EBC NT-proBNP and ET-1 between the groups, possibly due to the fact that all patients with PH were on appropriate treatment.

Hydrogen peroxide was significantly elevated in the EBCs of patients with COPD compared with PH and controls and was higher in PH patients compared with controls. 6-keto PGF<sub>1α</sub> was lower in PH and controls compared with COPD. Nitrogen oxides were elevated in COPD compared with controls. Levels of 8-isoprostane were similar in EBCs from the three groups. No significant difference was found among the EBC pH of the three patient groups.

Figure 3. Comparison of ET-1 and NT-proBNP levels in plasma of PH patients, COPD patients and controls



\*p<0.05; \*\*p<0.01  
COPD = chronic obstructive pulmonary disease; ET-1 = endothelin-1; NT-proBNP = N-terminal pro B-type BNP; PH = pulmonary arterial hypertension

Table 3. Mean levels of ET-1 and NT-proBNP in plasma

	PH	COPD	Control
ET-1 (fmol/mL)	2.7 (0.74)	5.42 (3.70)	5.83 (3.14)
NT-proBNP (fMol/mL)	825 (152)	1068 (218)	490 (117)

Data are presented as means (standard errors)  
COPD = chronic obstructive pulmonary disease; ET-1 = endothelin-1; NT-proBNP = N-terminal pro B-type BNP; PH = pulmonary hypertension

No correlation between levels of ET-1 and NT-proBNP in plasma and EBC was observed (Figure 2 and Table 2 vs Figure 3 and Table 3). Mean (SEM) levels of eNO were 11.9 (1.3) ppb, 10.1 (1.5) ppb and 9.3 (2.4) ppb for PH patients, COPD patients and controls, respectively. Therefore, there was no significant difference among the groups with respect to eNO. No correlation between any of the markers and measures of functional capacity or echocardiographic and right catheter parameters.

## Summary and conclusion

- NT-proBNP is detectable in EBC, suggesting that this technique can be feasibly used for biomarker detection in PH.
- There was no significant difference in levels of NT-proBNP and ET-1 between the EBC of patients with PH and those with COPD or healthy controls.
- Levels of NT-proBNP and ET-1 did not correlate between EBC and plasma nor with pulmonary arterial pressures.
- Lung diseases significantly affect EBC biomarker results, and this factor needs to be taken into account when interpreting EBC biomarker levels in PH.

## Acknowledgements

We are most grateful to Professor Anne Keogh for her advice and to Actelion Endothelin Research Awards (AERA) for their support.

## References

- Nootens M, et al. *J Am Coll Cardiol* 1995;26:1581-5.
- Torbicki A, et al. *Circulation* 2003;108:844-8.
- Allanore Y, et al. *Arthritis Rheum* 2003;48:3503-8.