

Hemodynamic and Clinical Correlates of Endothelin-1 in Chronic Thromboembolic Pulmonary Hypertension

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Background In non-thromboembolic pulmonary hypertension, endothelin (ET)-1 levels are increased and correlate with the hemodynamic severity of the disease. Whether such correlations exist in chronic thromboembolic pulmonary hypertension (CTEPH) is unknown, nor whether ET-1 levels correlate with hemodynamic outcome after pulmonary endarterectomy (PEA).

Methods and Results ET-1 levels were determined by ELISA. ET-levels were increased in 35 CTEPH patients (1.62 ± 0.21 pg/ml) compared with healthy controls ($n=11$: 0.75 ± 0.06 pg/ml, $p < 0.02$). ET-1 levels correlated (all $p < 0.0001$) with mean pulmonary artery pressure (mPAP) ($r=0.70$), cardiac index ($r=-0.76$), total pulmonary resistance ($r=0.72$), mixed venous oxygen saturation ($r=-0.87$), and the distance walked in the 6-min walk test ($r=-0.59$; $p < 0.005$; $n=23$). Three months after PEA, ET-1 levels had decreased ($p < 0.002$), and were similar between patients with and without residual pulmonary hypertension ($p=0.4$). Preoperative ET-1 levels, however, were higher in patients with bad postoperative outcome; that is, patients who either died because of persistent pulmonary hypertension or had residual pulmonary hypertension after PEA (2.68 ± 0.48 pg/ml, and 1.13 ± 0.15 pg/ml, respectively; $p < 0.002$). The levels also correlated with hemodynamic outcome after PEA (mPAP: $r=0.67$, $p < 0.0001$). By receiver-operator characteristic curve analysis, ET-1 > 1.77 pg/ml detected a bad postoperative outcome with a sensitivity and specificity of 79% and 85%, respectively, and a likelihood ratio of 5.2.

Conclusion ET-1 levels in CTEPH closely correlated with the hemodynamic and clinical severity of disease in a large cohort of patients. Preoperative ET-1 levels may be useful for better identification of patients at risk for persistent pulmonary hypertension after PEA. (Circ J 2006; 70: 1058–1063)

Key Words: Chronic thromboembolic pulmonary hypertension; Endothelin-1; Pulmonary endarterectomy

Chronic thromboembolic pulmonary hypertension (CTEPH) results from incomplete resolution of the vascular obstruction associated with pulmonary embolism (PE).¹ CTEPH is considered to develop in 0.5–4% of Caucasian patients after acute PE.^{1,2} In Japanese patients, the incidence may be even higher.³ Left untreated, a gradual hemodynamic and symptomatic decline will occur. Progression of disease may involve recurrent thromboembolism or in situ pulmonary artery thrombosis; however, in many patients, it appears to be related to the development of a secondary arteriopathy in the small pre-capillary pulmonary vessels.⁴

Endothelin (ET)-1, an endothelium derived 21-residue peptide, is considered to play a role in the pathophysiology of various forms of pulmonary arterial hypertension (PAH). ET-1 is a potent vasoconstrictor peptide and, in vitro, a smooth-muscle mitogen, and might therefore contribute to the increase in vascular tone and pulmonary vascular remodeling observed in pulmonary hypertension.⁵ In patients with non-thromboembolic pulmonary hypertension, elevated

ET-1 levels, correlating with the hemodynamic severity of the disease, have been demonstrated.^{6–11} In CTEPH, plasma levels of big ET-1, the non-functional precursor of ET-1,¹² and mature ET-1¹¹ have been elevated in studies involving small numbers of patients. In both studies, however, no correlation between ET-1 levels and hemodynamic severity of disease was documented. In addition, upregulation of the ET-B receptor gene in the hyperplastic media of pulmonary arterial biopsies of CTEPH patients has been demonstrated.¹² ET-B receptor activation on smooth muscle cells is considered to contribute to vasoconstriction¹³ and vascular remodeling.¹⁴ Taken together, these observations indicate that ET-1 may also play a role in CTEPH, but so far no correlation with pathophysiology has been shown.

Our main objective was to study ET-1 levels in relation to the hemodynamic and clinical severity of disease in patients with CTEPH. In addition, ET-1 levels were assessed in relation to hemodynamic outcome after pulmonary endarterectomy (PEA).

Methods

Thirty-five consecutive patients (11 males, 24 females; mean age, 48 years) diagnosed with CTEPH, referred to the Academic Medical Center of the University of Amsterdam, were studied. The diagnosis of CTEPH was established on the basis of previously reported procedures.^{15,16} Diagnosis and cardiopulmonary hemodynamics were determined by pulmonary angiography and right heart catheterization, as part of the routine (preoperative) work-up for PEA. Pulmo-

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Table 1 Clinical and Hemodynamic Patient Characteristics at Baseline

	mPAP >25 mmHg (n=28)	mPAP ≤25 mmHg (n=7)
<i>Characteristics</i>		
Age (mean (range); years)	48 (16–78)	
Gender (M/F)	11/24	
NYHA II	10	
NYHA III	19	
NYHA IV	6	
<i>Hemodynamics</i>		
mPAP (mmHg)	48±3	21±1
CI (L·min ⁻¹ ·m ⁻²)	2.1±0.1	3.1±0.2
TPR (dynes·s ⁻¹ ·cm ⁻⁵)	1,025±87	276±19
PVR (dynes·s ⁻¹ ·cm ⁻⁵)	760±78	158±21
	(n=24)	
PCWP (mmHg)	10±1	9±1
	(n=24)	
RAP (mmHg)	10±1	6±1
Sv ₂ O ₂ (%)	58±1	70±2
	(n=19)	(n=6)

Values are mean ± SEM.

mPAP, mean pulmonary artery pressure; NYHA, New York Heart Association functional class; CI, cardiac index; TPR, total pulmonary resistance; PVR, pulmonary vascular resistance; PCWP, pulmonary capillary wedge pressure; RAP, right atrial pressure; Sv₂O₂, mixed venous oxygen saturation.

nary hypertension was defined as a mean pulmonary artery pressure (mPAP) >25 mmHg at rest (n=28), or >30 mmHg during a standardized exercise test on a cycle ergometer (n=7).¹⁷ In all patients, normal left ventricular function was documented by echocardiography. In addition, coronary angiography was routinely performed in all patients over the age of 40 years. All patients received oral anticoagulation therapy for at least 3 months prior to referral to hospital. Postoperative hemodynamics were determined on the first or second day following PEA, before removal of the Swan-Ganz catheter.

The study was approved by the Medical Ethics Committee of the Academic Medical Center, and informed consent was given by all subjects.

Blood Sampling and Assays

In all patients, blood samples were analyzed for ET-1. Eleven age matched, healthy volunteers (mean age (range), 45 (25–62) years) served as controls. Samples were obtained from the brachial vein, left to coagulate for 1 h at room temperature, centrifuged at 3,000 rpm for 10 min at 4°C, and subsequently stored at –80°C until analysis. ET-1 serum levels were measured with ELISA (Quantiglo®; Human ET-1 Immunoassay, R&D systems, Minneapolis, MN, USA) by luminescence detection (Victor™ Wallac 1420, multilabel counter, Perkin Elmer, Turku, Finland) according to the manufacturer's instructions.

Classification of Functional Impairment

Each patient was functionally classified according to the New York Heart Association (NYHA) classification of the World Health Organization.¹⁸ In 23 patients, the 6-min walk test (6-MWT) was performed using a standard protocol according to the guidelines of the American Thoracic Society.¹⁹

Statistical Analysis

All data are expressed as mean ± SEM. SPSS 11.5 for windows was used to determine statistics (Chicago, IL,

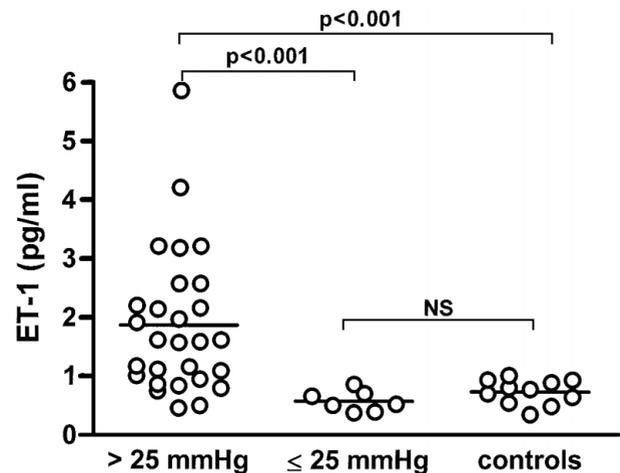


Fig 1. Endothelin (ET)-1 levels in patients with chronic thromboembolic pulmonary hypertension with resting mean pulmonary artery pressure (mPAP) >25 mmHg (n=28), mPAP ≤25 mmHg (n=7), and healthy controls (n=11).

USA). Differences in ET-1 levels between groups were analyzed with the nonparametric Kruskal-Wallis one-way ANOVA test. In the case of an overall statistical difference, the differences between 2 groups were further analyzed with the Mann-Whitney U test; p-values were corrected for multiple comparisons through the use of Dunn's multiple comparisons test. Spearman's rank correlation test was used to assess correlations between ET-1 levels and the hemodynamic parameters and the distance walked in the 6-MWT. The Jonckheere-Terpstra test was used to analyze ET-1 levels (continuous variable) in relation to the NYHA functional class (discontinuous variable).²⁰ The Wilcoxon signed rank test was used to analyze the effect of PEA on hemodynamic parameters and ET-1. To further investigate the correlation between preoperative ET-1 levels and bad postoperative outcome (ie, death from persistent pulmonary hypertension or residual pulmonary hypertension), a receiver-operator characteristic (ROC) curve analysis was performed. The cut-off value for the preoperative ET-1 levels was chosen from the optimal combined sensitivity vs specificity relation. A p-value of <0.05 was considered significant.

Results

All 35 patients underwent a full preoperative evaluation and subsequently, 30 underwent PEA (25 patients with pulmonary hypertension at rest, and 5 patients with exercise-induced pulmonary hypertension). The latter patients suffered from disabling impairment of exercise tolerance and had angiographic evidence for proximal chronic thromboembolic occlusion of at least 2 lobes. Three patients were considered to suffer from distal, inoperable CTEPH. In 2 of the 7 patients with exercise-induced pulmonary hypertension, PEA was postponed. Patient characteristics are summarized in Table 1.

Hemodynamic Characteristics

The hemodynamic characteristics of the patients are summarized in Table 1. Most patients with pulmonary hypertension at rest (n=28) suffered from moderate to severe pulmonary hypertension, with a median mPAP of 49 mmHg

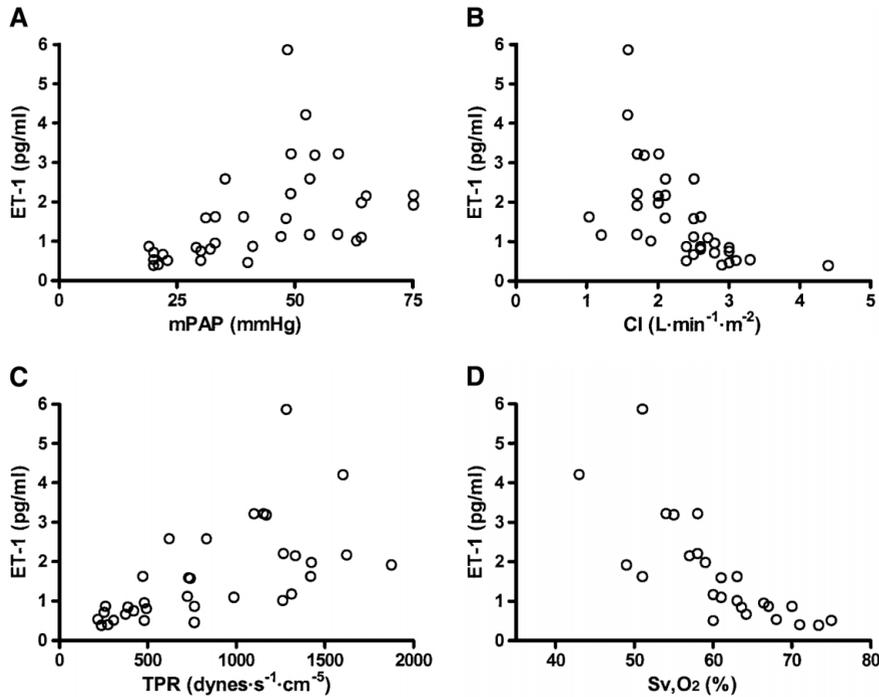


Fig 2. Correlations of endothelin (ET)-1 with hemodynamic parameters. (A) Mean pulmonary artery pressure (mPAP); Spearman $r=0.70$; $p<0.0001$. (B) Cardiac index (CI); Spearman $r=-0.76$; $p<0.0001$. (C) Total pulmonary resistance (TPR); Spearman $r=0.72$; $p<0.0001$. (D) Mixed venous oxygen saturation (Sv,O₂; $n=25$); Spearman $r=-0.87$; $p<0.0001$.

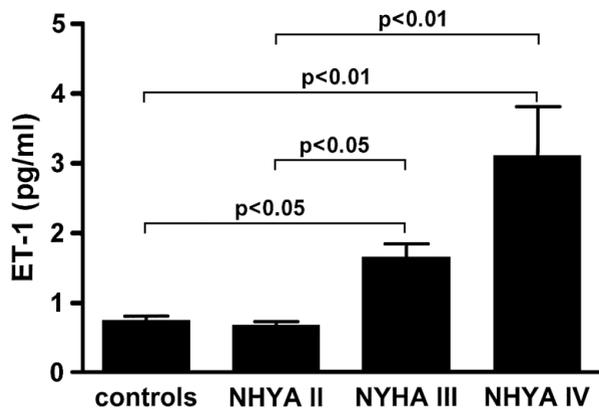


Fig 3. Relation between endothelin (ET)-1 levels and New York Heart Association (NYHA) functional class in patients with chronic thromboembolic pulmonary hypertension and healthy controls.

(range 29–75), and a median total pulmonary resistance (TPR) of $1,042 \text{ dynes} \cdot \text{s}^{-1} \cdot \text{cm}^{-5}$ (range 387–2,019). Asymptomatic coronary artery disease was present in 1 patient only, who suffered from a significant stenosis in the left anterior descending coronary artery.

Correlates of ET-1 in CTEPH Patients

ET-1 levels (Fig 1) were significantly elevated in the CTEPH patients compared with the healthy controls (mean 0.75 pg/ml ; 99% confidence interval $0.55\text{--}0.95 \text{ pg/ml}$). In all 7 patients with exercise-induced pulmonary hypertension, ET-1 levels were within normal range. In fact, in this study, ET-1 levels $\geq 1 \text{ pg/ml}$ had a positive predictive value of 100% for pulmonary hypertension at rest.

ET-1 levels showed highly significant correlations with all hemodynamic parameters reflecting severity of disease. Correlations of ET-1 levels with mPAP, cardiac index (CI), TPR and mixed venous oxygen saturation (Sv,O₂, $n=25$) are shown in Fig 2. In addition, ET-1 levels also correlated with

Table 2 Preoperative Clinical and Hemodynamic Characteristics of Patients in Relation to Postoperative Hemodynamic Outcome

	Postoperative mPAP $\leq 25 \text{ mmHg}$ ($n=20$)	Postoperative mPAP $> 25 \text{ mmHg}$ ($n=9$)
<i>Functional class</i>		
NYHA II	7	0
NYHA III	12	5
NYHA IV	1	4
<i>Hemodynamics</i>		
mPAP (mmHg)	37 ± 3	$58 \pm 3^*$
CI ($\text{L} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$)	2.5 ± 0.1	$1.9 \pm 0.3^\dagger$
TPR ($\text{dynes} \cdot \text{s}^{-1} \cdot \text{cm}^{-5}$)	691 ± 98	$1,259 \pm 115^\dagger$
PVR ($\text{dynes} \cdot \text{s}^{-1} \cdot \text{cm}^{-5}$)	488 ± 79	$1,034 \pm 97^\dagger$
	($n=19$)	
PCWP (mmHg)	10 ± 1	11 ± 1
	($n=19$)	
RAP (mmHg)	8 ± 1	$12 \pm 2^\S$
Sv,O ₂ (%)	64 ± 2	$56 \pm 1^*$
	($n=15$)	($n=8$)

Values are mean \pm SEM.

Abbreviations see in Table 1.

Mann Whitney-U test: $^\S p<0.05$; $^\dagger p<0.005$; $^* p<0.0001$.

the pulmonary vascular resistance ($n=31$; $r=0.74$, $p<0.0001$) and right atrial pressure (RAP; $n=35$; $r=0.49$, $p<0.005$). However, ET-1 levels did not correlate with pulmonary capillary wedge pressure ($n=31$; $r=0.20$, $p=0.3$). After exclusion of the 7 patients with exercise-induced PAH, significant correlations between ET-1 levels and hemodynamic parameters were demonstrated (mPAP: $r=0.48$, $p<0.01$; CI: $r=-0.64$, $p<0.0001$; TPR: $r=0.53$, $p<0.005$; RAP: $r=0.52$, $p<0.005$; Sv,O₂: $r=-0.80$, $p<0.0001$).

ET-1 levels increased in proportion to the preoperative NYHA functional class (Fig 3). In addition, in 23 patients in whom it was studied, the ET-1 level also showed a significant inverse correlation with the distance walked in the 6-MWT ($r=-0.59$, $p<0.005$).

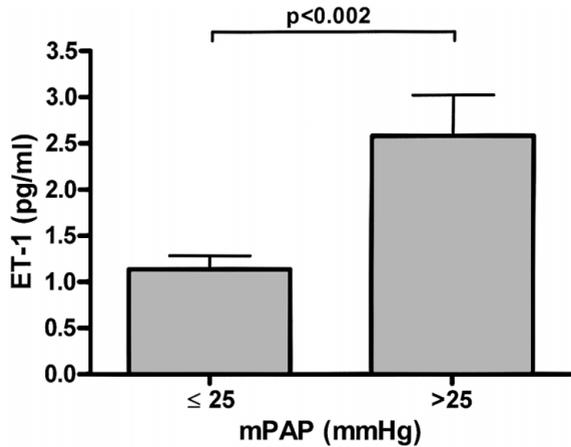


Fig 4. Preoperative endothelin (ET)-1 levels, in relation to postoperative hemodynamic outcome. mPAP, mean pulmonary artery pressure.

Effect of PEA

PEA (n=30) resulted in substantial hemodynamic improvement in 25 patients. Three patients died postoperatively. Two patients died 3 and 15 days, respectively, after PEA from right ventricular failure caused by persistent pulmonary hypertension despite removing organized thromboembolic material from the proximal segmental vessels. One died from postoperative massive alveolar hemorrhage. In the remaining 27 patients following PEA, the mPAP decreased from 44 ± 3 to 24 ± 1 mmHg ($p < 0.0001$), and the TPR from 837 ± 93 to 415 ± 28 dynes \cdot s $^{-1}$ \cdot cm $^{-5}$ ($p = 0.0001$). The CI rose from 2.3 ± 0.1 to 2.5 ± 0.1 L \cdot min $^{-1}$ \cdot m $^{-2}$ ($p = 0.7$). In 7 patients, residual pulmonary hypertension (ie, mPAP > 25 mmHg), was demonstrated shortly after PEA, of whom 2 patients had a mPAP > 30 mmHg. Preoperatively, the patients with bad postoperative outcome (ie, death because of persistent pulmonary hypertension or residual pulmonary hypertension) represented the more severely affected patients (Table 2).

ET-1 Levels in Relation to Hemodynamic Outcome

From 23 of 27 patients, blood samples 3 months after PEA were available for comparison of ET-1 levels with preoperative ET-1 levels. The ET-1 levels had decreased significantly from 1.62 ± 0.19 to 0.94 ± 0.07 pg/ml ($p < 0.002$). In fact, postoperative ET-1 levels did not differ significantly from the healthy controls (0.75 ± 0.06 pg/ml; $p = 0.12$). In addition, ET-1 levels 3 months postoperatively did not differ between patients with residual pulmonary hypertension and patients with normalized mPAP (0.91 ± 0.10 pg/ml, and 1.00 ± 0.11 pg/ml, respectively; $p = 0.4$).

Preoperative ET-1 levels, however, differed significantly between the patients (n=9) who had died because of persistent pulmonary hypertension or who had residual pulmonary hypertension after PEA, and the patients (n=20) with normalized pulmonary hemodynamics (Fig 4). In fact, the preoperative ET-1 levels correlated significantly with mPAP after PEA ($r = 0.67$, $p < 0.0001$; Spearman rank correlation test). By ROC curve analysis, the preoperative ET-1 was demonstrated to be a good predictor of bad postoperative outcome (Fig 5; area under the curve 0.87). An ET-1 level of 1.77 pg/ml had sensitivity and specificity of 79%

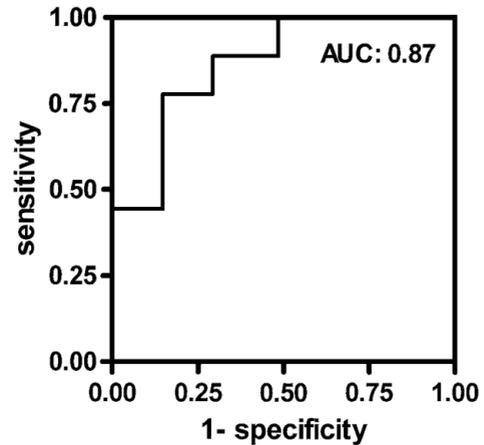


Fig 5. Receiver-operator characteristics curve (area under the curve (AUC)) of preoperative endothelin-1 level to predict death from persistent pulmonary hypertension or residual pulmonary hypertension (mean pulmonary artery pressure > 25 mmHg) after pulmonary endarterectomy.

and 85%, respectively, for bad outcome after PEA, with a likelihood ratio of 5.2.

Discussion

In this study of patients with CTEPH, we demonstrated increased serum ET-1 levels, which correlated significantly with both hemodynamic as well as functional parameters reflecting severity of disease. In addition, in the patients studied, preoperative ET-1 levels were associated with persistent or residual pulmonary hypertension after PEA.

Based on our data, ET-1 levels appear to reflect the hemodynamic severity of disease in CTEPH. After PEA, in which the surgically accessible chronic thromboembolic obstruction was removed, ET-1 levels decreased to normal values. Surprisingly, in the present study, postoperative ET-1 levels did not differ between patients with residual pulmonary hypertension and those with normalized pulmonary hemodynamics after PEA. At least in part, however, this can be explained by the fact that residual pulmonary hypertension observed after PEA in this series was relatively mild, with mPAP < 30 mmHg in all but 2 patients. Previously, Nagaya et al demonstrated that the level of plasma brain natriuretic peptide (BNP) correlated with clinical and hemodynamic severity of disease in CTEPH patients.²¹ In addition, the postoperative BNP level was shown to be a useful noninvasive marker for hemodynamic outcome after PEA. In patients with pulmonary hypertension, BNP is predominantly secreted by the right ventricle, and it is considered to reflect the functional impairment of the right ventricle.²² ET-1, however, which is predominantly produced by the pulmonary endothelium, more likely reflects the vascular (arteriopathic) component of the disease.²³ Taken together, these observations warrant future studies on the role of combined markers for pre- and postoperative assessment of CTEPH patients.

Our observations are similar to those in studies of non-thromboembolic pulmonary hypertension⁹⁻¹¹ and the ET-1 levels observed in the present study, in particular the levels in the more severely impaired patients, are in the range of ET-1 levels reported before in studies of idiopathic PAH (iPAH),^{10,11} and pulmonary hypertension associated with

venous congestion.⁶ Moreover, in patients with venous congestive pulmonary hypertension, it was demonstrated that elevated systemic ET-1 levels decreased after surgical correction of valvular heart disease.⁹ Our observations thus confirm and extend the notion that ET-1 elevation, observed in various forms of pulmonary hypertension, is likely to reflect a secondary phenomenon that is reversed after restoration of pulmonary hemodynamics.

Although ET-1 elevation in CTEPH is likely to represent a secondary phenomenon, this does not preclude ET-1 from being implicated in the pathogenesis of CTEPH and to play a role in the observed hemodynamic progression of disease. In a canine model of chronic embolic pulmonary hypertension, an increased plasma concentration of ET-1 was demonstrated, that coincided with increased ET-1 immunoreactivity in the thickened pulmonary arteries of the affected animals.²⁴ The development of vascular remodeling in this model could be attenuated by the administration of the dual ET-1 receptor antagonist bosentan. Recently, in 2 uncontrolled, open-label studies of patients with inoperable CTEPH, treatment with bosentan was associated with a significant hemodynamic improvement, as well as an increase in the distance walked in the 6-MWT.^{25,26} Taken together, these data support the notion that the ET-1 system is implicated in the pathogenesis of CTEPH and appears to be a therapeutic target in patients with inoperable disease or residual pulmonary hypertension after PEA.

ET-1 levels also correlated with the level of functional impairment as assessed by NYHA classification and the distance walked in the 6-MWT, which is in line with previous observations in iPAH patients in whom the ET-1 levels also correlated significantly with the distance walked in the 6-MWT.¹⁰

The observed correlations between ET-1 levels and the hemodynamic parameters were statistically robust. By inclusion of patients suffering from exercise-induced pulmonary hypertension we were able to study the whole spectrum of disease severity. Inclusion of these patients in the correlation between ET-1 levels and hemodynamic parameters at rest, however, may have affected the statistical significance of these correlations. Exclusion of these patients from the analyses, however, only modestly affected the statistical significance of the observed correlations.

Preoperative ET-1 levels correlated with hemodynamic outcome after PEA. Patients who either died because of persistent pulmonary hypertension or had residual pulmonary hypertension after PEA had significantly higher preoperative ET-1 levels than patients with normalization of pulmonary hemodynamics after PEA. Moreover, by ROC curve analysis, preoperative ET-1 was shown to be a good predictor of postoperative outcome. Residual pulmonary hypertension after PEA is considered to be, at least in part, the consequence of secondary arteriopathy. Failure to substantially lower the PVR after PEA is associated with postoperative hemodynamic instability, right ventricular failure and highly increased mortality.²⁷ Therefore, preoperative identification of patients at risk for persistent or residual pulmonary hypertension is of major importance. Although, in general, medical treatment prior to PEA is not indicated, these patients might benefit from preoperative medical treatment.^{28–33} Although our observations are based upon a relatively small number of patients and, therefore, confirmation in a larger cohort of patients is mandatory, the present study suggests that ET-1 levels might be useful as a noninvasive parameter for identifying these patients prior to PEA.

Conclusion

In CTEPH, ET-1 levels are increased and closely reflect the hemodynamic and functional impairment of the patients. Moreover, our observations warrant further studies evaluating the role of preoperative measurement of ET-1 levels, along with other indices that portend an unfavorable surgical outcome, to identify patients at risk for persistent or residual pulmonary hypertension after PEA.

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References

1. Fedullo PF, Auger WR, Kerr KM, Rubin LJ. Chronic thromboembolic pulmonary hypertension. *N Engl J Med* 2001; **345**: 1465–1472.
2. Pengo V, Lensing AW, Prins MH, Marchiori A, Davidson BL, Tiozzo F, et al. Incidence of chronic thromboembolic pulmonary hypertension after pulmonary embolism. *N Engl J Med* 2004; **350**: 2257–2264.
3. Nakamura M, Okada O, Sakuma M, Nakanishi N, Miyahara Y, Yamada N, et al. Incidence and clinical characteristics of chronic pulmonary thromboembolism in Japan compared with acute pulmonary thromboembolism: Results of a multicenter registry of the Japanese Society of Pulmonary Embolism Research. *Circ J* 2002; **66**: 257–260.
4. Moser KM, Bloor CM. Pulmonary vascular lesions occurring in patients with chronic major vessel thromboembolic pulmonary hypertension. *Chest* 1993; **103**: 685–692.
5. Goto K. Basic and therapeutic relevance of endothelin-mediated regulation. *Biol Pharm Bull* 2001; **24**: 1219–1230.
6. Chang H, Wu GJ, Wang SM, Hung CR. Plasma endothelin levels and surgically correctable pulmonary hypertension. *Ann Thorac Surg* 1993; **55**: 450–458.
7. Cody RJ, Haas GJ, Binkley PF, Capers Q, Kelley R. Plasma endothelin correlates with the extent of pulmonary hypertension in patients with chronic congestive heart failure. *Circulation* 1992; **85**: 504–509.
8. Giaid A, Yanagisawa M, Langleben D, Michel RP, Levy R, Shennib H, et al. Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. *N Engl J Med* 1993; **328**: 1732–1739.
9. Nootens M, Kaufmann E, Rector T, Toher C, Judd D, Francis GS, et al. Neurohormonal activation in patients with right ventricular failure from pulmonary hypertension: Relation to hemodynamic variables and endothelin levels. *J Am Coll Cardiol* 1995; **26**: 1581–1585.
10. Rubens C, Ewert R, Halank M, Wensel R, Orzechowski HD, Schultheiss HP, et al. Big endothelin-1 and endothelin-1 plasma levels are correlated with the severity of primary pulmonary hypertension. *Chest* 2001; **120**: 1562–1569.
11. Stewart DJ, Levy RD, Cernacek P, Langleben D. Increased plasma endothelin-1 in pulmonary hypertension: Marker or mediator of disease? *Ann Intern Med* 1991; **114**: 464–469.
12. Bauer M, Wilkens H, Langer F, Schneider SO, Lausberg H, Schafers HJ. Selective upregulation of endothelin B receptor gene expression in severe pulmonary hypertension. *Circulation* 2002; **105**: 1034–1036.
13. McCulloch KM, Docherty CC, Morecroft I, MacLean MR. Endothelin-B receptor-mediated contraction in human pulmonary resistance arteries. *Br J Pharmacol* 1996; **119**: 1125–1130.
14. Davie N, Haleen SJ, Upton PD, Polak JM, Yacoub MH, Morrell NW, et al. ET(A) and ET(B) receptors modulate the proliferation of human pulmonary artery smooth muscle cells. *Am J Respir Crit Care Med* 2002; **165**: 398–405.
15. Auger WR, Fedullo PF, Moser KM, Buchbinder M, Peterson KL. Chronic major-vessel thromboembolic pulmonary artery obstruction: Appearance at angiography. *Radiology* 1992; **182**: 393–398.
16. Viner SM, Bagg BR, Auger WR, Ford GT. The management of pulmonary hypertension secondary to chronic thromboembolic disease. *Prog Cardiovasc Dis* 1994; **37**: 79–92.
17. Barst RJ, McGoon M, Torbicki A, Sitbon O, Krowka MJ, Olschewski H, et al. Diagnosis and differential assessment of pulmonary arterial hypertension. *J Am Coll Cardiol* 2004; **43**: 40S–47S.

18. Simonneau G, Galie N, Rubin LJ, Langleben D, Seeger W, Domenighetti G, et al. Clinical classification of pulmonary hypertension. *J Am Coll Cardiol* 2004; **43**: 5S–12S.
19. American Thoracic Society. ATS statement: Guidelines for the six-min walk test. *Am J Respir Crit Care Med* 2002; **166**: 111–117.
20. Mahler JM, Magel RC. A comparison of tests for the k-sample, non-decreasing alternative. *Stat Med* 1995; **14**: 863–871.
21. Nagaya N, Ando M, Oya H, Ohkita Y, Kyotani S, Sakamaki F, et al. Plasma brain natriuretic peptide as a noninvasive marker for efficacy of pulmonary thromboendarterectomy. *Ann Thorac Surg* 2002; **74**: 180–184.
22. Mukoyama M, Nakao K, Hosoda K, Suga S, Saito Y, Ogawa Y, et al. Brain natriuretic peptide as a novel cardiac hormone in humans: Evidence for an exquisite dual natriuretic peptide system, atrial natriuretic peptide and brain natriuretic peptide. *J Clin Invest* 1991; **87**: 1402–1412.
23. Galie N, Manes A, Branzi A. The endothelin system in pulmonary arterial hypertension. *Cardiovasc Res* 2004; **61**: 227–237.
24. Kim H, Yung GL, Marsh JJ, Konopka RG, Pedersen CA, Chiles PG, et al. Endothelin mediates pulmonary vascular remodelling in a canine model of chronic embolic pulmonary hypertension. *Eur Respir J* 2000; **15**: 640–648.
25. Hoeper MM, Kramm T, Wilkens H, Schulze C, Schafers HJ, Welte T, et al. Bosentan therapy for inoperable chronic thromboembolic pulmonary hypertension. *Chest* 2005; **128**: 2363–2367.
26. Hughes R, George P, Parameshwar J, Cafferty F, Dunning J, Morrell NW, et al. Bosentan in inoperable chronic thromboembolic pulmonary hypertension. *Thorax* 2005; **60**: 707.
27. Jamieson SW, Kapelanski DP, Sakakibara N, Manecke GR, Thistlethwaite PA, Kerr KM, et al. Pulmonary endarterectomy: Experience and lessons learned in 1,500 cases. *Ann Thorac Surg* 2003; **76**: 1457–1462.
28. Nagaya N, Sasaki N, Ando M, Ogino H, Sakamaki F, Kyotani S, et al. Prostacyclin therapy before pulmonary thromboendarterectomy in patients with chronic thromboembolic pulmonary hypertension. *Chest* 2003; **123**: 338–343.
29. Bresser P, Fedullo PF, Auger WR, Channick RN, Robbins IM, Kerr KM, et al. Continuous intravenous epoprostenol for chronic thromboembolic pulmonary hypertension. *Eur Respir J* 2004; **23**: 595–600.
30. Ghofrani HA, Schermuly RT, Rose F, Wiedemann R, Kohstall MG, Kreckel A, et al. Sildenafil for long-term treatment of nonoperable chronic thromboembolic pulmonary hypertension. *Am J Respir Crit Care Med* 2003; **167**: 1139–1141.
31. Ono F, Nagaya N, Kyotani S, Oya H, Nakanishi N, Miyatake K. Hemodynamic and hormonal effects of beraprost sodium, an orally active prostacyclin analogue, in patients with secondary precapillary pulmonary hypertension. *Circ J* 2003; **67**: 375–378.
32. Kataoka M, Satoh T, Manabe T, Anzai T, Yoshikawa T, Mitamura H, et al. Oral sildenafil improves primary pulmonary hypertension refractory to epoprostenol. *Circ J* 2005; **69**: 461–465.
33. Sasayama S, Kunieda T, Tomoike H, Matsuzaki M, Shirato K, Kuriyama T, et al. Effects of the endothelin receptor antagonist bosentan on hemodynamics, symptoms and functional capacity in Japanese patients with severe pulmonary hypertension. *Circ J* 2005; **69**: 131–137.