

Pulmonary hypertension

Clinical and pathophysiological studies

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To my wife Dženana and my daughter Dejna

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ABSTRACT

Pulmonary hypertension (PH) is a common abnormality, most often associated with various cardiopulmonary diseases. Pulmonary arterial hypertension (PAH) is a devastating pulmonary vascular disease characterised by the proliferation of endothelial, smooth-muscle cells and fibroblasts. The processes that initiate the pathological changes seen in PH are still unknown. Pulmonary hypertension is defined by increased pulmonary artery mean pressure over 25 mm Hg at rest. Right heart catheterisation is required to confirm the diagnosis and to estimate the severity of PH.

The aims of this thesis were to evaluate whether Doppler echocardiography can be used to determine pulmonary vascular resistance (PVR) in patients with PAH; to evaluate the association between PH in patients with lung diseases awaiting lung transplantation (LTx) and mortality; to assess circulating levels of growth factors, vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), transforming growth factor β 1 (TGF- β 1), interleukin-6 (IL-6) and endothelin-1 (ET-1) across the lung circulation in patients with PAH and their association with the severity of disease; to examine the influence of intravenous epoprostenol on the arterial to venous ET-1 ratio in PAH patients.

Forty-two patients with PAH underwent Doppler echocardiography simultaneously (n=22) and non-simultaneously (n=60) with right heart catheterisation. Retrospectively, 177 patients with advanced lung disease accepted for lung transplantation were studied. Blood samples for the analysis of growth factors, ET-1 and IL-6 were obtained simultaneously from the pulmonary artery (PA) and the radial artery (RA) in patients with PAH (n=44) during right heart catheterisation and were compared with control subjects (n=20).

The correlation coefficient between catheter and simultaneous/non-simultaneous Doppler echocardiography was 0.93/0.92 for PVR. In multivariate analysis, PVR and forced vital capacity (FVC) % of predicted were independently associated with death in patients on the waiting list for LTx. Serum levels of VEGF, PDGF, TGF- β 1, ET-1 and IL-6 were significantly higher in patients with PAH as compared with controls. There was a consistent step-up of VEGF, PDGF and TGF- β 1 across the lungs in PAH patients whereas arterial and PA serum levels of growth factors, ET-1 and IL-6 were similar in the controls (p=NS). IL-6 appeared as a predictor of mortality in multivariate analysis. There were significant correlations between serum levels of ET-1, hemodynamic data and clinical variables.

In conclusion, Doppler echocardiography can be used for estimating of PVR in patients with PAH and may reduce the need for invasive follow-up in these patients. Patients with increased PVR and a lower FVC % of predicted awaiting LTx should be considered for a higher organ allocation priority. The finding of increased circulating levels of growth factors indicates increased release and/or decreased clearance of growth factors at the lung vascular level. These changes may contribute to vascular remodelling in PAH. IL-6 emerged as an independent predictor of adverse outcome in patients with PAH. The ET-1 RA/PA ratio of unity indicates that the clearance and release of ET-1 across the lungs are balanced in controls, PAH patients and during intravenous epoprostenol infusion in treatment-naïve PAH patients. ET-1 serum levels correlated with hemodynamic and clinical markers of PAH severity.

LIST OF PAPERS

This thesis is based on the following four papers, which will be referred to in the text by their Roman numerals:

- I. **Assessment of pulmonary vascular resistance by Doppler echocardiography in patients with pulmonary arterial hypertension.** Selimovic N, Rundqvist B, Bergh CH, Andersson B, Petersson S, Johansson L, Bech-Hanssen O.
J Heart Lung Transplant 2007 Sep; 26(9):927-34

- II. **Pulmonary hemodynamics as predictors of mortality in patients awaiting lung transplantation.** Selimovic N, Andersson B, Bergh CH, Martensson G, Nilsson F, Bech-Hanssen O, Rundqvist B.
Transpl Int 2008 Apr; 21(4):314-9.

- III. **Increased serum levels of growth factors and interleukin-6 across the pulmonary circulation in patients with pulmonary arterial hypertension.** Selimovic N, Andersson B, Bergh CH, Sakiniene E, Carlsten H, Rundqvist B.
Submitted

- IV. **Endothelin-1 across the lung circulation in patients with pulmonary arterial hypertension and influence of epoprostenol infusion.** Selimovic N, Bergh CH, Andersson B, Sakiniene E, Carlsten H, Rundqvist B.
Submitted.

ABBREVIATIONS

α 1 ATD	Alpha 1 antitrypsin deficiency
CI	Cardiac index
CO	Cardiac output
COPD	Chronic obstructive pulmonary disease
CVD	Collagen vascular disease
ET-1	Endothelin-1
FVC %	Forced vital capacity %
ILD	Interstitial lung disease
IL-6	Interleukin-6
IPAH	Idiopathic pulmonary arterial hypertension
LTx	Lung transplantation
NYHA	New York Heart Association
PA	Pulmonary artery
PADP	Pulmonary artery end-diastolic pressure
PAH	Pulmonary arterial hypertension
PAMP	Pulmonary artery mean pressure
PASP	Pulmonary artery peak systolic pressure
PCWP	Pulmonary capillary wedge pressure
PDGF-BB	Platelet-derived growth factor-BB
PVR	Pulmonary vascular resistance
RA	Radial artery
RAP	Right atrial pressure
SaO ₂	Arterial oxygen saturation
SVO ₂	Mixed venous oxygen saturation
TGF- β 1	Transforming growth factor beta 1
TPG	Transpulmonary gradient
VEGF	Vascular endothelial growth factor
WPW	Wolff-Parkinson-White
WU	Wood units

INTRODUCTION

Pulmonary hypertension (PH) is a frequently present in association with various cardiopulmonary diseases. Pulmonary vascular changes as a cause of PH was first describe in the late 19th century as a clinical-pathological syndrome characterized by obstruction of the small pulmonary arteries and right ventricular hypertrophy in patients presenting with dyspnoea and cyanosis ^{1,2}. After the development of right heart catheterisation in the second half of the 20-th century, it was found that many diseases could cause pulmonary hypertension.

PH is defined by a mean pulmonary pressure over 25 mm Hg at rest or over 30 mm Hg during activity ³. In 1951 Dresdale ⁴ et al. were the first investigators to use the term “primary PH” to describe a disease “distinguished from “secondary PH” by the absence of intrinsic heart or lung diseases in 39 patients with unexplained pulmonary hypertension. Because pulmonary hypertension can be caused by diverse aetiologies, a classification of the disorder has been desirable. During the World Health Organisation (WHO) World Symposiums on Pulmonary Hypertension held in Evian in 1998 and Venice in 2003, pulmonary hypertension was classified into five clinical categories. The first category, termed pulmonary arterial hypertension (PAH), included a first subgroup without identifiable cause, or so-called primary pulmonary hypertension (PPH). It incorporated both familial and sporadic forms of the disease. The second subgroup included a number of conditions or diseases of known cause that have in common the localization of lesions to the small pulmonary vascular arterioles. The Third WHO Conference (2003) introduced minor changes to the Evian classification ⁵. The most important modification in 2003 was replacing the term “primary PH” with “idiopathic PAH” (IPAH). The change was made because it had become evident that IPAH and forms of “secondary” PAH share histopathological features, natural history and response to therapy (Table 1)⁵.

The symptoms of PAH are non-specific and, as a result, patients with this disorder are frequently misdiagnosed and treated for more common conditions ^{6,7}. A median interval of 1.9 years between symptom onset and diagnosis was reported for a large case series of patients with primary PH from 1955 to 1977 ⁸. IPAH is a rare condition with an incidence of approximately one to two per million. An analysis of data from a comprehensive register in France has determined that the prevalence and incidence of PAH is 15 cases per million adult inhabitants and 2.4 cases /million of adult inhabitants/yr ⁹. The National Institutes of Health Prospective Trial, which looked at 187 patients with a diagnosis of IPAH in the USA, found that more than 90% of cases were sporadic, and 6% were familial¹⁰. Untreated survival

following diagnosis rarely exceeds three years ¹¹. Females are affected twice as often as males. Current treatments aim to reduce peripheral vascular resistance and increase exercise tolerance. It is, however, unclear if pharmacological treatment actually affects the underlying pulmonary vasculopathy.

Pulmonary venous hypertension is the most common cause of pulmonary hypertension in clinical practice. Because blood of necessity flows through the pulmonary vascular bed into the left heart, any elevation in the filling pressure in the left side of the heart will result in an increase in pulmonary artery pressure. A substantial proportion of patients with left sided heart diseases develop pulmonary venous hypertension. In patients with moderate to severe heart failure referred to transplant clinics, pulmonary hypertension with a PVR of > 3.5 WU is reported in between 19 to 35% of patients ^{12, 13}. Pulmonary hypertension carries a poor prognosis for patients with heart failure ¹⁴⁻¹⁷. At 28 months of follow-up, the mortality rate was 57% in patients with moderate pulmonary hypertension compared with 17% in normotensive patients ¹⁴.

Table 1.

Clinical classification of pulmonary hypertension – Venice 2003

1. Pulmonary arterial hypertension (PAH)

- 1.1 Idiopathic (IPAH)
- 1.2 Familial (FPAH)
- 1.3 Associated with (APAH):
 - 1.3.1 Connective tissue disease
 - 1.3.2 Congenital systemic to pulmonary shunts
 - 1.3.3 Portal hypertension
 - 1.3.4 HIV infection
 - 1.3.5 Drugs and toxins
 - 1.3.6 Other (thyroid disorders, glycogen storage disease, Gaucher's disease, hereditary haemorrhagic telangiectasia, haemoglobinopathies, myeloproliferative disorders, splenectomy)
- 1.4 Associated with significant venous or capillary involvement
 - 1.4.1 Pulmonary veno-occlusive disease (PVOD)
 - 1.4.2 Pulmonary capillary haemangiomatosis (PCH)
- 1.5 Persistent pulmonary hypertension of the newborn (PPHN)

2. Pulmonary hypertension associated with left heart diseases

- 2.1 Left-sided atrial or ventricular heart disease
- 2.2 Left-sided valvular heart disease

3. Pulmonary hypertension associated with lung respiratory diseases and/or hypoxia

- 3.1 Chronic obstructive pulmonary disease
- 3.2 Interstitial lung disease
- 3.3 Sleep disordered breathing
- 3.4 Alveolar hypoventilation disorders
- 3.5 Chronic exposure to high altitude
- 3.6 Developmental abnormalities

4. Pulmonary hypertension due to chronic thrombotic and/or embolic disease

- 4.1 Thromboembolic obstruction of proximal pulmonary arteries
- 4.2 Thromboembolic obstruction of distal pulmonary arteries
- 4.3 Non-thrombotic pulmonary embolism (tumour, parasites, foreign material)

5. Miscellaneous

Sarcoidosis, histiocytosis X, lymphangiomatosis, compression of pulmonary vessels (adenopathy, tumour, fibrosing mediastinitis)

BACKGROUND

Doppler echocardiography in pulmonary hypertension

Doppler echocardiography is an important non-invasive method to detect the presence of PH and to evaluate the effects of PH on cardiac function. A breakthrough in the non-invasive assessment of pulmonary artery pressures came with the development of Doppler echocardiography in the late 1970s. The pressure gradient (PG) driving blood through an orifice can be calculated according to a simplified Bernoulli equation as $PG=4v^2$ mmHg (v =peak velocity with continuous-wave Doppler measurements). Accordingly, tricuspid regurgitation permits calculation of the systolic pressure gradient between the contracting right ventricle and the right atrium (tricuspid insufficiency pressure gradient, TIPG). For calculations of pulmonary artery systolic pressure (PASP), right ventricular outflow stenosis must be excluded and an estimate of the right atrial pressure (RAP) should be added to the pressure gradient; $PASP = TIPG + \text{estimated RAP}$. The reliability of such an assessment of PASP has been extensively verified¹⁸⁻²¹. The reported correlation was invariably excellent ($r = 0.89-0.97$), but unfortunately, the standard error of estimation was relatively high (4.9-8.0 mm Hg), making precise estimations of PASP in an individual patient less reliable²². While the peak velocity of the jet tricuspid regurgitation is related to PASP, the end-diastolic velocity of the pulmonary insufficiency jet is related to diastolic pulmonary pressure (PADP)²³.

The Doppler flow profiles correlated well with the catheter measurements ($r = 0.95$ and $r = 0.95$ respectively) for the peak and end-diastolic pulmonary to RV pressure gradients²⁴. However, when directly compared in the same patient, Doppler-derived pressure calculations based on diastolic velocities across the pulmonary valve were less accurate than those based on tricuspid jet velocity measurement ($r = 0.83$ versus $r = 0.98$ respectively)²⁵. However, this method is limited by a low prevalence of pulmonary insufficiency^{23, 26}.

It has recently been demonstrated that Doppler echocardiography can be used for determining PADP in patients with left heart disease by measuring the tricuspid regurgitation (TR) velocity at the time of pulmonary valve opening^{27, 28} because, at this time point, the right ventricular pressure is equal to PADP²⁹.

Doppler echocardiography has significantly impacted clinical medicine by its ability to determine intracardiac hemodynamics non-invasively. Since flow, cardiac output (CO)^{30, 31} and pressure variables: PASP, PADP and left ventricular filling pressure^{32, 33} can be assessed, we hypothesised that an estimation of PVR might be accurately obtained by Doppler-derived variables in patients with PAH. Since PVR is an essential variable for the prognosis and for

the assessment of therapeutic effects in PH a non invasive method for estimation of PVR would further increase the clinical benefits of Doppler echocardiography in patients with PH. Echocardiography as a non-invasive method which permits the assessment of several independent variables related to right heart hemodynamics appears to be suited for the follow-up of patients with PH and reduces the need for invasive follow-up. Non-invasive assessments of pulmonary artery pressures with echocardiography should always take account of the co-existing clinical and pathophysiological context.

Pulmonary diseases, pulmonary hypertension and the heart

The term “cor pulmonale” is still widely used in medical literature, but its definition varies and there is currently no consensual definition. In 1963 the WHO expert defined cor pulmonale as “hypertrophy of the right ventricle resulting from diseases affecting the function and/or structure of the lungs, except when these pulmonary alterations are the result of disease that primarily affects the left side of the heart, as in congenital heart disease”.³⁴

Pulmonary hypertension complicating chronic respiratory disease is generally defined by the presence of a resting mean pulmonary artery pressure (PAP) of > 20 mm Hg. This is slightly different from the definition of PAH (PAP > 25 mm Hg). In patients with lung diseases PH is a common. The prevalence of PH in individuals with COPD is not known precisely, approximately 10-30 % of patients with moderate to severe COPD have elevated pulmonary pressures³⁵. In heterogeneous groups of patients with fibrotic lung diseases, with majority suffering from idiopathic pulmonary fibrosis in pre-transplant setting, PH is detected by right heart catheterisation in 28-46% of patients^{36, 37}. In nearly 50 % of the patients accepted for lung transplantation PVR was pathologically increased (≥ 3 Wood Units) while pulmonary hypertension, defined as a mean pulmonary pressure of ≥ 25 mm Hg was found in 33 % of the patients³⁸. The complex nature of interactions between the pulmonary and cardiovascular systems is becoming increasingly appreciated. Pulmonary vascular abnormalities are frequently present in patients with respiratory disorders, including chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, sarcoidosis, neuromuscular or chest wall disorders, and disorders of ventilatory control, including sleep apnea syndromes and obesity hypoventilation syndrome. The mechanisms behind PH in patients with lung diseases are not clear. The combined effects of chronic hypoxemia, hypercapnia, inflammation, endothelial cell dysfunction and angiogenesis appear to contribute to the development of PH associated with lung diseases^{36, 39-42}.

Pulmonary hypertension, classified as group III in the WHO classification schedule for PH, may result in severe right ventricular dysfunction caused by lung disease. The development of cor pulmonale is generally associated with a poorer prognosis and increased risk of death^{36, 43, 44}. However, the impact of altered pulmonary hemodynamics as a risk factor(s) for mortality during waiting time for lung transplantation is unclear^{36, 44, 45}.

Vascular remodelling in pulmonary arterial hypertension

Vasoconstriction, inflammation, thrombosis in situ and cell proliferation in the pulmonary vasculature are part of the pathogenesis of PAH. Accumulated evidence indicates that the cellular proliferation of endothelial, smooth muscle cells (SMC) and fibroblasts plays an important role in the vascular remodelling, characteristic of PAH⁴⁶⁻⁴⁹ (Figure 1). Pulmonary vascular remodelling involves structural and functional changes of the normal architecture of the walls of pulmonary arteries. This process can occur as a primary response to injury, or stimulus such as hypoxia. However, the pathogenesis of PAH is incompletely understood.

The different vascular abnormalities associated with PAH include the abnormal muscularisation of distal precapillary arteries, the medial hypertrophy of large pulmonary muscular arteries, the loss of precapillary arteries, the neointimal formation that is particularly occlusive in vessels (100-500 μm) and the formation of a unique vascular structure in PAH, plexiform lesions in these vessels⁵⁰. Various cell-derived growth factors, vasoactive peptides and cytokines are involved in modulation of the vascular remodelling process.

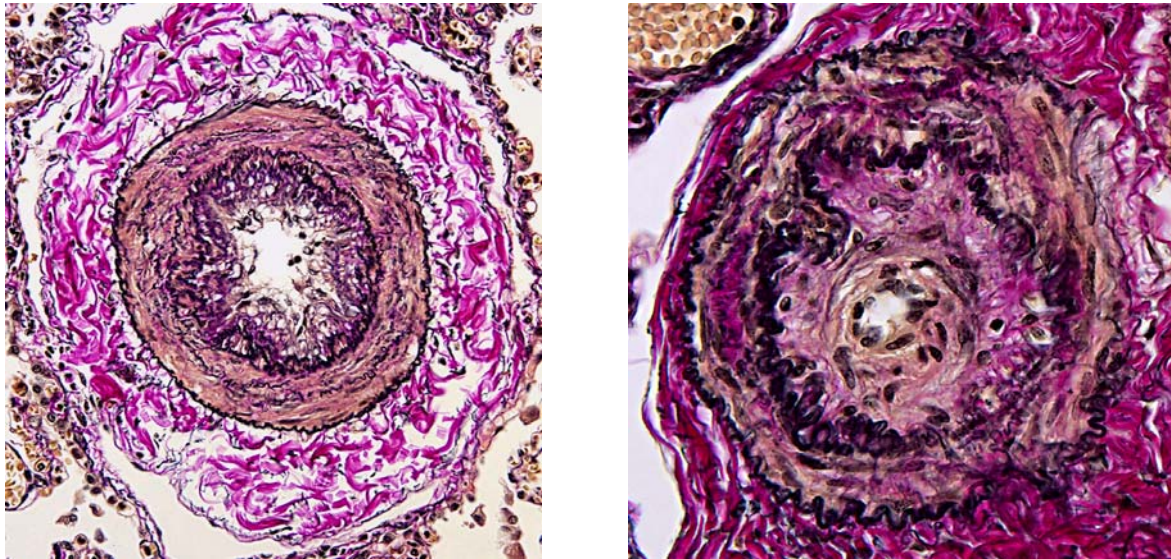


Figure 1. Histology of IPAH. Proliferation of endothelial, smooth muscle cells and fibroblasts with narrowing of pulmonary vessels (explanted lung from patients with IPAH at Sahlgrenska University Hospital) *

* Courtesy of Claes Nordborg MD, Dept. of Pathology Sahlgrenska University Hospital

Abnormalities have been identified in all three layers of the pulmonary arteriolar walls, but it is still not known which one plays a dominant role in the initiation of the disease. One possible candidate is the endothelium and the endothelin system. Endothelin-1 (ET-1) is a potent vasoconstrictor and mitogen released from endothelial cells acting in an paracrine way^{51, 52}. There are two ET-1 receptor subtypes, ET_A and ET_B. ET_A and ET_B receptors are found on SMCs of blood vessels, and both can mediate vasoconstriction, but ET_B receptors on endothelial cells may mediate vasodilatation and endothelin clearance particularly in microvessels.

Experimental studies in rats with hypoxia induced PH⁵³, coupled with clinical studies documenting an increase in expression of ET-1 in lungs of patients with PAH suggests that this vasoconstrictor that also promotes SMC proliferation and inflammation. The mechanism of action of ET-1 receptor antagonists may relate to its vasodilatory properties, although antimitogenic effects cannot be ruled out since numerous studies in animal models have found that ET antagonists can not only prevent development of, but also completely reverse vascular remodelling⁵³⁻⁵⁷. In several clinical trials, ET-1 receptor antagonists significantly increased exercise capacity and improved hemodynamics in patients with PAH⁵⁸⁻⁶¹, underscoring the pathophysiological role of the endothelin system in PAH.

There is evidence that inflammatory cells are present in the remodelled vasculature in pulmonary hypertension⁶². Some patients with PAH have circulating antinuclear antibodies and elevated circulating levels of IL-1 and IL-6. Lung histology studies have also revealed inflammatory infiltrates (mast cells, cluster of macrophages, T- and B-lymphocytes) in the range of plexiform lesions in severe PAH as well as an increased expression of chemokines RANTES and fractalkine⁶³⁻⁶⁶. Pro-inflammatory cytokines (interleukin-IL-1 β and IL-6 and tumor necrosis factor-TNF α) may also contribute to the proliferation of vascular cells^{67, 68}.

Proliferation of vascular cells is regulated by various growth factors. In particular, VEGF, PDGF and TGF- β 1, have been associated with vascular remodelling in both, experimental and human studies in PH^{63, 69-76}.

Vascular endothelial growth factor (VEGF) is an endothelial cell-specific mitogen and potent angiogenic peptide acting via two high-affinity tyrosine kinase receptors [VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1)]. The physiological role of VEGF in the lung is unknown. It has been proposed that VEGF supports pulmonary endothelial cells maintenance and survival. In patients with PAH, the VEGF expression is increased within the pulmonary vasculature, including plexiform lesions^{70, 77}. In patients with IPAH, VEGFR-1 expression is increased, whereas within the plexiform lesions it is VEGFR-2 is expressed⁷⁸. Hypoxia is a main stimulus for VEGF production and expression. Other growth factors, such as TGF- β and PDGF as well as cytokines IL-1 and IL-6 also have the potential to up-regulate VEGF expression⁷⁹.

Platelet-derived growth factor (PDGF) is a potent mitogen for fibroblasts and smooth muscle cells⁸⁰⁻⁸². The molecule consists of two peptide chains (termed 'A' and 'B') and is found as one of at least three possible isoforms, (AB, AA or BB)^{81, 82}. This growth factor is probably involved in a number of biologically important events including wound repair, inflammation, embryogenesis and development. The PDGF stimulates cell growth through the activation of cell surface receptors α and β . The PDGF receptors belong to family of transmembrane receptors tyrosine kinases that include the epidermal growth factor receptor and VEGF receptors. In vitro studies suggest that PDGF-B has affinity for both alpha and beta receptors, whereas PDGF-A shows affinity for only alpha receptors^{81, 82}. In a rat model of PH it has been shown that blockade of the PDGF receptor by imatinib could reverse vascular remodelling and cor pulmonale⁸³. In lung biopsies from patients with PAH, the PDGF-AA isoform was significantly increased⁸⁴ and pulmonary vascular expression of PDGF and its receptors was also found to be increased in explanted lungs from patients with PAH⁸⁵. The clinical effects of PDGF receptor blockade is now examined in patients with PAH^{86, 87}.

The transforming growth factor beta (TGF- β) superfamily consisted of TGF- β isoforms (TGF- β 1-5), bone morphogenetic proteins (BMPs), activins and inhibins⁸⁸. Mutations in the gene encoding the bone morphogenetic protein (BMP) type II receptor (BMPR-II) were recently identified in familial and some apparently sporadic cases of IPAH^{89, 90}. BMPR-II is present predominantly on the pulmonary vascular endothelium, and to a lesser extent on medial SMC⁹¹. Endothelial expression of BMPR-II mRNA and protein is reduced in cases of PPH, whether or not an identified mutation exists in BMPR-II gene coding sequence⁹¹. The role of BMPs in pulmonary vascular remodelling is not easy to predict, because the TGF- β family exerts complex effects on vascular cell function, which vary depending on the cell phenotype and the context. However, in general, TGF- β exerts antiproliferative effects on SMC and promotes differentiation. Similarly, BMPs tend to suppress proliferation of SMC from normal pulmonary arteries and from patients with secondary PH⁷⁶ but fail to suppress proliferation of smooth muscle cells from patients with IPAH⁷⁶. TGF- β is also known to increase production of extracellular matrix and increases elastin expression by stabilisation of elastin mRNA^{92, 93}.

AIMS OF THE THESIS

- To evaluate whether Doppler echocardiography can be used to determine pulmonary vascular resistance in patients with pulmonary arterial hypertension (Paper I)

- To examine the prognostic value of cardio-pulmonary hemodynamics for death in patients awaiting lung transplantation (Paper II)

- 1. To assess serum concentrations of the VEGF, PDGF, TGF- β 1 and Interleukin-6 across the pulmonary circulation in patients with PAH compared with control subjects
2. To correlate the serum levels of these growth factors and IL-6 with clinical and hemodynamic variables, and with outcome (Paper III)

- 1. To assess the transpulmonary endothelin-1 (ET-1) gradient in patients with different forms of pulmonary arterial hypertension and compare it with control subjects
2. To investigate the influence of intravenous epoprostenol during acute pharmacological intervention on the transpulmonary ET-1 gradient in PAH patients
3. To correlate circulating levels of ET-1 with hemodynamic and clinical variables (Paper IV)

METHODS

Study groups

All the patients and control subjects included in our studies were investigated at Sahlgrenska University Hospital, Göteborg. Study protocols were approved by the Institutional Review Board at the University of Gothenburg and all the patients and control subjects gave their informed consent to participate in the studies.

To determine whether Doppler echocardiography can be used to assess PVR in patients with PAH, we studied prospectively (Paper I) 42 consecutive patients with pulmonary vascular disease during evaluation for medical treatment or lung transplantation. Patients with left-sided heart disease or increased PCWP were excluded from the study. The mean age of patients was 53 (21-78) years, 69% female, and most patients were in functional class NYHA III (n=33). Thirty-two patients had PAH according to the WHO Clinical Classification. We included eight patients with chronic pulmonary embolism, one patient with sarcoidosis and one patient with multiple peripheral pulmonary artery stenosis due to the clinical and hemodynamic similarities with PAH. The patients underwent right heart catheterisation on 60 occasions including the baseline diagnostic investigation (n=42) and follow-up evaluation (n=18). The follow-up catheterisation was justified by the clinical situation. One patient underwent right heart catheterisation on four occasions, one on three occasions and thirteen patients on two occasions.

All the patients were in sinus rhythm. Doppler echocardiography was performed simultaneously during catheterisation (n=22), the same day (n=15) or during the preceding or following 24 hours (n=39). In six cases, there was a delay of more than 48 hours. Doppler echocardiography measurements were made with the observer blinded to the cardiac catheterisation data.

To identify risk factors for death while waiting time for LTx and especially to investigate the prognostic value of pulmonary hemodynamics and right heart function as compared with lung function tests and gas exchange, we retrospectively studied (Paper II) 233 patients with end-stage lung disease who were listed for bilateral or single lung transplantation at Sahlgrenska University Hospital from January 1990 to December 2003. Patients with IPAH or Eisenmenger's syndrome were excluded from the study. Data collected during the evaluation for LTx included age, sex, height, body weight, medical history, diagnosis, the results of

coronary angiography, invasive hemodynamics, echocardiography, radionuclide ventriculography and dynamic spirometric tests and arterial blood gas analysis, respectively. Only patients who had undergone right heart catheterisation were included in the final analysis (n = 177). The patients were divided into two groups: survivors (transplanted or still waiting) and non survivors. We separately compared patients with COPD/ α 1ATD and ILD (idiopathic pulmonary fibrosis, sarcoidosis, histiocytosis, lymphangiomyomatosis and others). All pre-transplant investigations were performed at Sahlgrenska University Hospital. Follow-up was complete and outcome was determined for all patients by the end of the study, 31 December, 2003.

To assess serum concentrations of VEGF, PDGF-BB, TGF- β 1, IL-6 and ET-1 across the pulmonary circulation in patients with PAH compared with control subjects, we prospectively studied (Papers III and IV) 44 consecutive patients with pulmonary hypertension during diagnostic or follow-up right heart catheterisation and compared them with controls [patients with left-sided Wolff-Parkinson White (WPW) syndrome, who were otherwise healthy n=20]. Blood samples were obtained simultaneously from the pulmonary artery (PA) and radial artery (RA) after hemodynamic recording and 10 minutes of rest in patients. Simultaneous blood sampling (PA/artery or PA/left atrium) in the control group was performed 30 minutes after the ablation procedure. Age, gender, etiology, NYHA class, six minute walking test, current medication, basic laboratory tests, hemodynamics and outcome (alive/dead) were documented for all PAH patients. The first patient was included in the study in February 2004. The follow-up was complete and outcome was determined for all patients by the end of the study, 31 December, 2007. The mean follow-up time for PAH patients was 814 ± 442 days.

A subgroup of patients with PH (n=13) received an intravenous epoprostenol infusion as a part of the clinical evaluation. After baseline measurements had been performed intravenous prostacycline was started with a dose of 2.5 ng/kg/min. The dose was increased by 2.5 ng/kg/min every 10 minutes until either a fall in mean arterial pressure for a least 15 mm Hg, but not below 60 mm Hg or symptomatic side effects (headache, jaw pain, severe flush, thoracic oppression) occurred.

Hemodynamic measurements (Papers I, II, III and IV)

Right heart catheterisation was performed at rest using the internal jugular vein approach, with a Swan-Ganz pulmonary artery catheter (7F, Baxter Health Care Corp, Edwards Div., Santa Ana, CA, USA) under fluoroscopic guidance. The following variables were measured or derived: PASP, pulmonary artery mean pressure (PAMP), PADP, PCWP, transpulmonary gradient (TPG), CO and PVR. The mean right atrial pressure (RAP) was measured and the RAP in end diastole was determined using the time interval from QRS to pulmonary valve opening.

Cardiac output (CO) was determined by the thermodilution method as the mean of three or five consecutive measurements not varying by more than 10%. The cardiac index was derived from cardiac output divided by the body surface area. The PVR, expressed in Wood units (WU), was calculated using the formula:

$$(1) \text{ PVR} = (\text{PAMP} - \text{PCWP}) / \text{CO}$$

A left radial artery catheter was used to measure systemic arterial blood pressure and systemic arterial oxygen saturation and for blood sampling.

Doppler echocardiography (Paper I)

Echocardiography was performed using the Vivid System Seven (GE/Vingmed, Milwaukee, Wisconsin, USA) ultrasound system. All Doppler echocardiography measurements were performed off line with a sweep speed of 100-200 mm/s. To obtain the best possible alignment between tricuspid regurgitant flow and the continuous-wave Doppler ultrasound beam, colour Doppler flow mapping was used. All the patients were examined using several non-standard projections in order to register the highest tricuspid regurgitant velocity (Figure 2). Most frequently, the highest velocity was obtained in a projection showing the right ventricle in a position between a standard apical four-chamber view and a parasternal view. Importantly, this was also the case when the colour Doppler mapping indicated a seemingly good alignment between flow and ultrasound beam in the apical four-chamber view. Pulmonary flow velocity was recorded by placing a 5 mm pulsed-wave Doppler sample volume in the right ventricular outflow tract at the level of the pulmonary valve. Blood flow velocity in the left ventricular outflow tract was recorded by pulsed-wave Doppler from an apical view. Mitral flow was recorded between the mitral leaflets in the four-chamber view. From the mitral velocity tracings, early flow velocity (E) and peak velocity during atrial systole (A) were measured. The E/A ratio was calculated. Pulmonary venous flow velocities

were obtained from the upper right pulmonary vein. Peak velocities during systole (S) and diastole (D) were measured. The S/D ratio was calculated.

The timing of pulmonary valve opening was determined as the time from the QRS complex and the onset of systolic flow in the pulmonary artery registered with pulsed-wave Doppler. This time interval was superimposed onto the velocity spectrum of the tricuspid regurgitant jet (*Figure 3*). We measured the RR intervals and did not accept a difference of more than 10% between the pulmonary artery pulsed-wave Doppler and tricuspid continuous-wave Doppler recordings. The velocity across the tricuspid valve at this moment was measured and the pressure gradient between the right ventricle and the right atrium was calculated by applying the simplified Bernoulli equation (pressure gradient=4 x velocity²). Peak pulmonary artery systolic pressure and PADP were obtained by adding the mean RAP to the pressure gradients. The mean RAP was estimated as 5, 10 or 15 mm Hg using the vena cava inferior dimension and collapsibility index with inspiration.

Pulmonary artery mean pressure was calculated as:

$$(2) \text{ PAMP} = \text{PADP} + 0,33 (\text{PASP}-\text{PADP})$$

The stroke volume was calculated as the product of the cross-sectional area of the left ventricular outflow tract and the velocity time integral. Left ventricular diastolic function was evaluated by integrating mitral and pulmonary venous flow profiles according to guidelines. Normal PCWP was assumed to be 9 mm Hg (normal range 6-12 mm Hg) in the non-invasive assessment of TPG (PAMP-PCWP) and PVR using the formula above (1).

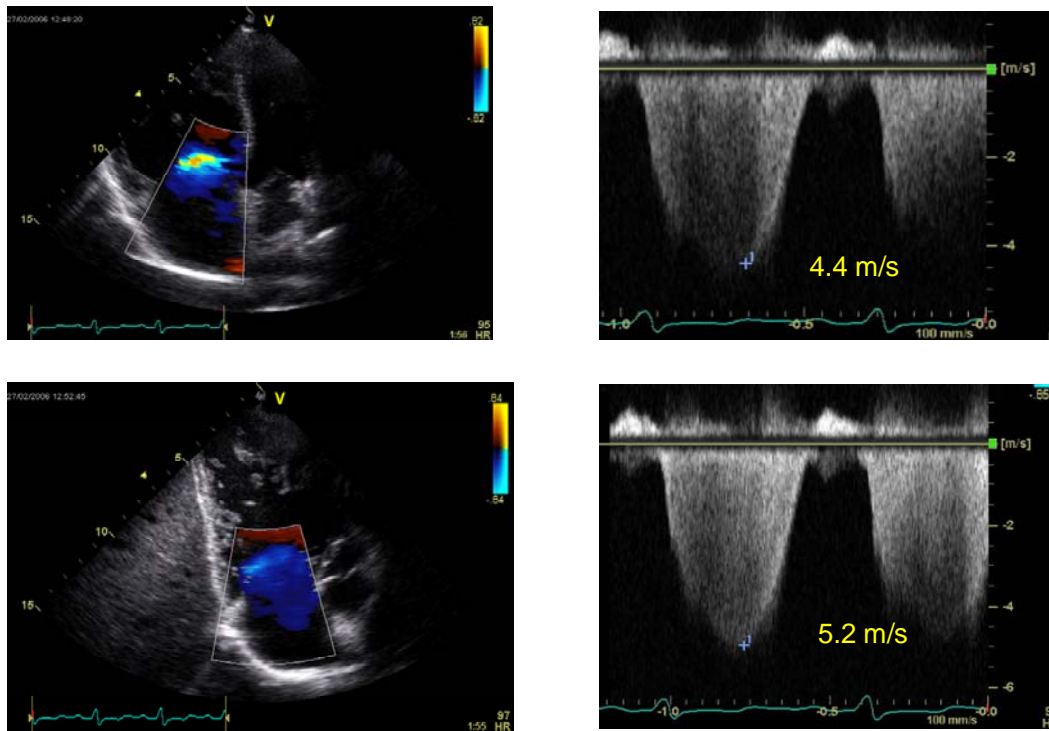


Figure 2.

Top: Standard apical four-chamber view showing mild TR and peak TR velocity of 4.4m/s with a peak gradient of 77.4 mm Hg. Bottom: Non-standard projection in the same patients and on the same occasion showing a higher TR velocity of 5.2 m/s, with a peak gradient of 108.2 mm Hg

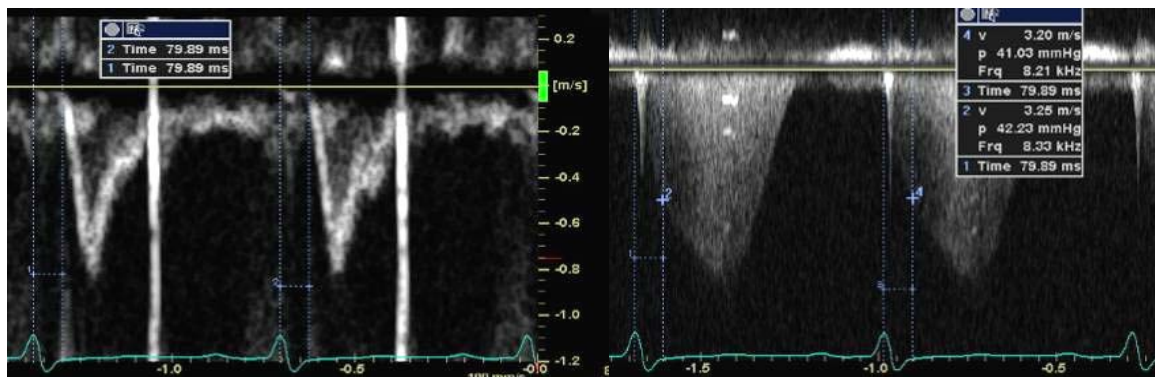


Figure 3.

The timing of onset of flow in the pulmonary artery is determined from the QRS (*left*) to the leading edge of the velocity spectrum sampled at the level of the pulmonary valve. This time interval ($1=80$ ms) is superimposed on the tricuspid regurgitant envelope (*right*) and the velocity ($2=3.25$ m/s) and the gradient is determined (42 mm Hg). With an estimated mean RAP of 5 mm Hg, the PADP would be 47 mm Hg.

Estimation of circulating levels of VEGF, PDGF-BB, TGF- β 1, ET-1 and IL-6 (Papers III and IV)

The transpulmonary gradient across the lungs was assessed by measuring the levels of the growth factors, ET-1 and IL-6 in blood samples taken simultaneously from the mixed venous blood of the pulmonary artery (venous samples) and radial artery/left atrium/femoral artery (arterial samples).

Serum was prepared by drawing 9 ml of blood in Venosafe tubes containing a clot activator (Terumo Europe N.V., Leuven, Belgium) and then allowing the tubes to stand for 60 min at 22°C to ensure full clotting of serum. These samples were centrifuged shortly after clot formation. All samples were stored at -70°C in aliquots and thawed only before measurement. The levels of the VEGF, PDGF-BB, TGF- β 1, ET-1 and IL-6 were assessed using an enzyme-linked immunosorbent assay for these factors (Quantikine, R&D Systems Minneapolis, MN). The minimum detectable level of VEGF, PDGF-BB and TGF- β 1 was 9 pg/ml, 15 pg/ml and 4.61 pg/ml respectively. The minimum detectable level of ET-1 ranged from 0.023-0.102 pg/ml (the mean value was 0.064 pg/ml). The minimum detectable level of IL-6 was less than 0.7 pg/ml.

STATISTICAL ANALYSIS

Data were entered in an electronic database and analysed using an SPSS program (version 12.0.1 and 15.0.1 for Windows, SPSS Inc., Chicago, IL, USA). Normally distributed continuous variables are expressed as the mean \pm SD. The relationship between two methods was assessed by linear regression and Bland-Altman analyses⁹⁴. A paired Student's t-test was used to compare continuous data. Comparisons between groups were performed using an independent - samples Student's t- test.

If variables did not follow the normal distribution, statistical analysis was performed using non-parametric methods and summary measures were presented as the median and interquartile range. The differences between the levels of these variables among PAH patients versus controls were tested using the Mann-Whitney U-test. The differences between the levels of growth factors and IL-6 in the radial artery versus the pulmonary artery in the same group (PAH patients/controls) were tested using Wilcoxon's signed-rank test.

Potential risk factors were initially analysed for significant association with mortality on the waiting list using the Cox proportional hazards model for continuous variables. Risk factors with a level of significance defined as $p < 0.2$ (<0.05 in paper II) in the univariate analysis

were included in the multivariable model. Actuarial survival was determined by the Life Table method. Kaplan-Meier graphs were used in the survival analysis and the log rank test was used to test for statistically significant differences between the curves.

Spearman's rank correlation was used to examine the correlation coefficient. The standard multiple regression model was used to assess the opportunity to predict the serum levels of ET1 using different hemodynamic and clinical variables.

In order to evaluate the inter-individual variability, measurements were made by two different investigators on the same recording. The variability was described by the coefficient of variation, which was expressed as the mean value of differences divided by the mean value of two measurements.

A probability value of < 0.05 was considered statistically significant.

RESULTS

Doppler echocardiography versus catheterisation (Paper I)

Doppler echocardiography performed simultaneously with catheter investigations showed good correlations and small absolute differences between catheter and Doppler (Table 2, Figures 4-5). The agreement between non-simultaneous Doppler echocardiography and the corresponding catheter hemodynamic measurements is illustrated in *Table 3*. The correlation between catheter and non-simultaneous Doppler data was good and the mean differences were small, except for PADP.

Table 2.

Comparison between catheter hemodynamic assessment and simultaneous Doppler echocardiographic data (Doppler)

Variable	MRAP (mmHg) n = 22	PASP (mmHg) n = 22	PADP (mmHg) n = 20	PAMP (mmHg) n = 20	TPG (mmHg) n = 20	CO (l/min) n = 16	PVR (WU) n = 15
Catheter	6 ± 6	73 ± 22	27 ± 12	46 ± 15	38 ± 13	4.8 ± 1.5	9.2 ± 5.6
Simultaneous Doppler	8 ± 4	72 ± 19	30 ± 7	44 ± 11	35 ± 11	4.5 ± 1.4	9.0 ± 5.3
Mean difference ± SD	-1.5 ± 3.1	0.7 ± 7.8	-2.6 ± 6.4	1.4 ± 5.8	2.9 ± 5.1	0.3 ± 0.8	0.3 ± 2.1
Correlation coefficient-R	0.88	0.94	0.89	0.95	0.92	0.86	0.93
p-value catheter /Doppler	0.04	0.69	0.08	0.31	0.02	0.1	0.65

Abbreviations as in Table 3.

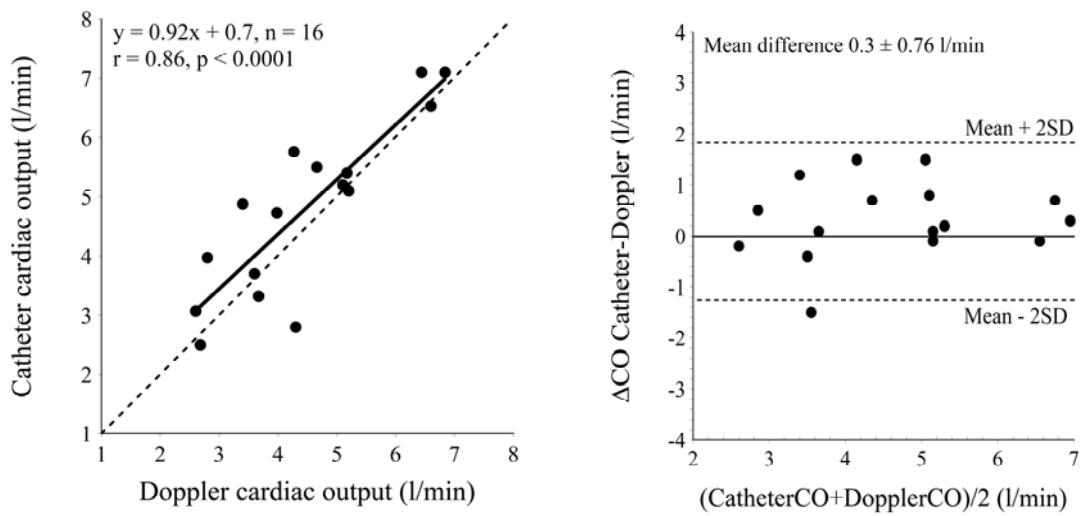


Figure 4. Correlation between simultaneous catheter and Doppler cardiac output (CO) (*left*). The Bland-Altman plot (*right*) shows the mean difference (solid line) and ± 2 SD (dotted lines).

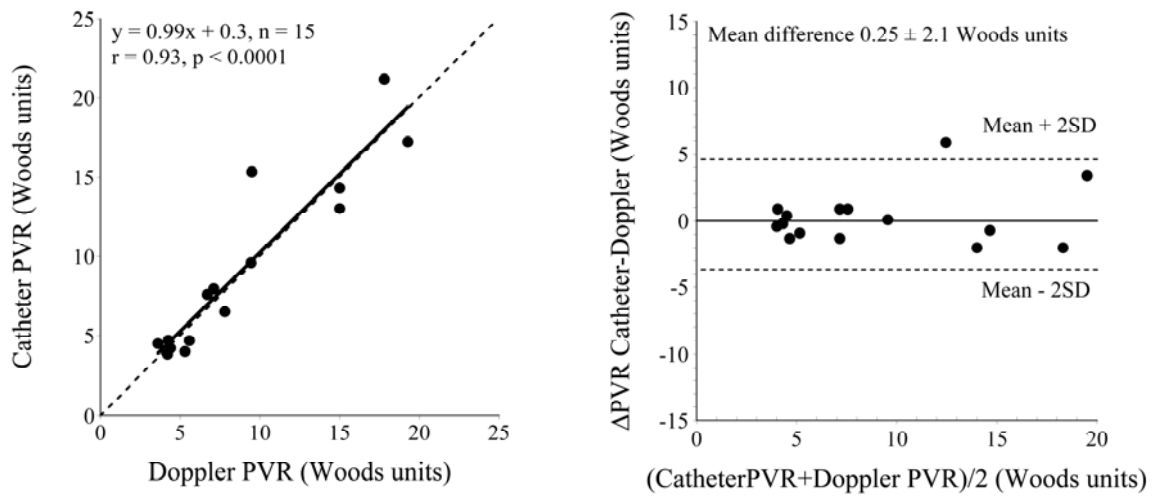


Figure 5. Correlation between simultaneous catheter and Doppler pulmonary vascular resistance (PVR) (*left*). The Bland-Altman plot (*right*) shows the mean difference (solid line) and ± 2 SD (dotted lines).

Table 3.

Comparison between catheter hemodynamic assessment and non-simultaneous Doppler echocardiographic data (Doppler)

Variable	MRAP (mmHg) n = 60	PASP (mmHg) n = 56	PADP (mmHg) n = 60	PAMP (mmHg) n = 56	TPG (mmHg) n = 55	CO (l/min) n = 57	PVR (WU) n = 52
Catheter	7 ± 6	83 ± 26	31 ± 13	52 ± 18	43 ± 17	4.7 ± 1.5	10.9 ± 6.1
Non-simultaneous Doppler	8 ± 4	84 ± 25	38 ± 15	54 ± 17	44 ± 17	4.9 ± 1.5	10.1 ± 5.4
Mean difference ± SD	-1.1 ± 3.2	-1.7 ± 12.3	-7.0 ± 7.3	-2.0 ± 7.2	-1.2 ± 7.4	-0.3 ± 1.1	0.8 ± 2.4
Correlation coefficient-R	0.82	0.88	0.87	0.91	0.90	0.75	0.92
p-value catheter vs. Doppler	0.01	0.3	0.0001	0.04	0.22	0.06	0.02

Mean (SD). MRAP, mean right atrial pressure; PASP, pulmonary artery peak systolic pressure; PADP, pulmonary artery end diastolic pressure; PAMP, pulmonary artery mean pressure; TPG, transpulmonary gradient; CO, cardiac output; PVR, pulmonary vascular resistance; WU, Wood units.

The predictors of mortality while awaiting lung transplantation (Paper II)

The outcome for patients accepted for lung transplantation is shown in Table 4. The mean waiting time for patients transplanted during the study period was 371±326 days and the mean time to death on the waiting list was 445±476 days. Patient characteristics, hemodynamics, lung function data and gas exchange values for non-survivors and survivors respectively are presented in Table 5.

The hemodynamic variables that appeared to be relevant in the univariate analysis were PVR, systemic vascular resistance, mixed venous oxygen saturation (SVO₂) and right ventricular ejection fraction (RVEF) during exercise. The results of the multivariate analysis are presented in Table 6. When COPD/α1ATD and ILD were compared, patients with ILD had a mortality rate that was twice as high on the waiting list (Figure 6). In patients with ILD, pulmonary artery mean pressures (PAMP) and PVR were higher than in patients with COPD/

α 1ATD (27 ± 12 vs. 22 ± 6 mm Hg, $p=0.002$ and 4.3 ± 2.9 vs. 3.2 ± 1.5 WU, $p=0.002$ respectively), whereas RVEF during exercise was lower in the ILD group (0.36 ± 0.1 vs. 0.42 ± 0.1 , $p=0.005$). When survivors and non-survivors with ILD were analysed separately, survivors had lower PVR (3.6 ± 1.9 vs. 6.2 ± 4.3 WU, $p=0.007$) and higher CI (3.0 ± 0.8 vs. 2.3 ± 0.6 L/min/m², $p=0.01$). However, lung function and gas exchange results were similar among survivors and non-survivors in the ILD group. The probability of survival was lower among ILD patients with PVR > 3 Wood units ($p=0.01$; Figure 7). All deaths in the ILD group occurred within twenty months after listing (83% during the first year).

In patients with COPD/ α 1ATD, there were no statistically significant differences in pulmonary hemodynamics between survivors and non-survivors (PAMP 22 ± 4 vs. 22 ± 6 mm Hg, $p=0.78$; CI 3.1 ± 0.6 vs. 2.9 ± 0.6 L/min/ m², $p=0.22$; PVR 2.7 ± 4.3 vs. 3.3 ± 1.6 WU, $p=0.18$), but there were significant differences in spirometric data (FVC% of predicted and FEV1% of predicted were lower in non-survivors ($p=0.01$ $p<0.0001$) respectively).

Table 4.

Outcome for patients listed for Ltx between 1990- 2003 who underwent right heart catheterisation

Diagnosis	Accepted for LTx (n)	Transplanted (n)	Mortality on the waiting list (n)
α 1 ATD	56	49	7
COPD	61	50	9
CF	14	12	2
ILD	46	32	12
Total	177	143	30

α 1 ATD- alpha 1 antitrypsin deficiency; COPD- Chronic obstructive pulmonary disease; CF- Cystic fibrosis; ILD- Interstitial lung disease

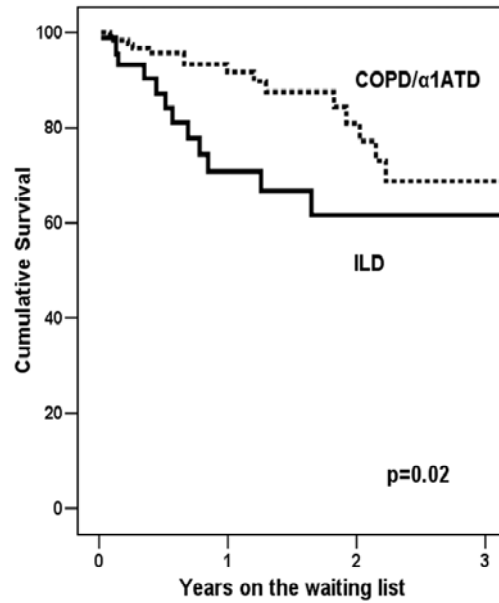


Figure 6. Differences in survival between patients with COPD/ α 1ATD and ILD

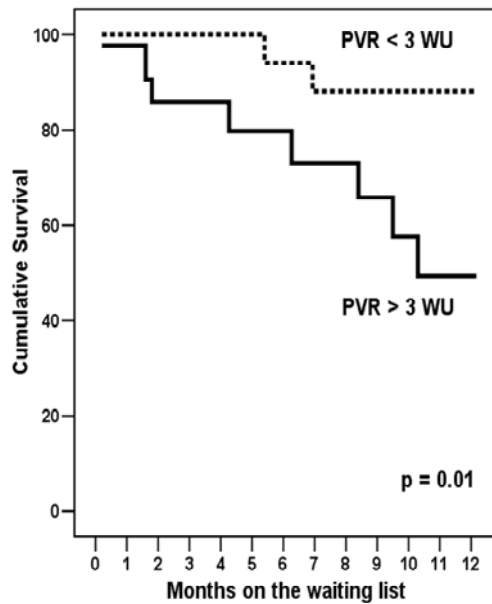


Figure 7. Probability of survival of patients with ILD on the waiting list stratified by PVR

Table 5.

Patients' characteristics, hemodynamics, dynamic spirometric indices and gas exchange at time of referral for transplantation

	Non-survivors		Survivors		p value
	Value	n	Value	n	
<i>General characteristics</i>					
Age, yr	50 ± 8	30	49 ± 9	147	0.44
Gender,F;no (%)	16(53)	30	91(62)	147	0.33
BMI	21 ± 4.5	30	21.3 ± 4.2	147	0.69
<i>Hemodynamics</i>					
Heart rate	93 ± 12	29	90 ± 14	144	0.28
RAP, mm Hg	3 ± 5	30	3 ± 2	147	0.60
PAMP, mm Hg	25 ± 11	29	23 ± 8	147	0.24
CI, L/min/m ²	2.8 ± 0.8	30	3.0 ± 0.7	147	0.36
PVR, Wood units	4.2 ± 3.3	30	3.3 ± 1.7	147	0.03
SVR, Wood units	21 ± 8.4	23	19 ± 5.2	117	0.13
SVO ₂ (%)	67 ± 12	20	71 ± 5	73	0.03
RVEF - at rest (%)	38 ± 11	24	40 ± 9	130	0.37
RVEF - exercise (%)	34 ± 14	16	41 ± 10	99	0.03
<i>Dynamic spirometric tests</i>					
FVC%, predicted	42 ± 14	30	51 ± 17	147	0.01
FEV ₁ %, predicted	27 ± 15	30	25 ± 14	147	0.47
<i>Gas exchange</i>					
P _a O ₂ , kPa	7.8 ± 1.7	30	8.8 ± 7.8	143	0.48
P _a CO ₂ ,kPa	6.3 ± 1.4	30	5.9 ± 1.1	143	0.08

All values, except sex are the mean ± SD. Abbreviations: BMI = Body mass index; RAM = Right atrial mean pressure; PAMP = Pulmonary artery mean pressure; CI = Cardiac index; PVR = Pulmonary vascular resistance; SVR = Systemic vascular resistance, RVEF = Right ventricular ejection fraction (radionuclide ventriculogram); SVO₂ = Mixed venous oxygen saturation, FVC% = Forced vital capacity %, FEV₁ % = Forced expiratory volume %, P_aO₂ = arterial oxygen partial pressure, P_aCO₂ = arterial carbon dioxide partial pressure.

Table 6.

Multivariate analysis of predictors of mortality on the waiting list

Variable	Hazard ratio	95% confidence interval	p value
PVR	1.23	1.06 - 1.42	0.005
FVC%, predicted	0.96	0.94 - 0.99	0.004
Diagnosis (ILD / COPD/ α 1ATD)	1.36	0.58 - 3.19	0.48

For abbreviations, see Table 5.

Transpulmonary gradient of VEGF, PDGF-BB, TGF- β 1, IL-6 and ET-1 in patients with PAH (Papers III and IV)**Study population**

The distribution of different forms of PAH and patient characteristics are shown in Table 7. NYHA functional class, six minute walking distance and the hemodynamic variables of patients with PAH are presented in Table 8. There were no significant differences regarding age between control subjects and patients with idiopathic pulmonary hypertension (39 ± 16 vs. 48 ± 18 years, $p=0.13$), but patients with PAH associated with collagen vascular diseases were older than controls (62 ± 12 vs. 39 ± 16 years, $p<0.001$).

Growth factors, IL-6 and ET-1 across the lung circulation in patients with PAH

The median serum levels of VEGF, PDGF-BB, TGF- β 1, IL-6 and ET-1 in arterial and venous blood samples were significantly higher in patients with PAH than in control subjects (Figure 8, A-E).

When the group of patients with IPAH was compared with the group of patients with collagen vascular diseases, there were no significant differences regarding arterial and mixed venous levels of VEGF, PDGF-BB, TGF- β 1, IL-6 and ET-1 (Table 9).

Table 7. Patients' characteristics

Diagnosis	n	Gender (F/M)	Age (mean; range) year
IPAH	16	13/3	48 (20-77)
PAH associated with collagen vascular disease:	24	19/5	62 (30-76)
a/ Scleroderma	13	9/4	63 (37-76)
b/ Systemic lupus erythematosus	3	3/0	51 (44-63)
c/ Rheumatoid Arthritis	4	3/1	59 (30-73)
d/ Mixed connective tissue disease	3	3/0	68 (63-72)
e/ Dermatomyositis	1	1/0	69
Others:	4	3/1	38 (20-58)
a/ Portopulmonary hypertension	1	1/0	34
b/ Eisenmenger syndrome	2	2/0	50 (41-58)
c/ Cong. peripheral pulmonary stenosis	1	1/0	20
Total	44	35/9	55 (20-77)
Controls / Wolff-Parkinson-White syndrome/	20	8/12	39 (19-63)

IPAH, idiopathic PAH, Cong, congenital

Table 8. Clinical and hemodynamic characteristics of patients with PAH (n = 44)

Clinical and hemodynamic characteristics	Data
NYHA functional class;	
I/II/III/IV (No)	2/4/35/3
6 MWD, m	342 (211-422)
RAP, mm Hg	5 (3-9)
MPAP, mm Hg	44 (33-56)
PCWP, mm Hg	7 (6-11)
SaO ₂ , %	93.9 (90.3-97.2)
CI, L/min/m ²	2.99 (2.7-3.3)
PVR, WU	7.2 (4.5 -12.3)

NYHA, New York Heart Association functional class; 6 MWD, six minutes walk distance; RAP, right atrial pressure; MPAP, mean pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; SaO₂, arterial oxygen saturation; CI, cardiac index; PVR, pulmonary vascular resistance; WU, Wood units

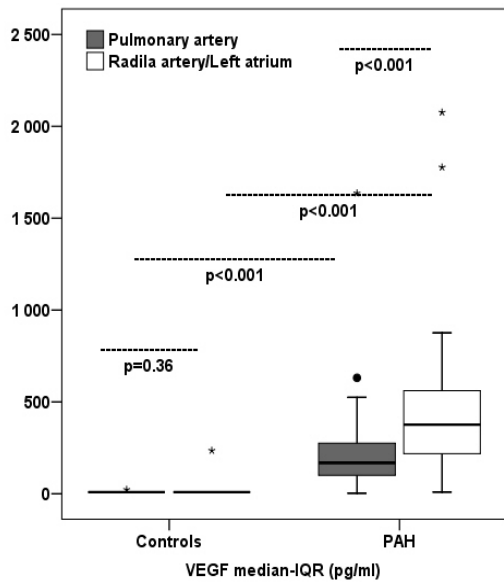


Figure 8-A.
Serum levels of VEGF in controls (n=20) and in patients with PAH (n=44)

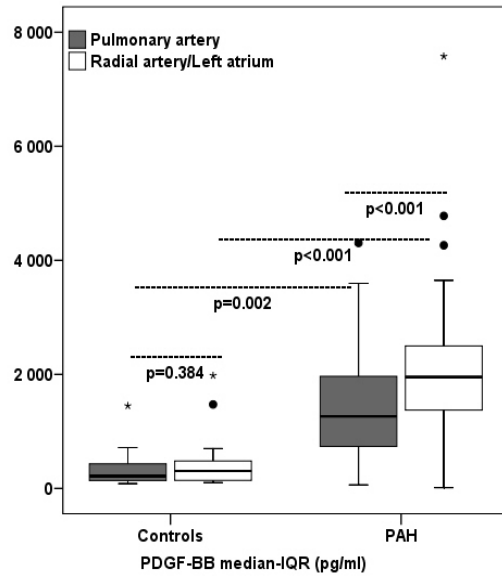


Figure 8-B.
Serum levels of PDGF-BB in controls (n=20) and in patients with PAH (n=44)

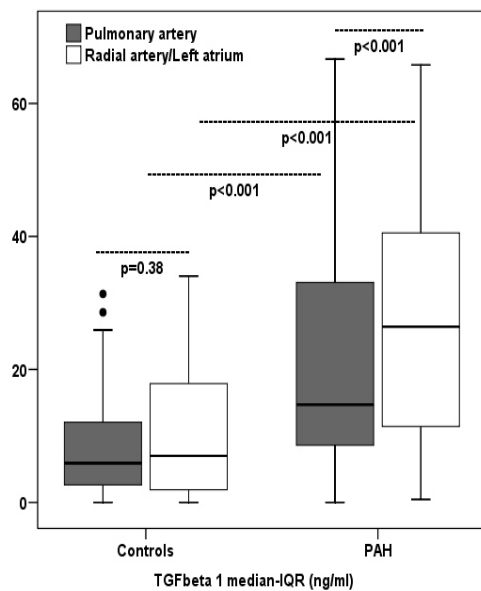


Figure 8-C.
Serum levels of TGF- β 1 in controls (n=20) and in patients with PAH (n=44)

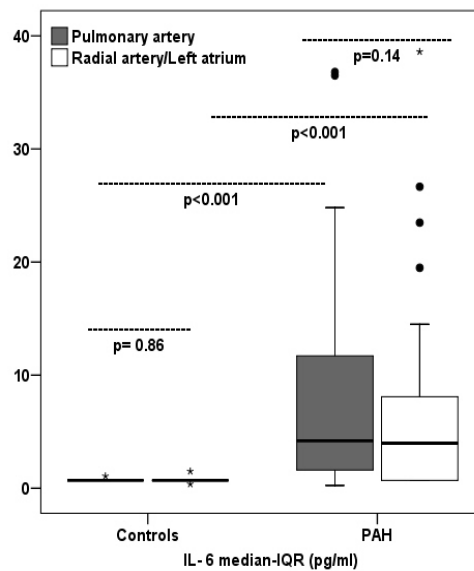


Figure 8-D.
Serum levels of IL-6 in controls (n=20) and in patients with PAH (n=44)

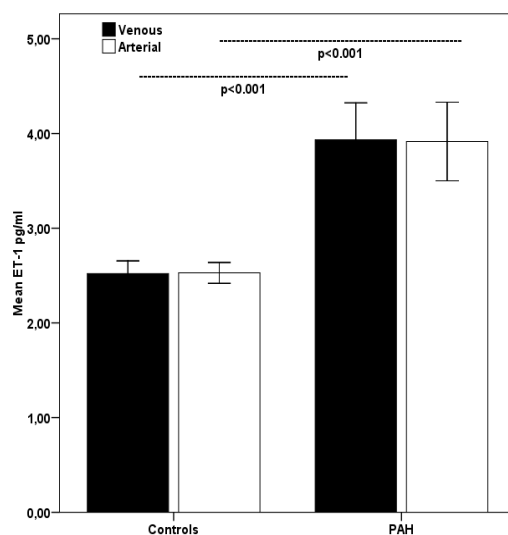


Figure 8-E.
Serum levels of ET-1 in controls
n=20) and patients with PAH (n=39)

Table 9.

IL-6, growth factors and ET-1 levels in patients with idiopathic PAH and in PAH associated with collagen vascular disease*

Variables		IPAH patients n = 16	PAH associated with CVD n = 24	p -value
IL-6 RA	pg/ml	4.3 (1.3-7.4)	3.3 (0.7-11.1)	0.744
IL-6 PA	pg/ml	4.1 (1.4-8.5)	4.5 (1.2-16.9)	0.391
VEGF RA	pg/ml	397 (141-679)	377 (247-502)	0.868
VEGF PA	pg/ml	176 (52-343)	159 (113-208)	0.814
PDGF-BB RA	pg/ml	1634 (1353-2150)	2004 (1385-2608)	0.44
PDGF-BB PA	pg/ml	1440 (954-1952)	977 (603-1713)	0.172
TGF-β1 RA	ng/ml	21.4 (11-42.3)	29.4 (12 -39)	0.619
TGF- β1 PA	ng/ml	14.8 (7.4-37.3)	14.7 (8.3-32.2)	0.868
ET-1 RA/LA	pg/ml**	4.2 ± 1.2, n=14	3.8 ± 1.5, n=18	0.39
ET-1 PA	pg/ml **	4.3 ± 0.9, n=14	3.8 ± 1.5, n=18	0.26

*The data are presented as the median (IQR); IPAH, idiopathic pulmonary arterial hypertension, CVD, collagen vascular disease, RA, radial artery, PA, pulmonary artery

**The data are presented as the mean± SD

Patients receiving specific PAH therapy had arterial and mixed venous blood levels of PDGF-BB, TGF- β 1 and IL-6 respectively than were similar to those of untreated patients ($p=0.41, 0.44; 0.48, 0.49; 0.9, 0.9$; respectively). There was a tendency towards higher arterial levels of VEGF in the treated group compared with the untreated patients (569 ± 549 vs. 363 ± 190 pg/ml, $p=0.09$). We found significantly higher arterial serum levels of VEGF in patients on specific treatment with prostanoids (881 pg/ml; $n=8$) in comparison to untreated PAH patients (363 pg/ml; $n=25$, $p=0.002$). There was no significant correlation between the levels of growth factors, levels of IL-6, and clinical and hemodynamic variables reflecting the severity of pulmonary hypertension. A significant correlation was found between IL-6 levels in arterial and mixed venous samples and CRP levels (peripheral vein) ($r=0.59$, $p=0.004$ and $r=0.44$, $p=0.04$ respectively). TGF- β 1 correlated significantly with PDGF-BB in the pulmonary artery ($r=0.58$, $p<0.001$) but not in the systemic circulation ($r=0.27$, $p=0.07$). A weak correlation was found between VEGF and PDGF-BB levels in the pulmonary artery ($r=0.36$, $p=0.017$).

Association between the levels of growth factors and IL-6 with outcome

The 15 patients that died during follow-up, displayed a tendency towards lower arterial VEGF serum levels than those who survived (311 ± 48 vs. 525 ± 85 pg/ml; $p=0.09$). The results of the univariate analysis of potential risk factors associated with mortality during this period are shown in Table 10. The result of the multivariate analysis is presented in Table 11. Elevated serum levels of IL-6 emerged as an independent risk factor for mortality during the observation period.

Table 10.

Univariate analysis of possible predictors of mortality in patients with PAH

Variable	Hazard ratio	95% confidence interval	p value
IL-6 RA pg/ml	1.07	1.02-1.13	0.012
VEGF RA pg/ml	0.99	0.99-1.0	0.06
PDGF-BB RA pg/ml	1.0	0.99-1.0	0.12
TGF- β 1 RA ng/ml	1.0	0.97-1.03	0.84
Age (years)	1.05	1.01-1.09	0.026
PVR (WU)	1.03	0.93-1.13	0.586
6MWD m	0.997	0.99-1.01	0.128
SaO ₂ %	0.94	0.879-1.01	0.11

RA, radial artery, PVR, pulmonary vascular resistance, 6 MWD, 6 minute walking distance

Table 11.

Multivariate predictors of mortality in patients with PAH

Variable		Hazard ratio	95% confidence interval	p value
IL-6 RA	pg/ml	1.08	1.02-1.15	0.012
VEGF RA	pg/ml	0.99	0.99-1.01	0.152
PDGF-BB RA	pg/ml	1.0	0.99-1.0	0.285
Age	(years)	1.03	0.98-1.09	0.207

RA, radial artery

Serum ET-1 levels across the lung circulation in patients with PAH

The arterial to venous ratio of ET-1 (serum ET-1 levels in the systemic circulation divided by serum ET-1 levels in the pulmonary circulation- transpulmonary gradient) was similar in patients with PAH and control subjects (p=0.65).

When the group of patients with IPAH was compared with the group of patients with collagen vascular diseases, there were no significant differences regarding arterial and mixed venous levels of ET-1, although patients with IPAH had a significantly higher MPAP and PVR (Table 12). There were no significant differences in the transpulmonary gradient of ET-1 between these two groups of patients.

Table 12.

ET-1 levels in patients with idiopathic PAH and in PAH associated with CVD*

Variables	IPAH n = 14	PAH associated with CVD n = 18	p-value
ET-1 RA/LA pg/ml	4.2 ± 1.2	3.8 ± 1.5	0.39
ET-1 PA pg/ml	4.3 ± 0.9	3.8 ± 1.5	0.26
Δ ET-1 (RA-PA) pg/ml	- 0.04 ± 0.59	0.04 ± 0.28	0.63
RA/PA ratio	0.98 ± 0.13	1.01 ± 0.07	0.45
RAP mm Hg	10 ± 7	6 ± 4	0.12
MPAP mm Hg	58 ± 19	40 ± 14	0.008
PVR Wood units	12.7 ± 6.3	7.4 ± 3.7	0.01

* The data are presented as the mean ± SD

Transpulmonary ET-1 gradient during with epoprostenol infusion

Acute pharmacological intervention with intravenous epoprostenol did not change the transpulmonary ET-1 gradient, even though there was a significant increase in the cardiac index (CI) and a significant decrease in PVR (Table 13).

Transpulmonary ET-1 gradient with chronic specific PAH treatment

There was significant increase in serum ET-1 levels in both the systemic and the pulmonary circulation but the balance between the clearance and release of ET-1 across the lung circulation was unaltered (Table 14).

Correlation between hemodynamic and clinical variables and ET-1 serum levels

There was a significant correlation between ET-1 levels and clinical and hemodynamic parameters associated with the severity of PAH (Table 15). A significant relationship was observed between estimated glomerulus filtration rate (eGFR) and arterial and mixed venous level of ET-1 for the whole study population (PAH patients and control subjects) ($r=-0.59$, $p<0.001$; $r=0.57$, $p<0.001$ respectively). Patients with reduced renal function had significantly higher levels of ET-1 in their arterial circulation and pulmonary circulation ($p=0.004$ and $p=0.003$). Controls had a normal kidney function with a mean eGFR of 121 ± 25 ml/min/1.73 m². Patients who died during this study had a significantly lower eGFR (59 ± 12 vs. 84 ± 33 ml/min/1.73 m², $p=0.018$).

Predictions of serum ET-1 levels

Only serum creatinine and pulmonary vascular resistance made a significant contribution to the prediction of the dependent variable, ET-1 ($p<0.001$ and $p=0.009$ respectively) using a multiple regression model, including RAP, MPAP, PVR, age, BMI, 6MWT, s-NT proBNP and sCr. This model explains 62% of the variance in arterial ET-1 levels.

Table 13. The levels ET-1 in RA and PA at baseline and during epoprostenol infusion*

Variables	Baseline n = 13	Epoprostenol n = 13	p-value
ET-1 RA , pg/ml	3.3 ± 0.7	3.3 ± 0.6	0.6
ET-1 PA , pg/ml	3.4 ± 0.7	3.5 ± 0.9	0.51
Δ ET-1 (RA-PA), pg/ml	-0.08 ± 0.25	-0.22 ± 0.51	0.38
RA/PA ratio	0.98 ± 0.07	0.96 ± 0.09	0.52
HR, beat/min	81 ± 9	91 ± 13	0.004
MPAP, mm Hg	46 ± 12	44 ± 14	0.26
CI, L/min/m ²	2.6±0.6	3.3±0.7	<0.001
PVR, Wood Units	8.4 ± 3.1	6.2 ± 2.7	0.001
MAP mm Hg	94 ± 16	81 ± 11	0.003

*The data are presented as the mean ± SD

HR, heart rate, MAP, mean arterial pressure

Table 14.

Arteriovenous ET-1 gradient with chronic specific PAH treatment*

Variables	Baseline n = 9	Follow-up n = 9	p - value
ET-1 RA , pg/ml	3.5 ± 1.0	4.5 ± 0.9	0.05
ET-1 PA , pg/ml	3.5 ± 0.79	4.5 ± 0.89	0.02
Δ ET-1 (RA-PA), pg/ml	0.02 ± 0.49	-0.02 ± 1.8	0.83
RA/PA ratio	1.00 ± 0.12	0.99 ± 0.04	0.91
MPAP, mm Hg	54 ± 10	50 ± 18	0.59
CI , L/min/m ²	2.7±0.4	2.9±0.6	0.46
PVR, Wood units	9.1 ± 2.4	8.5 ± 4.4	0.69

*The data are presented as the mean ± SD

Table 15.

Correlations of ET-1 serum levels with hemodynamics and clinical variables

Correlations	Radial Artery		Pulmonary Artery	
	r	p-value	r	p-value
RAP	0.56	<0.001	0.62	<0.001
MPAP	0.39	0.013	0.42	0.008
CI	-0.55	<0.001	-0.60	<0.001
PVR	0.53	<0.001	0.56	<0.001
SaO ₂	-0.17	0.31	-0.17	0.29
SvO ₂	-0.52	0.001	-0.55	<0.001
6 MWD	-0.29	0.08	-0.27	0.10
NYHA class	0.36	0.02	0.36	0.02

SVO₂, mixed venous oxygen saturation, BMI, body mass index, for the other abbreviations see table 3.

DISCUSSION

Doppler echocardiography in the assessment of pulmonary hemodynamics (Paper I)

This study demonstrates that a comprehensive haemodynamic assessment, including the transpulmonary gradient and PVR, can be obtained by Doppler echocardiography in patients with PAH. This finding may be of clinical relevance as the prognosis in patients with PAH is closely related to hemodynamic indices of right ventricular function.¹¹

Catheter versus Doppler derived pulmonary artery pressure and PVR

There were strong correlations with the catheter investigations for both simultaneous and non-simultaneous Doppler-estimated pulmonary artery pressures and volume flow. Non-simultaneous estimated pressures showed wider limits of agreement, illustrating the importance of day-to-day variations in hemodynamics. Further, an overestimation of the PADP pressure using Doppler echocardiography that cannot be explained by day-to-day variations. Two possible reasons may explain this overestimation of PADP. Firstly, the overestimation is probably related to the addition of estimated mean RAP to Doppler-derived PADP. From our findings, we know that the RAP at the time of pulmonary valve opening is lower than the mean RAP (mean difference 2 mmHg, range -2 to 7 mmHg, $p < 0.0001$). In addition, the semi-quantitative assessment of mean RAP in itself tended to overestimate the catheter mean RAP (mean difference 1 mmHg, range -6 to 8 mmHg, $p = 0.03$). Secondly, the overestimation of PADP might be explained by the non-simultaneous assessment of the timing of pulmonary valve opening. Low velocities were removed by filtering to reduce noise and this might have caused a systematically overestimated interval from QRS to valve opening.

Previous investigators have shown a close agreement between catheter and Doppler echocardiography during simultaneous measurements of PASP⁹⁵, but they reported an underestimation by Doppler echocardiography in a large group of patients with PAH²². It is important to note that, in the present study, great care was taken to find the highest tricuspid regurgitation velocities in order to reduce underestimation due to angle errors and in most cases a non-standard projection was used. This approach was not used by Hinderliter et al. and may explain the closer agreement between Doppler echocardiography and catheter-derived data in the present study.

Cardiac output and left ventricular filling pressure

In patients with PAH, CO is an important prognostic variable reflecting right ventricular function. Cardiac output determination with Doppler echocardiography has been used in clinical trials evaluating treatment effects⁹⁶. However, to our knowledge, CO determination with Doppler echocardiography has not been validated against thermodilution or direct Fick methodology in patients with PAH. In patients with PAH, right ventricular pressure is usually markedly elevated, which affects the geometry of the left ventricle^{97, 98}. The agreement between Doppler echocardiography and thermodilution in the present study was, however, similar to that in a previous report on healthy subjects and on patients with left heart diseases, with a mean difference of 0.3 l/min⁹⁹.

It is unclear whether changes within the left ventricle and abnormal diastolic ventricular interaction in patients with PAH also affect the accuracy of Doppler echocardiography assessments of PCWP. In our study, we used the pulmonary and mitral flow profile to assess left ventricular filling pressures and PCWP. The majority of the patients had relaxation abnormality of the left ventricle, a finding that is in agreement with those of other investigators^{32, 33}, but all the patients were found to have a pattern of mitral and pulmonary flow indicating normal PCWP. However, only patients with normal PCWP were included and we are therefore unaware of the ability of Doppler echocardiography to identify increased PCWP in patients with PAH. On the other hand, patients with established PAH have normal PCWP (<15 mmHg)¹⁰⁰ and increased mean pulmonary artery pressure by definition. Inaccuracies in Doppler echocardiography assessments of PCWP will therefore have a limited impact on the calculation of the transpulmonary pressure gradient and PVR.

Predictors of the survival of patients on the waiting list for lung transplantation (Paper II)

The increased PVR was an independent predictor of pre- transplant mortality in patients with end-stage lung diseases waiting for LTx. Furthermore, mortality was found to be twice as high in patients with ILD compared with COPD/ α 1ATD patients which is consistent with previous studies^{101, 102}.

Pulmonary artery mean pressure, PVR and definition of pulmonary hypertension

Interestingly, pulmonary artery pressure was not associated with outcome in the present study. However and importantly, mean PA pressure reflects only one aspect of pulmonary hemodynamics, while PVR is coupled to cardiac output, which is in turn dependent on cardiac function. This association is supported by the finding in the present study that reduced right ventricular performance during exercise was found to be associated with an adverse outcome

in the univariate analysis. Almost all the patients (95%) in the present study had normal left ventricular ejection fraction and left heart filling pressures, which argues against left ventricular dysfunction as a contributory factor to the increased PVR. Similar findings with a stronger influence on survival by PVR compared with pulmonary artery pressure have been reported in patients with pulmonary arterial hypertension^{103, 104}. In nearly 50% of the patients in the present study PVR was pathologically increased (≥ 3 Wood units), while pulmonary hypertension, defined as a mean pulmonary artery pressure of ≥ 25 mm Hg, was found in only 33% of the patients. A similar relationship has previously been reported in other studies in patients with severe lung diseases^{36, 105}.

Pulmonary hypertension in different lung diseases

In previous studies, PVR has not been reported separately as a predictor of pre-transplant outcome in patients with lung diseases awaiting LTx. The findings in the present investigation are supported by Lettieri et al.³⁶ who recently found that, in patients with severe idiopathic lung fibrosis, pulmonary hypertension was associated with an adverse outcome. In other investigations in which pulmonary hemodynamics were reported, PVR or pulmonary artery pressures did not correlate with outcome in multivariate analysis^{39, 44, 45}. However, those studies differ from the present one in terms of study population and diagnoses which makes comparisons difficult. Our findings are similar to those in previous studies in which pulmonary artery pressures did not differ significantly between patients who died and who survived to LTx^{39, 45}.

The mechanisms behind pulmonary hypertension and increased PVR in patients with lung diseases are not clear. Whether this is due to endothelial and vascular smooth muscle cell dysfunction, cytokine derangements, genetic factors, progressive fibrosis, hypoxemia, hypercapnia, or vascular remodelling remains uncertain^{36, 40-42}. Correlations between PVR and variables reflecting the severity of the pulmonary disease such as SaO₂, vital capacity and six minute walking distance have been reported³⁶. However, other reports and the present study did not find a robust relationship between dynamic spirometric indices and PVR⁴⁵. Similarly, in a large population of patients with severe COPD investigated using right heart catheterisation¹⁰⁶, the prevalence of severe pulmonary hypertension was 2.7%, but only 1.1% had COPD as the only identifiable cause of pulmonary hypertension. The changes in pulmonary hemodynamics were not explained by the degree of pulmonary dysfunction. The prevalence of pulmonary hypertension in patients with ILD is higher than in patients with COPD. Shorr and colleagues¹⁰⁷ found that approximately a quarter of more than 2,000

patients with idiopathic pulmonary fibrosis who were listed for LTx in the UNOS registry had pulmonary hypertension.

The serum levels of the growth factors and IL-6 across the lung circulation in patients with PAH (Paper III)

The serum levels of VEGF, PDGF-BB, TGF- β 1 and IL-6 were significantly higher in patients with pulmonary hypertension compared with controls. Furthermore, there was a consistent step-up of growth factors across the pulmonary vasculature in PAH patients, which was not found in the control group, suggesting an increased release of these mediators. Both arterial and mixed venous levels and the transpulmonary gradients of growth factors were similar among patients with idiopathic PAH and in patients with PAH associated with connective tissue diseases, indicating that the pulmonary vasculopathy is also a source of the high serum levels of growth factors in systemic autoimmune diseases. A concomitant increase in all three growth factors in patients with PAH suggests common stimuli for an increase in their production or reduced clearance. However, we did not find any significant relationship between the levels of VEGF, PDGF-BB and TGF- β 1 with clinical or hemodynamic variables such as pulmonary artery pressures or pulmonary vascular resistance reflecting the severity of PAH.

In the present study, elevated arterial IL-6 concentrations were independently associated with mortality in the multivariate analysis, suggesting that IL-6 may be a biomarker for the prognosis of PAH. However, there was no step-up of IL-6 across the lungs in either of the study groups, suggesting that systemic levels of IL-6 are not determined by the lungs in PAH.

Vascular endothelial factor and PAH

VEGF is strongly expressed in the plexiform lesions in the lungs of patients with severe idiopathic and secondary forms of PAH^{62, 108}. Experimental studies have shown that chronic hypoxia increases lung tissue VEGF expression and that VEGF is probably a modulator of chronic hypoxia-induced pulmonary vascular remodelling and may also provide protection from disease progression¹⁰⁹. In the current study, we did not find any correlation between VEGF and arterial or mixed venous oxygen saturation. Our results regarding circulating levels of VEGF agree with the findings in a previous report of elevated serum VEGF levels in peripheral venous blood samples from patients with PAH¹¹⁰. In contrast, Benisty et al. found no significant differences in circulating VEGF levels in peripheral venous blood samples between patients and control subjects¹¹¹. In that study, plasma VEGF was measured, and this

may have resulted in an underestimation of circulating VEGF levels, since VEGF can be stored in platelets. We found significantly higher arterial serum levels of VEGF in patients on specific treatment with prostanoids compared with untreated PAH. This is in agreement with a previous study showing that intravenous epoprostenol therapy increased the levels of VEGF in serum and platelet lysate ¹¹⁰. This may be one mechanism behind the beneficial effect of prostacyclin therapy.

Platelet-derived growth factor and PAH

Patients with PAH in the current study had a four to five times higher serum level of PDGF-BB with a significant transpulmonary gradient as compared with control subjects. This finding is consistent with a recent report which demonstrated the increased expression of PDGF in explanted lungs obtained from PAH patients compared with donor lung tissue ⁸⁵. The same investigators also showed that increased PDGF expression was localised to SMC and endothelial cells in small remodelled pulmonary arteries. On the other hand, Eddahibi et al observed that circulating PDGF was slightly decreased in patients with PAH compared with control subjects ¹¹⁰. In their study, blood samples were taken from peripheral veins, which may not reflect the pulmonary circulation. This makes their findings difficult to compare with our results.

In an animal model the PDGF receptor inhibitor (STI571-imatinib mesylate) was recently shown to reverse pulmonary hypertension ⁸³. In small clinical studies, treatment with the PDGF receptor antagonist-imatinib had beneficial effects in patients with advanced PAH. This underscores the hypothesis that PDGF may play an important pathophysiological role in PAH ^{86, 87}.

Transforming growth factor beta 1 and PAH

There is growing evidence that abnormalities in TGF- β superfamily signalling are linked to the pathogenesis of severe PAH ^{70, 75}. In the present study, we found significantly higher levels of TGF- β 1 in PAH patients in comparison to control subjects. Failure of the growth inhibitory effects of bone morphogenetic proteins (BMPs) could contribute to the pulmonary vascular remodelling in PAH. Morrel et al. showed that TGF- β 1 and BMPs inhibited the serum-stimulated proliferation of pulmonary artery smooth muscle cells (PASMC) from normal subjects and patients with secondary PAH (Eisenmenger patients). In contrast, TGF- β 1 enhanced the serum-stimulated proliferation of PASMCs from IPAH patients ⁷⁶. This

raises additional questions about the interactions of the TGF β /BMP pathways with other pathways known to be involved in the regulation of PASMC growth.

We observed a significant correlation between circulating levels of TGF- β 1 and PDGF-BB and VEGF and PDGF-BB in the lung circulation. Our findings agree with published data on the interactions and correlations between these growth factors ¹¹⁰⁻¹¹⁵.

Interleukin -6 and PAH

Patients with PAH associated with connective tissue diseases displayed a tendency towards higher levels of circulating IL-6 compared with patients with IPAH, but the differences were not statistically significant. Similar findings of increased IL-6 serum concentrations in patients with severe PAH were reported by Humbert et al.⁶⁷. In contrast, Hoeper et al. did not find any significant increase in serum IL-6 in patients with PAH ¹¹⁶. In that study blood samples were obtained from a peripheral vein, which makes the result difficult to compare. From the available data, it is also impossible to clarify whether the various study populations were comparable. IL-6, a pro-inflammatory and vasodepressor cytokine, showed a significant association with mortality in patients with PAH. Similar findings have been reported in patients with congestive heart failure ¹¹⁷ and in patients with acute coronary syndromes ¹¹⁸. IL-6 is produced not only by leukocytes ¹¹⁹ but also by the vascular tissue endothelial and smooth muscle cells, myocytes and fibroblasts in various organs, including the lung. These data suggests that IL-6, as a systemic mediator of immune and inflammatory response ¹¹⁹, plays a role in the pathophysiology of different cardiovascular diseases such as acute coronary syndromes, congestive heart failure and pulmonary hypertension ^{117, 118, 120}.

A balance between the clearance and production of ET-1 across the lung circulation

(Paper IV)

The increased levels of circulating ET-1 in patients with PAH in the present study were similar to data published in previous studies ¹²¹⁻¹²³. We did not find any significant differences in the arterial/venous ratio (AV) of ET-1 between patients with PAH and control subjects, similar to the findings in a recent report ¹²². On the other hand, Stewart et al. showed that arterial ET-1 levels were consistently higher than venous levels, with an increased AV ET-1 gradient in patients with IPAH, but in the control group the mean AV ratio was below unity ¹²¹. In patients with secondary pulmonary hypertension, the AV ET-1 gradient in their study was not significantly different from unity. These differences, as compared to the results in the present study, may be explained by different sample sites and different populations of patients

with pulmonary hypertension. In that study, paired samples of arterial blood and venous blood were primarily obtained from the radial artery and from the antecubital vein.

Intravenous epoprostenol did not alter the balance between the clearance and release of ET-1 in the lung circulation in patients with PAH measured as an AV ET-1 ratio, despite significant changes in hemodynamics, suggesting that prostacyclin does not alter ET-1 release or clearance acutely. Wilkens et al. have assessed the influence of inhaled iloprost on the AV ratio of Big ET-1 and observed a significant decrease in the AV ratio of Big ET-1. In their study, seven of 15 patients were receiving long-term treatment with iloprost, whereas all the patients in our intervention with epoprostenol were not receiving any specific therapy for PAH. Furthermore, in the present study, most patients had associated PAH, in contrast to the iloprost intervention trial in which almost all the patients had IPAH. These differences in study populations and treatment make it difficult to compare their results with the findings in the current study and to interpret the inconsistent results. During tilt tests, peripheral ET-levels increase in healthy subjects and, as a result, a possible confounding effect of baroreflex activation effecting ET-1 release during prostacyclin infusion can-not be ruled out in these studies ¹²⁴. Interactions between prostacyclin and the endothelin system have been demonstrated in experimental studies, but there is little information about the effects of prostacyclin on the endothelin system in PAH patients ^{125, 126}. In vitro studies have demonstrated that prostacyclin (PGI₂) and prostaglandin E₂ (PGE₂) cause inhibition of the basal ET-1 secretion of about 40% and an inhibition of serum-stimulated ET-1 secretion of 50% in a dose-related and time-course fashion by possibly stimulating guanylate cyclase ¹²⁷. There is one study showing an influence of chronic intravenous epoprostenol on the transpulmonary gradient of endothelin in patients with PAH ¹²⁸. In that study, intravenous prostacyclin-therapy for three months increased the percentage of patients with an ET-1 AV ratio of < 1 compared with patients receiving conventional treatment. Similar to the data reported by Wilkens et al. ¹²³, they did not find any significant changes in the absolute values of ET-1 during treatment. The AV ratio may therefore be a more sensitive measure of pulmonary clearance and release than the absolute values of endothelin. Our findings have shown that a balance between the clearance and production of ET-1 was maintained during acute vasoreactivity testing with intravenous epoprostenol in untreated PAH patients.

The findings in the present study are consistent with previous observations which indicate that circulating levels of ET-1 reflect the hemodynamic and clinical status of PAH patients. Correlations were observed between ET-1 levels and hemodynamic parameters indicating

severity of pulmonary hypertension, such as pulmonary vascular resistance and right atrial pressure, as different from the findings reported by Stewart et al. and Dupuis and colleagues^{121, 129} but similar to the data reported by Rubens et al. and Nootens et al.^{122, 130}. It is worth noting that the correlation between ET-1 levels and right atrial pressure is of interest, since high venous pressure could be a major mechanism stimulating ET-1 release in peripheral veins and could contribute to the high ET-1 levels in mixed venous blood¹³¹.

Study limitations

I. The paper would have been strengthened if the number of simultaneous investigations was higher. The frequency response of fluid filled pulmonary catheters is inadequate for the measurement of instantaneous pressures but this type of catheter is routinely used for clinical decision making. Another potential source of error is the fact that tricuspid and pulmonary velocities are not recorded simultaneously with calculation of PADP. Inaccuracies in assessment of wedge pressure would probably limit the accuracy of non-invasive calculated PVR.

II. There are several limitations in the present study. It is a retrospective review of patients who were evaluated for transplantation at a single centre. A selection of patients with more advanced disease may be a limitation for an objective analysis. In addition, not all patients underwent right heart catheterisation during the evaluation for LTx, which may have led to selection bias. Another limitation is that data are missing for patients who admitted for Ltx evaluation but were not accepted for LTx for various reasons.

III. The sample size in the present study is small with respect to survival analysis. The predictive value of IL-6 for mortality in patients with PAH should therefore be interpreted with caution. Another limitation of the study is that arterial blood samples were taken from the left atrium or the femoral artery in control subjects, but from the radial artery in PAH patients. However, blood from the radial artery/femoral artery should be a reliable substitute for an aortic sample because of the rapid transient time and low surface area of the large arterial tree. In addition, pulmonary blood flow was not measured in the control group, which hampers the estimation of a potential flow-dependent wash-out of growth factors and IL-6 from the pulmonary vasculature. However, no correlation between the levels of growth factors and cardiac output was found in PAH patients.

IV. One limitation could be a small group of patients on long-term specific treatment and treatment dominated by the ET-R antagonist-bosentan. The circulating levels of ET-1 must be interpreted with caution, because ET-1 is a paracrine mediator diffusing into the vascular media, with some spillover into the blood, hence correlations between hemodynamic parameters and measured ET-1 levels may be variable and poor¹³².

Summary and conclusions

Paper I. Strong correlations and, in absolute terms, small differences between catheter and simultaneous Doppler echocardiography measurements indicate that Doppler echocardiography can provide hemodynamic assessments in groups of patients that are comparable with invasive data. The patients that were included had PAH, but the method that is described should be applicable to all patients with severe pulmonary hypertension regardless of etiology.

Paper II. This study demonstrates that, in patients with severe lung disease, PVR was an independent predictor of death during the waiting time for LTx. Increased PVR, elevated pulmonary pressures and reduced cardiac index were most common in the ILD population. Patients with ILD had a two-fold increase in mortality compared with patients with COPD/ α 1ATD. Furthermore, our findings indicate that pulmonary hypertension defined by increased PVR (≥ 3 WU) was associated with an adverse outcome, but not pulmonary hypertension defined as mPAP (≥ 25 mmHg at rest), underscoring the importance of measuring not only PA pressures but also cardiac output.

Paper III. Serum levels of the growth factors VEGF, PDGF-BB and TGF- β 1 were significantly higher in both arterial and pulmonary arterial samples from patients with PAH compared with control subjects. Furthermore, there was a significant step-up of all growth factors across the lungs in PAH patients. These findings indicate the increased release or/and reduced clearance of these mediators at the lung vascular level. IL-6 serum levels were higher among PAH patients and IL-6 was an independent predictor of adverse outcome. With a shift from the focus on vasoconstriction to abnormal vascular cellular proliferation in the small vessels of the lungs in clinical PAH, the findings in the present study support the hypothesis that growth factors contribute to vascular remodelling in PAH.

Paper IV. This study showed a balanced release and clearance of ET-1 across the lung circulation in control subjects, in patients with different forms of PAH, in patients with PAH

during acute epoprostenol infusion and in patients with PAH on chronic specific treatment. The findings in the present study suggest that the pulmonary extraction and production of ET-1 is balanced.

Clinical perspective

Paper I. Our study indicates that Doppler echocardiography can provide hemodynamic assessments in groups of patients that are comparable with invasive data. This should be of interest in clinical trials monitoring the effects of medical treatment. By combining the approach used in the present study with other echocardiography variables, such as right heart dimensions and the presence of pericardial effusion, the evaluation of therapeutic interventions improves and hence could reduce the need for follow-up catheterisation in patients with PAH.

Paper II. These results may have implications for prioritisation of patients on the lung transplant waiting list regarding organ allocation. These findings also support an invasive strategy to determine pulmonary hemodynamics during the pre-transplant evaluation, at least in patients with ILD. Recently, several new pharmacological interventions for the treatment of pulmonary arterial hypertension have been shown to be effective. Further studies are needed to establish whether patients with advanced lung diseases and increased PVR may benefit from treatment with these drugs as a bridge to lung transplantation.

Papers III and IV. With a shift from the focus on vasoconstriction to abnormal vascular cellular proliferation in the small vessels of the lungs in clinical PAH, the findings in the present study support the hypothesis that growth factors and ET-1 contribute to vascular remodelling in PAH. These pathways may be a target for new therapeutic strategies.

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