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Pulmonary hypertension: insights from population and patient studies

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Pulmonary hypertension: insights from population and patient studies

Lessen uit translationeel onderzoek
Pulmonale hypertensie: inzichten uit populatie- en patiëntgebonden onderzoek

Thesis

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„Vom Ganzen über die Details zurück zum Ganzen."

brought to me by my teacher Dr. Widar Lehnemann, modified from Goethe:

„Willst du dich am Ganzen erquicken, so musst du das Ganze im Kleinsten erblicken."

For my parents Ellinor and Helmut Gall

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Introduction

Pulmonary Hypertension at the population level

Pulmonary hypertension (PH) is a disease of the pulmonary vasculature, characterized by a mean pulmonary arterial pressure (mPAP) ≥ 25 mmHg measured by right heart catheterization (RHC).[1]

On the population level, aging combined with smoking and other unhealthy lifestyle habits leads to an increase in incidence of non-communicable diseases, including left heart disease and chronic obstructive pulmonary disease (COPD), two important diseases underlying PH.[2] As a consequence, pulmonary hypertension might become more prevalent in the future, especially in the elderly.

The prevalence of PH in the population is not known, and as patients suffer from unspecific symptoms, diagnosis is challenging and often delayed.[3] The gold standard for diagnosing PH is RHC. However, RHC cannot be performed on a population basis because of its invasiveness and cost. Screening for PH in the general population may be done by means of echocardiography, as echocardiography can be used to estimate pulmonary artery systolic pressure (PASP).[4,5 6] Besides the clinical extreme of PH, an increased PASP in the general population has been associated with poor health outcomes, such as an increased mortality.[7] Even though the extreme fully developed PH is rare, an increase in pressure within the normal spectrum may have consequences at the population level.

Existing evidence from epidemiological studies and patient registries does not yet enable us to describe the missing link between factors that predispose to the development of PH and the actual disease. In this thesis we combine insights from population-based studies and registry data from a PH-referral clinic to address research questions about the prevalence and associated factors in the general population, as well as diagnosis, prognosis, and treatment of patients with PH.

Pulmonary Hypertension risk factors and etiology

PH is a heterogeneous phenotype. Various subgroups of PH can be distinguished based on underlying etiology, and adequate treatment will depend on the subgroup. After a diagnosis of PH, a diagnostic algorithm is initiated to determine the etiologic group of PH for each individual patient. A detailed list of etiologic groups is shown in Table 1.[8] The overall reported prevalences of the etiologic groups vary substantially between PH registries.[9] Only a small proportion of PH patients are classified as having pulmonary arterial hypertension (PAH, group I). An etiologic classification is important to optimize treatment for individual patients, but also to identify groups of persons at risk of developing PH. Risk factors for PH include several non-communicable diseases such as COPD, left ventricular dysfunction, liver cirrhosis, and infectious diseases such as Schistosomiasis

and HIV.8 Genetic characteristics may also pre-dispose to or protect from pulmonary vascular disease. [10]

Table 1 Etiologic classification for pulmonary hypertension, according to the world symposium on pulmonary hypertension in Nice 2013

1.		Pulmonary Arterial Hypertension (PAH)
	1.1	Idiopathic PAH
	1.2	Hereditable PAH: BMPR2,ALK1, ENG, SMAD9, CAV1, KCNK3, unknown
	1.3	Drugs and toxins induced PAH
	1.4	PAH associated with: Connective tissue diseases, HIV infection, Portal hypertension, Congenital heart disease, Schistosomiasis
	1´	Pulmonary veno-occlusive disease and/or pulmonary capillary hemangiomatosis
	1´´	Persistent pulmonary hypertension of the newborn
2.		Pulmonary hypertension due to left heart disease
	2.1	Systolic dysfunction
	2.2	Diastolic dysfunction
	2.3	Valvular disease
	2.4	Congenital /acquired left heart inflow/outflow tract obstruction and congenital cardiomyopathies
3.		Pulmonary hypertension due to lung diseases and/or hypoxia
	3.1	Chronic obstructive pulmonary disease
	3.2	Interstitial lung disease
	3.3	Other lung disease with mixed obstructive and restrictive pattern
	3.4	Sleep-disordered breathing
	3.5	Alveolar hypoventilation disorders
	3.6	Chronic exposure to high altitude
	3.7	Developmental abnormalities
4.		Chronic Thromboembolic pulmonary hypertension (CTEPH)
5.		Pulmonary Hypertension with unclear and/or multifactorial mechanisms
	5.1	Hematologic disorders: chronic hemolytic anemia, myeloproliferative disorders, splenectomy
	5.2	Systemic disorders: Sarcoidosis, pulmonary histiocytosis, lymphangioleiomyomatosis
	5.3	Metabolic disorders: Glycogen storage disease, Gaucher disease, thyroid disorders
	5.4	Others: tumoral obstruction, fibrosing mediastinitis, chronic renal failure, segmental PH

BMPR2=bone morphogenic protein receptor type II; CAV1=caveolin-1; ALK1= activin-like receptor kinase-1; ENG=endoglin; HIV=human immunodeficiency virus; SMAD9= decapentaplegic 9; KCNK3= potassium channel super family K member-3 [8]

Pulmonary hypertension diagnosis, treatment and prognosis

PH patient registries, which collect demographic, medical, and treatment data, have helped to improve understanding of many aspects of the disease, especially diagnosis, prognosis, and treatment. [11] Globally, there are a few well-established registries, with some focusing on the full spectrum of patients with PH, and others with a more specific focus on a particular subgroup, such as PAH or chronic thromboembolic PH (CETPH). [9,12-14] In Germany there is only one single center registry in Giessen. Other PH centers take part in multi-center and multi-national registries such as the Compera registry. [15] The registry in Giessen is the largest single center registry worldwide that has included all patients with PH, irrespective of their etiological group. Additionally, patient data are linked to a large biobank which contains blood samples from each patient taken at the time of diagnosis and throughout the course of the disease. This large data source provides a unique opportunity and resource to identify new diagnostic and prognostic tools that may lead to a better understanding of the disease. [16-18] Response to treatment can be studied in different etiological groups and modern read out techniques can be applied to identify possible targets for future treatment.

Given the complexity of diagnosing PH, not all patients who are referred with suspected PH will be diagnosed with the disease. [4] To date, no minimally invasive, low cost, and highly accurate diagnostic test is available to exclude or prove PH. The gold standard for the diagnosis of PH is RHC. [19] Having a more practical diagnostic marker would facilitate the diagnosis of PH and could lower the associated risks. As the Giessen registry contains all patients referred to the Giessen University Hospital with suspected PH who underwent RHC for exclusion of PH, this is an excellent setting to study the value of novel and alternative diagnostic tests.

Despite the availability of several treatment options, the prognosis for patients who have PH is still poor.20 Patients with PH tend to suffer from progressive right heart failure and die soon after diagnosis.[21] The 1-, 3-, and 5-year survival rates for patients with PAH are 79-87%, 51-71%, and 48-58%, respectively. 9 Prognostic research has identified several factors associated with clinical worsening or mortality, including hemodynamic measurements, exercise tests results, echocardiographic parameters, and biomarkers, such as changes in glucose metabolism.[22] Such changes are well-documented in patients with PAH.[23] However, no association of glucose metabolism with survival has been established, sp it remains unclear if markers of glucose metabolism may be used in prognostication of patients with PH.[24]

After establishing the diagnosis, treatment should be targeted to the individual patient to improve efficacy and prognosis. Therapeutic options include general measures, supportive therapy, and specific treatment based on the underlying etiology. While an operation for desobliteration of the pulmonary arteries may be curative in selected patients, especially

those with chronic-thromboembolic pulmonary hypertension (CTEPH), medical treatment is generally the best option for most patients.[25]
The available drugs for the treatment of PH can be divided into four groups according to their mechanism of action: endothelin receptor antagonists (ERAs), phosphodiesterase type 5 inhibitors (PDE5i) and soluble guanylate cyclase (sGC) stimulators in the nitric oxide (NO) pathway, prostacyclin, and tyrosine kinase inhibitors.[26] Most of these available drugs that may be used to treat PH are approved for treatment of PAH only. Patients with PH of other etiologies do not have equivalent pharmacological options. Personalized treatment and combination therapy are two important aspects in the management of PH patients remain major challenges for patients, clinicians, and researchers.

Aims and outline of this thesis

This thesis will focus on PH from complementary perspectives with the overall aim of providing a comprehensive view of PH at the population and clinical level. The ultimate aim is to provide valuable information about the etiology, diagnosis, treatment, prognosis and population burden of the disease. In **chapter 2** PH is investigated from a population perspective. **Chapter 2.1** gives insight into the prevalence and associated factors of PH in the Rotterdam Study, a large population-based cohort study. The association of left heart parameters and pulmonary artery pressure is evaluated in the same population in **chapter 2.2**. **Chapter 3** changes the perspective to that of the patient registry. In **chapter 3.1** the Giessen PH registry is introduced and predictors of mortality are described. **Chapter 3.2** presents two new potential diagnostic biomarkers for PH, sFlt-1 and PlGF, studied in the Giessen PH registry. The prognostic implications of markers of glucose metabolism in patients with PH are presented in **chapter 3.3**. In **chapter 4** clinical and therapeutical aspects are discussed. In **chapter 4.1** the right ventricular contractile reserve is presented including its prognostic relevance. **Chapter 4.2** compares sildenafil with inhaled nitric oxide for acute vasodilator testing in PAH patients. **Chapter 4.3** gives insight into survival with sildenafil and inhaled iloprost in a cohort of patients with pulmonary hypertension. Finally, **chapter 5** is a general discussion and conclusion.

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2.1 |

Prevalence of Pulmonary Hypertension in the General Population: the Rotterdam Study

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Abstract

Background: Pulmonary hypertension is characterized by increased pulmonary artery pressure and carries an increased mortality. Population-based studies into pulmonary hypertension are scarce and little is known about its prevalence in the general population. We aimed to describe the distribution of echocardiographically-assessed pulmonary artery systolic pressure (ePASP) in the general population, to estimate the prevalence of pulmonary hypertension, and to identify associated factors.

Methods: Participants (n=3381, mean age 76.4 years, 59% women) from the Rotterdam Study, a population-based cohort, underwent echocardiography. Echocardiographic pulmonary hypertension was defined as ePASP>40 mmHg.

Results: Mean ePASP was 26.3 mmHg (SD 7.0). Prevalence of echocardiographic pulmonary hypertension was 2.6% (95%CI: 2.0; 3.2). Prevalence was higher in older participants compared to younger ones (8.3% in those over 85 years versus 0.8% in those between 65 and 70), and in those with underlying disorders versus those without (5.9% in subjects with COPD versus 2.3%; 9.2% in those with left ventricular systolic dysfunction versus 2.3%; 23.1% in stages 3 or 4 left ventricular diastolic dysfunction versus 1.9% in normal or stage 1). Factors independently associated with higher ePASP were older age, higher BMI, left ventricular diastolic dysfunction, COPD and systemic hypertension.

Conclusion: In this large population-based study, we show that pulmonary hypertension as measured by echocardiography has a low prevalence in the overall general population in the Netherlands, but estimates may be higher in specific subgroups, especially in those with underlying diseases. Increased pulmonary arterial pressure is likely to gain importance in the near future due to population aging and the accompanying prevalences of underlying disorders.

Key words: Hypertension, pulmonary; Echocardiography; Epidemiology; Population.

Introduction

Pulmonary hypertension (PH) is a severe disorder defined by a mean pulmonary artery pressure of ≥ 25 mmHg at rest [1-4]. Pulmonary hypertension can occur as an isolated disease or as a consequence of a number of underlying diseases and conditions, such as heart failure and chronic obstructive pulmonary disease (COPD) [4-11]. Although higher levels of pulmonary pressure have been associated with increased mortality both in patients and in the general populations, general population prevalence estimates are scarce [7, 10, 12-15].

The diagnostic method of choice for PH is right-heart catheterization [1]. However, its invasive nature renders it unsuitable in population-based studies. Transthoracic Doppler echocardiography is a non-invasive tool used in clinical practice for screening and monitoring of PH progression. Although some studies describe under- or overestimation of pulmonary arterial pressures by echocardiography, a meta-analysis has shown it to have good sensitivity (83%), reasonable specificity (72%) and a correlation 0.7 with invasively acquired measurements [16, 17]. Most deviations from measurements by right heart catheterization seem to occur in patients with very high pressure estimates [18]. In echocardiograms, the pulmonary artery systolic pressure is the most frequently used parameter. We aimed to describe the distribution of echocardiographic pulmonary artery systolic pressure (ePASP) and to estimate the prevalence of pulmonary hypertension measured by echocardiography (ePH) in the general population. Furthermore, we sought to identify factors independently associated with ePASP.

Material and methods

Setting

This study was embedded in the Rotterdam Study, an ongoing population-based, prospective cohort study, which started in 1990-1993 in a suburb of Rotterdam, the Netherlands. The design and rationale has been described in detail elsewhere [19]. Briefly, the original subcohort (RS-I) enrolled 7983 participants aged 55 years or older. Two additional subcohorts (RS-II, n = 3011 participants of 55 years and older; and RS-III, n = 3768 participants of 45 years and older) were recruited into the study in 2000-2001 and 2006-2008, respectively. Every 3-4 years the participants are invited for re-examination. Information routinely collected includes anthropometry, cardiovascular risk factors, medication use, and extensive functional and imaging tests [19]. Left- and right-sided echocardiographic measurements are available only for the most recent follow-up round of RS-I (fifth follow-up) and RS-II (third follow-up) (from 2009 to 2012). Participants from those two subcohorts who visited the research centre were eligible for this study.

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the "Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)". All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

Echocardiography

Three trained echocardiographers obtained resting transthoracic echocardiograms in all participants visiting the research center. The standardized protocol included 2-dimensional scanning in the parasternal long and short axis views, apical and subcostal views. In addition, left ventricular dimensions were measured using 2-dimension guided M-mode. Tricuspid regurgitation peak velocity (TRV) was measured using Continuous Wave Doppler. Tissue Doppler imaging was done in the apical 4-chamber view.
Echocardiograms were made using a commercially available ultrasonography system (Vivid I, GE Healthcare, Little Chalfont, UK), with a 2.5 MHz transducer. All images obtained were digitally stored and assessed offline by the echocardiographers. Abnormal findings were confirmed by clinical experts and communicated to the participants and their general practitioners according to a pre-defined protocol.

Pulmonary Artery Systolic Pressure

Pulmonary artery systolic pressure was calculated as the sum of the estimated right atrial pressure (RAP) and the pressure gradient over the tricuspid valve [20] as: ePASP = $4*TRV^2$ + RAP. The pressure gradient was computed from the highest Doppler tricuspid regurgitation velocity gathered from several windows using the simplified Bernoulli equation ($4*TRV^2$) [20]. RAP was estimated according to the guidelines of the American Society of Echocardiography: if the inferior vena cava diameter was ≤ 21 mm and its forced inspiratory collapse ("sniff test") was > 50%, RAP was estimated to be 3 mmHg; if the diameter was > 21 mm and the collapse < 50%, RAP was estimated as 15 mmHg; in intermediate cases, a value of 8 mmHg was assigned[20].
Participants were deemed to have ePH if they had ePASP > 40 mmHg[2, 20, 21]. If data on RAP was missing, a tricuspid pressure gradient > 36 mmHg (TRV > 3.0 m/s) criterion was used instead. Participants in whom TRV was too small to measure or absent were included in the prevalence analyses as non-cases, as we they were most likely to have normal pulmonary pressures.

Left ventricular function

As ejection fraction was not available in our cohort, we assessed left ventricular (LV) function through LV fractional shortening. LV fractional shortening (%) at the endocardium was calculated by: (LV end-diastolic diameter - LV end-systolic diameter) / LV end-diastolic diameter * 100% [22]. LV systolic dysfunction was defined as a LV fractional shortening < 29% [23].

Pulsed Doppler recordings of transmitral filling velocities were obtained in the apical 4-chamber view, with the sample volume placed in the mitral valve orifice near the tips of the leaflets. Doppler peak E and peak A velocities were averaged over three cycles. Early mitral valve velocity deceleration time was measured as the time between the peak E wave and the upper deceleration slope extrapolated to the zero baseline. The E/E' ratio was calculated by dividing E-top velocity by early diastolic longitudinal velocity of the septal mitral annulus E'-top. LV diastolic function was categorized in line with previous publications [24, 25], using an algorithm which takes into account E/A ratio, E/E' ratio and E', dividing participants into 3 categories of diastolic function: normal or relaxation abnormality (stage 1), pseudonormal (stage 2), and restrictive (including both reversible [stage 3] and fixed [stage 4] LV diastolic dysfunction). For participants with atrial fibrillation, the categorization was based on mitral valve inflow deceleration time instead of the E/A ratio [24, 25].

Other covariates

Anthropometric and laboratory measurements were assessed in a standardized manner during the same visit as the echocardiography. Body mass index (BMI) was calculated as weight (kg) / height squared (m^2). Systemic hypertension was defined as blood pressure ≥ 140/90 mmHg or use of anti-hypertensive medication (Anatomical Therapeutic Chemical [ATC] codes C02, C03, C07, C08 and C09). Blood pressure was averaged between two measurements, taken with the participant in sitting position. Diabetes mellitus was defined as either fasting glucose level > 7.0 mmol/L, or a non-fasting glucose level > 11.0 mmol/L (if fasting serum was unavailable) or use of anti-diabetic medication (ATC code A10) [26, 27]. Smokers were classified into never, former and current smokers. COPD was diagnosed by an obstructive spirometry (proportion of the forced vital capacity exhaled in the first second [FEV_1/FVC] < 70%) at the research centre or by a pulmonologist or general practitioner. Participants with a spirometry suggestive of a restrictive syndrome and asthma patients were not considered to have COPD.

Sensitivity analysis

We also estimated ePH prevalence using 2 alternative definitions. The first one included those with a dilated right-ventricle (basal right ventricular end-diastolic diameter > 42 mm) in addition to those with ePASP > 40 mmHg[20]. This was done to take into account variables suggestive of PH beyond ePASP, as recommended by the European Society of Cardiology [1]. The second definition used a more stringent ePASP threshold of 50 mmHg or a tricuspid pressure gradient of 46 mmHg (TRV > 3.4 m/s) instead of the 40 mmHg cut-off [1], without taking right ventricular diameter into account.

Statistical analyses

Prevalence estimates with confidence intervals (Wald) of ePH were calculated for the full population and for subgroups defined by age, sex, smoking status, BMI, and presence of LV systolic dysfunction, LV diastolic dysfunction, COPD, systemic hypertension and diabetes mellitus.

To identify factors associated with ePASP, we created 2 linear regression models. In model A, each factor was adjusted for age and sex. Model B was a multivariate model in which all variables that were significant in model A were entered simultaneously.

Missing data (maximum proportion of missing values per covariate: 6%) was imputed with a multiple imputation procedure (5 imputations) using the Markov Chain Monte Carlo method. TRV, RAP and right ventricular end-diastolic diameter were used as predictors in the imputation model, but were not imputed. Missing data on smoking and COPD was dealt with using the last observation carried forward method.

We used Stata version 12 (StataCorp. College Station, TX, U.S.) and IBM SPSS Statistics version 20.0 (IBM Corp. Armonk, NY, U.S.) for the statistical analysis.

Results

The response-rate for the analysed rounds of examination was 63.4% for RS-I and 75.1% for RS-II, yielding a total of 3381 participants eligible for the study. Of those, we excluded 558 (16.5%) due to the fact that no data on the tricuspid regurgitation jet were recorded. The excluded population was younger, with a higher proportion of men, diabetics and current or past smokers, had better ventricular diastolic and lower systolic function and a higher BMI (table 1). The final study population was composed of 2823 participants with a mean age of 76.4 years (SD 6.2), 59% women, 10.4% had COPD and 3.5% had LV diastolic dysfunction stages 3 or 4 (table 1).

Table 1. Participant characteristics

	Study population (n = 2823)		Excluded population (n = 558)		
	n	Mean (SD) or %	n	Mean (SD) or %	p
Age, years	2823	76.4 (6.2)	558	74.7 (5.8)	<0.001
Women	2823	59%	558	53%	0.01
Body mass index, kg/m^2	2811	27.2 (4.0)	548	29.3 (4.7)	<0.001
Smoking	2823		558		0.004
Never	997	35.3%	158	28.3%	
Former	1566	55.5%	336	60.2%	
Current	260	9.2%	64	11.5%	
TRV, m/s	2153	2.3 (0.3)		NA	NA
RAP, mmHg	2473	3.9 (2.2)	368	3.7 (1.8)	0.02
ePASP, mmHg	1945	26.3 (7.0)		NA	NA
RVEDD, mm	2558	33.0 (4.4)	380	32.7 (4.1)	0.16
FS, %	2788	41.1 (5.9)	497	40.4 (6.3)	0.01
LV systolic dysfunction	2788	4.2%	497	4.0%	0.89
LV diastolic dysfunction	2790		510		0.002
Normal and stage 1	1716	61.5%	353	69.2%	
Stage 2	977	35.0%	147	28.8%	
Stages 3 and 4	97	3.5%	10	2.0%	
COPD	2823	10.4%	558	9.7%	0.60
Diabetes mellitus	2706	12.8%	519	21.8%	<0.001
Systemic hypertension	2773	87.1%	545	89.7%	0.08

TRV = tricuspid regurgitation peak velocity; RAP = right atrial pressure; ePASP = pulmonary artery systolic pressure; RVEDD = right ventricular end-diastolic diameter; FS = left ventricular fractional shortening; LV = left ventricle; COPD = chronic obstructive pulmonary disease.

Prevalence of ePH

The overall prevalence of ePH in our study was 2.6% (95%CI 2.0; 3.2). Older participants, and those with COPD, LV systolic or diastolic dysfunction had a significantly higher prevalence of ePH than their counterparts (table 2).

Table 2. Prevalence of echocardiographic pulmonary hypertension, overall and in subgroups

		ePH prevalence (95% CI)	p
Overall		2.6% (2.0; 3.2)	
Sex	Women	2.6% (1.8; 3.4)	0.93
	Men	2.7% (1.7; 3.6)	
Age	65 to 70 years	0.8% (0.02; 1.6)	**<0.001**
	70 to 75 years	1.6% (0.7; 2.5)	
	75 to 80 years	1.8% (0.9; 2.8)	
	80 to 85 years	4.1% (2.4; 5.9)	
	85 years or older	8.3% (5.0; 11.6)	
Smoking	Never	2.7% (1.7; 3.7)	0.32
	Former	2.8% (2.0; 3.6)	
	Current	1.2% (0.0; 2.5)	
Body mass index	< 25 kg/m^2	2.4% (1.4; 3.4)	0.66
	≥ 25 kg/m^2	2.7% (2.0; 3.4)	
COPD	Yes	5.9% (3.1; 8.5)	**0.001**
	No	2.3% (1.7; 2.8)	
Systemic hypertension	Yes	2.8% (2.1; 3.5)	0.15
	No	1.4% (0.2; 2.7)	
Diabetes mellitus	Yes	4.1% (2.0; 6.2)	0.07
	No	2.4% (1.8; 3.0)	
LV systolic dysfunction	Yes	9.2% (4.0; 14.4)	**< 0.001**
	No	2.3% (1.8; 2.9)	
LV diastolic dysfunction	Normal or stage 1	1.9% (1.2; 2.5)	**< 0.001**
	Stage 2	2.8% (1.7; 3.8)	
	Stages 3 and 4	23.1% (11.3; 34.9)	

COPD = chronic obstructive pulmonary disease; LV = left ventricle; ePH = pulmonary hypertension.

An enlarged right ventricle was strongly associated with higher ePASP, even after adjustments for age, sex and BMI ($p < 0.001$). Sensitivity analyses accounting for it (that is, using a definition of ePH based on either an ePASP of > 40 mmHg or a right ventricular end-diastolic dimension > 42 mm) yielded an overall prevalence of 4.5% (95%CI 3.7; 5.2) with a significantly higher prevalence in men than women (6.2% vs 3.2%, $p < 0.001$; table 3). A more stringent ePASP threshold of 50 mmHg, regardless of RV size, yielded an overall prevalence of 0.5% (95%CI: 0.2; 0.8), and no sex difference ($p = 0.91$).

Table 3. Prevalence of echocardiographic-defined pulmonary hypertension, alternative diagnostic criteria

	Diagnostic criteria					
	ePASP > 40 mmHg		ePASP > 40 mmHg or right ventricle > 42 mm		ePASP > 50 mmHg	
	(n = 2823)	p	(n = 2828)	p	(n = 2823)	p
Overall	2.6% (2.0; 3.2)		4.5% (3.7; 5.2)		0.5% (0.2; 0.8)	
Women	2.6% (1.8; 3.4)	0.93	3.2% (2.4; 4.0)	**<0.001**	0.5% (0.1; 0.9)	0.91
Men	2.7% (1.7; 3.6)		6.2% (4.7; 7.6)		0.5% (0.2; 0.8)	

Data are shown as prevalences (95% confidence interval)
RAP = right atrial pressure; TRV = tricuspid regurgitation velocity; ePASP = pulmonary artery systolic pressure, calculated as $4*TRV^2 + RAP$. If RAP could not be estimated, the case definition was based on TRV > 3.0 m/s and 3.4 m/s to correspond to ePASP > 40 mmHg and 50 mmHg, respectively
Participants with absent or too-small-to-measure TRV were included as non-cases.

Factors independently associated with ePASP

In 208 participants, data on right atrial pressure were missing and in 670 participants, we could not measure tricuspid regurgitation velocity because the regurgitation jet was either absent or too small to measure. Hence, ePASP could only be estimated in 1945 of the 2823 participants. In those, mean ePASP was 26.3 mmHg (SD 7.0) and the median was 25.3 mmHg (inter-quartile range 21.5; 29.8). It followed a slightly right-skewed distribution (Fig. 1).

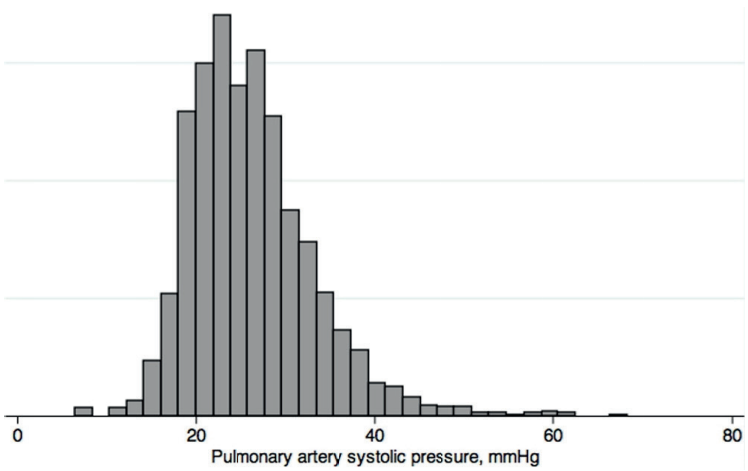

Figure 1. Distribution of pulmonary artery systolic pressure in 1945 participants in whom it could be estimated

In the fully adjusted model, a 10-year increase in age was associated with a 2.2 mmHg (95%CI 1.8; 2.7) higher ePASP. A 5 kg/m^2 increase in BMI was associated with a 0.7 mmHg (95%CI 0.3; 1.1) pressure increment (table 4). In comparison with participants with normal LV diastolic function or stage 1 LV diastolic dysfunction, participants with LV diastolic dysfunction stages 3 or 4 had higher ePASP estimates (7.1 mmHg change, 95%CI 5.0; 9.1). Sex, LV fractional shortening and diabetes mellitus were significantly associated with ePASP in model A, adjusting for age and sex, but were not after further adjustments. Systemic hypertension was significantly associated with higher ePASP even after adjusting for other factors (1.3 mmHg change; 95%CI 0.4; 2.1).

Table 4. Associations with pulmonary artery systolic pressure in linear regression models in 1945 participants in whom ePASP could be estimated

	Model A		Model B	
	Change in ePASP, mmHg		Change in ePASP, mmHg	
	(95%CI)	p	(95%CI)	p
Age, per 10 years	2.6 (2.1; 3.1)	**<0.001**	2.2 (1.8; 2.7)	**<0.001**
Women	-0.7 (-1.3; -0.1)	**0.03**	-0.6 (-1.2; 0.004)	0.05
Body mass index, per 5 kg/m^2	0.8 (0.4; 1.1)	**<0.001**	0.7 (0.3; 1.1)	**0.001**
Smoking		0.67		
Never	Reference			
Former	-0.3 (-1.0; 0.4)			
Current	-0.2 (-1.4; 0.9)			
LV fractional shortening, per 10%	-1.0 (-1.5; -0.5)	**<0.001**	-0.5 (-1.0; 0.06)	0.09
LV diastolic dysfunction		**<0.001**		**<0.001**
Normal and stage 1	Reference		Reference	
Stage 2	1.1 (0.5; 1.7)		1.1 (0.4; 1.7)	
Stages 3 and 4	7.8 (5.7; 9.8)		7.1 (5.0; 9.1)	
COPD	2.3 (1.3; 3.3)	**<0.001**	2.4 (1.4; 3.4)	**<0.001**
Diabetes mellitus	1.0 (0.003; 2.0)	**0.05**	0.3 (-0.7; 1.3)	0.56
Systemic hypertension	1.8 (0.9; 2.7)	**<0.001**	1.3 (0.4; 2.1)	**0.01**

Dependent variable is ePASP (mmHg).
LV = left ventricular; COPD = chronic obstructive pulmonary disease; 95%CI = 95% confidence interval.
In model A, each variable is adjusted for age and sex.
In model B, adjustments were made for all the variables which had $p < 0.05$ in model A.

Discussion

Overall, we found that pulmonary hypertension as assessed by echocardiography has a prevalence of 2.6% in the general population in the Netherlands. The prevalence of ePH was higher in older persons, and in those with COPD, LV systolic or diastolic dysfunction. Factors independently associated with higher ePASP in a multivariate model were older age, higher BMI, left ventricular diastolic dysfunction, COPD and systemic hypertension.

Prevalence of ePH

Increased levels of pulmonary pressure has been associated with increased all-cause mortality in the general population, as well as increased admission rates in heart failure patients [15, 28]. Yet, data on the prevalence of increased ePASP in the general population is scarce [29]. Given the population aging and the known association of PH with heart and lung diseases, an increasing PH prevalence could be expected [29]. The prevalence of ePASP > 40 mmHg has recently been estimated to be 8% in healthy volunteers aged > 50 years in Italy and ePH was found in 6.8% of participants in an African-American population [30, 31]. A study in Armadale reported a prevalence of 9.1%[21]. However, this estimate may be inflated, as the study included participants referred for echocardiography, likely representing a population with increased risk as compared to the general population. Still, our estimates are low, considering that the populations in the other studies were likely to be as healthy or healthier than ours based on age and comorbidities [30, 31]. Our case-definition method (that is, including those with absent or unmeasurable TRV as non-cases, instead of excluding them) may have led to conservative estimates. Removal of those participants from the analyses, hence basing the definition of ePH on ePASP or TRV (in those without RAP) measurements only, yields a 3.4% prevalence estimate. Additional exclusion of participants without an RAP estimate, thus basing the cases only on ePASP, yields a prevalence of 3.6%, still leaving part of the difference unexplained.

The prevalence of pulmonary hypertension in patients with COPD has been reported to be as high as 47-49% in clinical studies [6, 7]. Such difference from our estimates (5.9%) may be explained by differences in study populations (patient cohorts versus general population) [6, 7]. Our population likely does not include the most severe cases of COPD, as they may have been unable to visit the research centre.

Since the European Society of Cardiology suggests the inclusion of “additional echocardiographic variables suggestive of PH” in the echocardiographic assessment of PH, secondary analyses were done with an additional criterion (dilated right ventricle) [1]. This increased prevalence estimates to 4.5% with a significant difference between men and women (6.2% versus 3.2%, $p < 0.001$). The overall larger size of the right ventricle in men probably underlies this difference. We also performed analyses with a more stringent threshold of 50 mmHg, which yielded a prevalence of 0.5% and no sex-related differences.

ePASP and associated factors

Our ePASP estimates (median 25.3 mmHg and mean 26.3 mmHg) were similar to values reported in three previously published studies, even though the other population were younger and overall healthier [15, 30, 32]. As ePASP is known to increase with older age, we had expected a greater difference [6, 15, 30, 33]. Patterns of age-related ePASP increase may be variable across generations and locations, therefore longitudinal studies would serve well in elucidating the trajectories of pulmonary pressures across the life course [15, 30, 33].

We did not find sex-related differences in the prevalence of ePH, and sex was not independently associated with ePASP. Previous literature is divided on this point, as some studies have found differences and others have not [6, 7, 15, 31, 33].

LV fractional shortening was not found to be independently associated with ePASP, despite our finding of a significantly higher ePH prevalence in those with LV systolic dysfunction (9.2% versus 2.3%, $p < 0.001$). Others also could not demonstrate consistent associations between ejection fraction and ePASP[10, 15]. Instead, literature suggests that ePASP is more associated with LV diastolic function, as we also demonstrated[10].

Strengths of this study are the large sample size and the population-based nature. Other strong points are a structured and standardized echocardiographic assessment and the availability of large number of covariates. One of the limitations is the lack of the diagnostic gold standard for PH, right heart catheterization[1, 2]. Right heart catheterization is unsuitable for a general-population setting because of its invasive nature. Echocardiography is frequently used as the first step in PH evaluation[2]. Although echocardiographic estimates of pulmonary arterial pressure may over or underestimate invasive measurements, they deviate most at the very high end of the pulmonary arterial pressure spectrum, particularly in poor quality exams [34]. In this study, the vast majority of the population have normal pulmonary pressures and only 2% of the exams were deemed of poor quality. Furthermore, echocardiography has a sensitivity of 83%, a specificity of 72%, and a correlation of 0.7 with invasively acquired estimates [16, 17]. Thus, although echocardiography should not be used for clinical diagnosis of PH, it is arguably one of the best exams available for epidemiologic research. We only had ePASP estimates for 57% of the cohort, but we were able to include 83% of it in the ePH prevalence analyses. In comparison, a similar study reported that 69% of their participants had analyzable jets [15]. Lastly, our population is elderly and predominantly white, so caution should be taken to generalize it to other age groups and ethnicities.

Conclusions

Pulmonary hypertension as measured by echocardiography has low prevalence in the overall general population in the Netherlands, but estimates may be higher in specific subgroups, especially in those with left ventricular dysfunction or COPD. BMI and systemic hypertension were associated with ePASP independently of heart or lung disease.

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2.2 |

Left Ventricular and Left Atrial Echocardiographic Measures and Pulmonary Arterial Pressure in the General Population: the Rotterdam Study

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Abstract

Background: The relation between left heart parameters and pulmonary arterial pressures has rarely been studied in the general population. Our objective was to study the associations of left heart parameters with pulmonary artery systolic pressure in a large population-based study.

Methods: This study was conducted within the population-based Rotterdam Study. In 2592 participants with a mean age of 72.6 years (61.4% women), we studied associations of left heart structure and systolic and diastolic function with echocardiographically measured pulmonary artery systolic pressure (ePASP).

Results: ePASP was associated with E/A ratio (ePASP (95% CI) per 1-SD increase: 0.96 (0.71, 1.21) mmHg), left atrial diameter (ePASP per 1-SD increase: 0.77 (0.48, 1.07) mmHg), E/E' ratio (ePASP per 1-SD increase: 0.65 (0.39, 0.92) mmHg), and left ventricular volume (ePASP per 1-SD increase: 0.57 (0.21, 0.94) mmHg), fractional shortening (ePASP per 1-SD increase: 0.49 (0.21, 0.77) mmHg), aortic root diameter (ePASP per 1-SD increase: -0.52 (-0.81, -0.24) mmHg), and mitral valve deceleration time (ePASP per 1-SD increase: -0.34 (-0.60, -0.08) mmHg). Results did not materially differ when restricting the analyses to participants free of shortness of breath symptoms.

Conclusion: Structural and functional left heart echocardiographic parameters in the normal range are associated with pulmonary arterial pressure in the general population.

Keywords: Echocardiography; pulmonary artery systolic pressure; pulmonary hypertension; epidemiology; population-based

Introduction

Pulmonary hypertension (PH) can occur idiopathically or, most frequently, in association with a number of common clinical conditions, including left ventricular dysfunction and chronic obstructive pulmonary disease [1, 2]. PH represents the extreme form of increased pulmonary artery systolic pressure (PASP). Both PH and increased PASP within the normal range are associated with significant morbidity and mortality, both in patients with underlying disorders and in the general population [1-3]. However, increased PASP often remains undetected until it is in an advanced stage, most likely due to its nonspecific symptoms, such as shortness of breath and fatigue[1-4].

Left ventricular failure represents a severe clinical phenotype on the extreme end of a continuum of left ventricular function. Data on the associations of left heart parameters within the normal range with pulmonary artery pressure in the general ageing population are scarce and not much is known about the increase in PASP related to subclinical abnormalities in structure and function of the left-sided heart [2, 5, 6].

Therefore, we aimed to determine the associations of echocardiographically measured left heart parameters across the full spectrum with PASP in the general population.

Material and methods

Study Population and Setting

This study was conducted within the Rotterdam Study, an ongoing prospective population-based cohort study in the city of Rotterdam, the Netherlands. The rationale and design of the Rotterdam Study have been described in detail elsewhere [7, 8]. In short, the Rotterdam Study started in 1990-1993 with a single cohort (RS-I, n=7983 participants of 55 years and older). Two additional cohorts (RS-II, n=3011 participants of 55 years and older, and RS-III, n=3932 participants of 45 years and older) were recruited in 2000-2001 and 2006-2008, respectively.

Every 3-4 years, participants undergo a home interview and clinical examinations at the Rotterdam Study research center. All 4423 participants who visited the research center during the fifth examination of RS-I (2009-2011), the third examination of RS-II (2011-2012) and the second examination of RS-III (2012) were eligible for this analysis. We excluded participants who did not undergo echocardiography (mostly due to logistic reasons, such as non-availability of the echocardiographers or the ultrasound machine), those with poor quality of echocardiography, and those in whom tricuspid valve regurgitation values were too small to measure or missing, making it impossible to calculate ePASP. We also excluded participants without measurements of resting heart rate or body surface area (BSA), which were used as covariables. Our final analyses included 2592 participants (Figure 1).

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Dutch Ministry of Health, Welfare and Sport, implementing the "Wet Bevolkings Onderzoek: ERGO (Population Screening Act: Rotterdam Study). All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

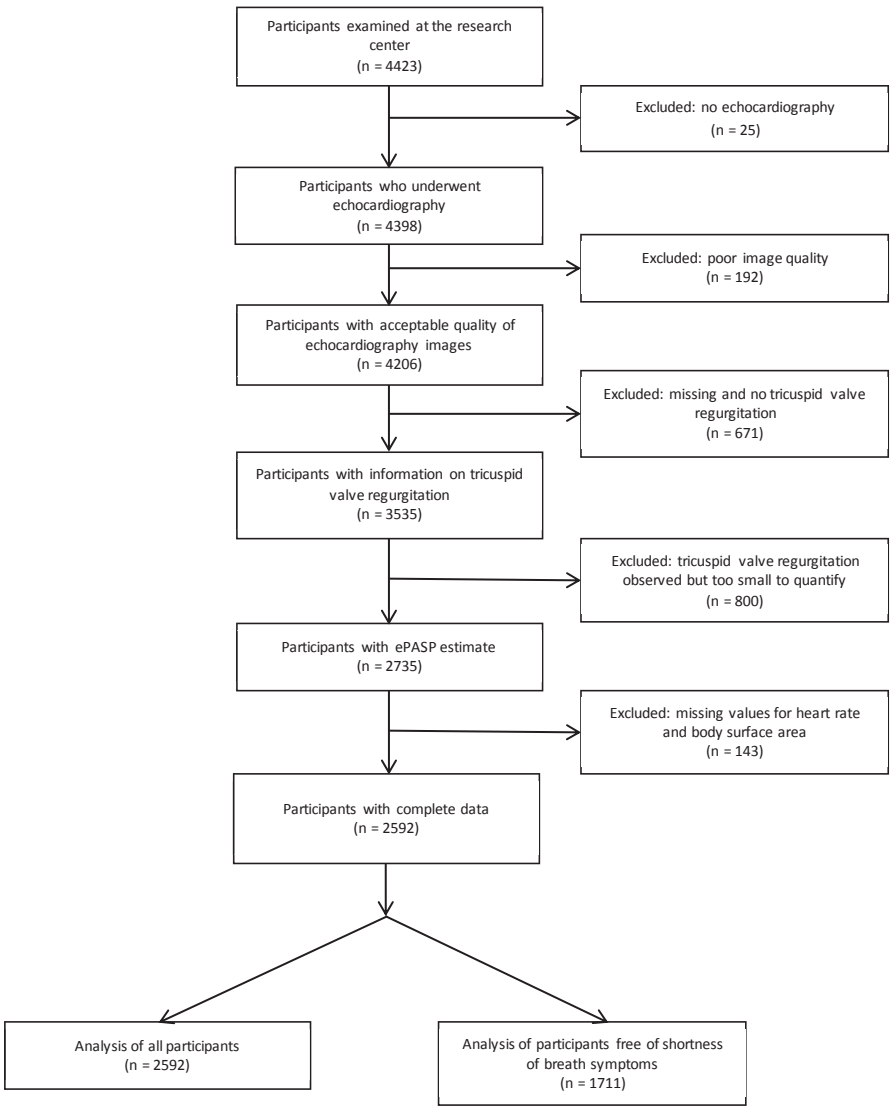

Figure 1. Flow chart of study participants

Echocardiography

Resting transthoracic 2-dimensional echocardiography was performed by experienced echocardiographers, using an identical standardized protocol for all participants. Echocardiography was obtained using a commercially available ultrasonography system (Vivid I, GE Healthcare, Little Chalfont, UK), with a 2.5 MHz transducer. All images were digitally stored and assessed offline by the echocardiographers.

Left-sided measurements were aortic root diameter (AoD), left atrial diameter (LAD), left ventricular end diastolic diameter (LVEDD), left ventricular end systolic diameter (LVESD), interventricular septum thickness (IVST), and left ventricular posterior wall thickness (LVPWT). Left ventricular mass (LVmass) in grams was calculated according to the formula by Devereux and colleagues as $0.8 * \{1.04 * ([LVEDD + IVST + LVPWT]^3 - LVEDD^3)\} + 0.6$ [9]. Left ventricular volume (LVvol) in milliliters was calculated according to the formula by Teichholz and colleagues as $7 * LVEDD^3 / (2.4 + LVEDD)$ [10]. Left ventricular fractional shortening (LVFS) was calculated using the formula: LVFS = (LVEDD-LVESD)/LVEDD * 100% [11]. Pulsed wave Doppler was used to measure the early transmitral ventricular diastolic filling velocity (E wave) and late diastolic filling velocity (A wave) during 3 cardiac cycles. Tissue Doppler was used to measure the early diastolic longitudinal filling velocity of the septal mitral annulus (E') during 3 cardiac cycles. The means of the E wave, A wave and E' were calculated and used to calculate E/A ratio and E/E' ratio. Mitral valve deceleration time was measured as the time between the peak E-top wave and the upper deceleration slope extrapolated to the zero baseline.

Echocardiographically measured pulmonary artery systolic pressure (ePASP) was calculated as the sum of the estimated right atrial pressure (RAP, based on inferior vena cava diameter and forced inspiratory collapse) and the pressure gradient over the tricuspid valve. RAP was estimated according to the algorithm recommended by the ASE/EAE/CSE [12]: if the inferior vena cava diameter was ≤ 21 mm and its forced inspiratory collapse ("sniff test") was > 50%, RAP was considered to be 3 mmHg; if the diameter was > 21 mm and the collapse < 50%, RAP was considered as 15 mmHg; in intermediate cases, a value of 8 mmHg was assigned. Tricuspid regurgitation peak velocity (TRV) was measured using Continuous Wave Doppler. The tricuspid pressure gradient was estimated using the simplified Bernoulli formula [12]: $4*TRV^2$. Thus, ePASP was estimated as $4*TRV^2 + RAP$. All 232 participants with measured TRV values but missing RAP were assigned a conservative RAP of 3 mmHg.

Shortness of Breath

We collected information about shortness of breath during the home interview, which included the following question: “Do you ever have shortness of breath?”. If the answer was yes, the participant was asked “Do you have shortness of breath at rest?”. If the answer was no, the participant was asked “Do you have shortness of breath when walking on a flat surface?”. If the answer was no, the participant was asked “Do you have shortness of breath when climbing stairs?”. If participants answered no to the first question, or if they answered yes to the first question but no to the other 3, they were classified as being free of significant shortness of breath. This is in line with the New York Heart Association (NYHA) validity class 1 [13].

Covariables

Information routinely collected in every follow-up round of the Rotterdam Study includes anthropometrics, cardiovascular risk factors, and medication use. BSA in m^2 was calculated as: BSA = 0.007184 * (height in cm)$^{0.725}$ * (weight in kg)$^{0.425}$ [14]. Resting heart rate was measured during blood pressure measurement using an Omron M7 pulse blood pressure monitoring device. Heart rate was measured twice with a time-interval of 5 minutes between both measurements. The mean of the 2 measurements was used in the analyses. Medication use was coded according to the Anatomical Therapeutical Chemical coding system of the World Health Organization [15].

Data Analysis

We fitted basic multivariable linear regression models to study the association of ePASP as the dependent variable with each of the following independent echocardiographic variables separately: LAD, AoD, mitral valve deceleration time, IVST, LVPWT, LVmass, LVvol, LVEDD, left ventricular fractional shortening, E/A ratio, and E/E’ ratio. All basic models were adjusted for sex, age, resting heart rate, and BSA. We also fitted a large multivariable linear regression model with ePASP as the dependent variable and LAD, AoD, mitral valve deceleration time, LV mass, LVvol, left ventricular fractional shortening, E/A ratio, and E/E’ ratio, as well as sex, age, resting heart rate, and BSA. LVvol and LVmass are calculated based on the measured LVEDD, IVST, and LVPWT. Therefore, we chose to use the clinically more relevant measures of LVvolume and LVmass in the larger model instead of the underlying measurements. To calculate the percentage of variance in ePASP explained by the left-sided measurements, we used an F-test to compare a model which only included age, sex, resting heart rate, and BSA, with a model which additionally included LAD, AoD, mitral valve deceleration time, LVmass, LVvolume, left ventricular fractional shortening, E/A ratio and E/E’ ratio. We also analyzed the effect of clinical

categories of E/A ratio and E/E' ratio instead of using the continuous measures. E/A ratio was divided into 3 categories: ≤ 0.75, > 0.75 and < 1.50, and ≥ 1.50. We divided E/E' ratio also into 3 categories: ≤ 8, > 8 and < 15, and ≥ 15[16].

We tested for potential interaction between the echocardiographic variables and sex by adding multiplicative interaction terms to the regression models. The interaction terms were not significant after Bonferroni correction for multiple testing (P-value cutoff was 0.00625), thus we did not stratify by sex. To study whether our results were driven by symptomatic individuals we repeated the analysis in participants free of significant shortness of breath.

Effect estimates are presented as ßs (both per 1-unit increase and per 1-standard deviation (SD) increase in the left-sided variable) with 95% confidence intervals (CIs). We considered P-values of < 0.05 statistically significant. All analyses were done using IBM SPSS Statistics version 21.0 (IBM Corp., Somers, NY, USA).

Results

Participant characteristics and echocardiographic measurements are presented in Table 1. The mean age was 72.6 (SD 8.7) years and 61.4% of the participants were women. The mean ePASP was 25.4 (SD 6.7) mmHg.

In the basic model, all parameters studied were significantly related to ePASP, except for IVST. Table 2 and Figure 2a show the results per unit and per 1-SD increase, respectively. The variables with the strongest associations with ePASP were LAD, E/A ratio, E/E' ratio, and LVvol.

In the large multivariable model, ePASP was associated with E/A ratio (ePASP (95% CI) per 1-SD increase: 0.96 (0.71, 1.21) mmHg), left atrial diameter (ePASP per 1-SD increase: 0.77 (0.48, 1.07) mmHg), E/E' ratio (ePASP per 1-SD increase: 0.65 (0.39, 0.92) mmHg), and left ventricular volume (ePASP per 1-SD increase: 0.57 (0.21, 0.94) mmHg), fractional shortening (ePASP per 1-SD increase: 0.49 (0.21, 0.77) mmHg), aortic root diameter (ePASP per 1-SD increase: -0.52 (-0.81, -0.24) mmHg), and mitral valve deceleration time (ePASP per 1-SD increase: -0.34 (-0.60, -0.08) mmHg). Table 3 and Figure 3a show the results per 1-unit and per 1-SD increase in measurements, respectively. The directions of effect for all associations remained the same as in the basic models (Table 2 and Figure 2a), except for LVFS, which was associated with a lower ePASP in the basic model and with a higher ePASP in the large model.

The echocardiographic parameters combined explained 8.7% of the variance in ePASP in addition to the variance explained by age, sex, resting heart rate and BSA, ($p < 0.001$).

Table 1. Demographic, clinical and echocardiographic characteristics of included participants, n = 2592

	n measured	Mean (SD) or n (%)
Age (years)	2592	72.6 (8.7)
Women	2592	1591 (61.4%)
Height (cm)	2592	167 (9)
Weight (kg)	2592	75.0 (13.4)
Body surface area (m^2)	2592	1.83 (0.19)
Body mass index (kg/m^2)	2592	26.9 (4.0)
Systolic blood pressure (mmHg)	2591	147 (22)
Diastolic blood pressure (mmHg)	2591	84 (11)
Resting heart rate (bpm)	2592	68 (11)
Chronic obstructive pulmonary disease	2592	267 (10.3%)
Atrial Fibrillation	2369	163 (6.9%)
Shortness of breath *	2337	
Validity class 1		1711 (73.2%)
Validity class 2		220 (9.4%)
Validity class 3		271 (11.6%)
Validity class 4		135 (5.8%)
Smoking	2590	
Never		944 (36.4%)
Former		1377 (53.2%)
Current		269 (10.4%)
Diabetes mellitus	2520	243 (9.6%)
Medication use **	2022	
Blood pressure lowering drugs		1131 (55.9%)
Diuretics		419 (20.7%)
Potassium sparing agents		46 (2.3%)
ß-blockers		639 (31.6%)
Calcium blockers		232 (11.5%)
RAAS inhibitors		640 (31.7%)
Other blood pressure lowering drugs		15 (0.7%)
Serum lipid reducing agents		617 (30.5%)
Echocardiography	2592	
Pulmonary arterial systolic pressure (mmHg)	2592	25.4 (6.7)
Left ventricular mass (g)	2575	130 (38)
Left ventricular volume (ml)	2582	126 (31)
Interventricular septum thickness (mm)	2578	8 (2)

Table 1. Continued

	n measured	Mean (SD) or n (%)
Left ventricular posterior wall thickness (mm)	2581	7 (1)
Diameter of left atrium (mm)	2586	42 (6)
Left ventricular fractional shortening (%)	2575	42 (6)
Left ventricular end-diastolic dimension (mm)	2582	51 (5)
Diameter of the aortic root (mm)	2586	34 (4)
E/A ratio	2457	0.93 (0.35)
≤ 0.75	2475	741 (29.9%)
> 0.75, and < 1.50	2457	1636 (66.1%)
≥ 1.50	2475	98 (4.0%)
E/E' ratio	2548	11.0 (4.8)
≤ 8	2548	620 (23.1%)
> 8, and < 15	2548	1714 (63.8%)
≥ 15	2548	352 (13.1%)
Mitral valve inflow deceleration-time,	2566	204 (42)

bpm: beats per minute; E/A ratio: E wave divided by A wave; E/E' ratio: E wave divided by E'; HDL: high density lipid protein; RAAS: renin-angiotensin-aldosterone system

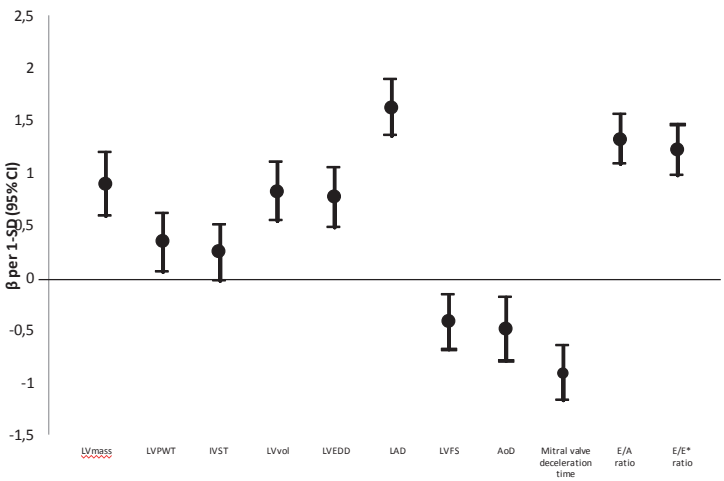

Figure 2a. Basic model: Associations of individual left heart echocardiographic parameters with ePASP in the Rotterdam Study, n = 2592[a,b]

[a] Number of participants for which we had at least information of one left heart echocardiographic parameter and ePASP

[b] Adjusted for age, sex, resting heart rate, and body surface area

LVmass: left ventricular mass; LVPWT: left ventricular posterior wall thickness; IVST: interventricular septum thickness; LVvol: left ventricular volume; LVEDD: left ventricular end-diastolic diameter; LAD: left atrial diameter; LVFS: left ventricular fractional shortening; AoD: aortic root diameter; E/A ratio: E wave divided by A wave; E/E' ratio: E wave divided by E'

Table 2. Basic model: associations of individual left heart echocardiographic parameters with ePASP in the Rotterdam Study

	Full study population, n = 2592 [a, b]		Restricted to free of shortness of breath, n = 1711 [a, b]	
	Difference in ePASP per 1-unit increase (95% CI)	p -value	Difference in ePASP per 1-unit increase (95% CI)	p -value
Left ventricular mass (per 10 grams)	0.23 (0.15 to 0.30)	<0.001	0.19 (0.10 to 0.29)	<0.001
Left ventricular volume (per 10 mL)	0.26 (0.27 to 0.35)	<0.001	0.22 (0.11 to 0.34)	<0.001
Left ventricular posterior wall thickness (mm)	0.28 (0.05 to 0.51)	0.016	0.28 (0.00 to 0.55)	0.048
Interventricular septum thickness (mm)	0.15 (-0.02 to 0.32)	0.078	0.12 (-0.09 to 0.32)	0.264
Diameter of left atrium (mm)	0.27 (0.22 to 0.31)	<0.001	0.25 (0.19 to 0.30)	<0.001
Left ventricular fractional shortening (%)	-0.07 (-0.12 to -0.03)	0.002	-0.05 (-0.11 to 0.01)	0.102
Left ventricular end-diastolic dimension (mm)	0.14 (0.09 to 0.20)	<0.001	0.13 (0.06 to 0.19)	<0.001
Diameter of the aortic root (mm)	-0.12 (-0.20 to -0.05)	0.001	-0.15 (-0.24 to -0.07)	0.001
Mitral valve inflow deceleration-time (per 10 msec)	-0.22 (-0.28 to -0.15)	<0.001	-0.20 (-0.27 to -0.13)	<0.001
E/A ratio	3.94 (3.23 to 4.65)	<0.001	3.60 (2.67 to 4.53)	<0.001
≤ 0.75	0.00 (reference)		0.00 (reference)	
> 0.75, and < 1.50	1.55 (0.98 to 2.12)	<0.001	1.47 (0.79 to 2.15)	<0.001
≥ 1.50	6.24 (4.94 to 7.54)	<0.001	5.69 (4.05 to 7.32)	<0.001
E/E' ratio	0.27 (0.22 to 0.32)	<0.001	0.27 (0.19 to 0.35)	<0.001
≤ 8	0.00 (reference)		0.00 (reference)	
> 8 and < 15	1.08 (0.46 to 1.70)	0.001	1.09 (0.36 to 1.83)	0.003
≥ 15	3.36 (2.44 to 4.28)	<0.001	3.36 (2.24 to 4.48)	<0.001

[a] Number of participants for which we had at least information of one left heart echocardiographic parameter and ePASP

[b] Adjusted for age, sex, resting heart rate, and body surface area

ePASP: echocardiographic pulmonary artery systolic pressure; SD: standard deviation; CI: confidence interval; E/A ratio: E wave divided by A wave; E/E' ratio: E wave divided by E'

Table 3. Large model: Multivariable analysis of associations of left heart echocardiographic parameters and ePASP in the Rotterdam Study

	Full study populationbll, n = 2400 [a, b]		Restricted to free of shortness of breath, n = 1604 [a, b]	
	ß per -1unit (%95 CI)	p - value	ß per -1unit (%95 CI)	p - value
Left ventricular mass (per 10 grams)	0.06 (-0.03 to 0.16)	0.272	0.07 (-0.05 to 0.18)	0.202
Diameter of left atrium (mm)	0.13 (0.08 to 0.18)	<0.001	0.15 (0.09 to 0.20)	<0.001
Left ventricular fractional shortening (%)	0.09 (0.04 to 0.14)	0.036	0.07 (0.00 to 0.13)	0.001
Left ventricular volume (per 10 mL)	0.18 (0.07 to 0.30)	0.015	0.18 (0.04 to 0.33)	0.002
Diameter of the aortic root (mm)	-0.13 (-0.20 to -0.06)	<0.001	-0.17 (-0.26 to -0.08)	<0.001
Mitral valve inflow deceleration-time, per 10 msec	-0.08 (-0.14 to -0.02)	0.059	-0.07 (-0.15 to 0.00)	0.011
E/A ratio	2.87 (2.12 to 3.62)	<0.001	2.75 (1.77 to 3.73)	<0.001
≤ 0.75	0.00 (reference)	NA	0.00 (reference)	NA
> 0.75, and < 1.50	1.10 (0.53 to 1.67)	0.001	1.15 (0.47 to 1.83)	<0.001
≥ 1.50	4.40 (3.07 to 5.73)	<0.001	4.21 (2.53 to 5.89)	<0.001
E/E' ratio	0.14 (0.09 to 0.20)	0.001	0.15 (0.06 to 0.23)	<0.001
≤ 8	0.00 (reference)	NA	0.00 (reference)	NA
> 8, and < 15	0.45 (-0.13 to 1.04)	0.087	0.61 (-0.09 to 1.31)	0.131
≥ 15	1.58 (0.66 to 2.50)	0.001	1.97 (0.84 to 3.10)	0.001

[a] Number of participants for which we had at least information of one left heart echocardiographic parameter and ePASP

[b] Adjusted for age, sex, resting heart rate, body surface area, and all other continuous left heart echocardiographic parameters in this table

ePASP: echocardiographic pulmonary artery systolic pressure; SD: standard deviation; CI: confidence interval; E/A ratio: E wave divided by A wave; E/E' ratio: E wave divided by E'

When restricting the analysis to the 1604 participants free of shortness of breath and with full data on all covariates in the large model, point estimates were similar to those obtained in the full study population. Table 3 and Figure 3b show the results per 1-unit and per 1-SD increase in the left heart measurements, respectively. In the large model, all echocardiographic parameters remained significantly associated with ePASP, except LVmass and mitral valve deceleration time. In this model the strongest associations with ePASP were found for E/A ratio, LAD, and AoD.

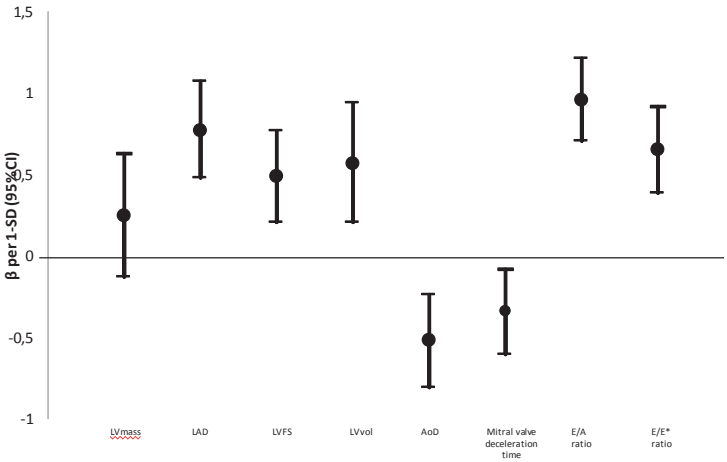

Figure 3a. Large multivariable model: Associations of left heart echocardiographic parameters with ePASP in the Rotterdam Study, n = 2400[a,b]

[a] Number of participants for which we had at least information of one left heart echocardiographic parameter and ePASP

[b] Adjusted for age, sex, heart rate, and body surface area and all other left heart echocardiographic parameters in this figure

LVmass: left ventricular mass; LAD: left atrial diameter; LVFS: left ventricular fractional shortening; LVvol: left ventricular volume; AoD: aortic root diameter; E/A ratio: E wave divided by A wave; E/E' ratio: E wave divided by E'

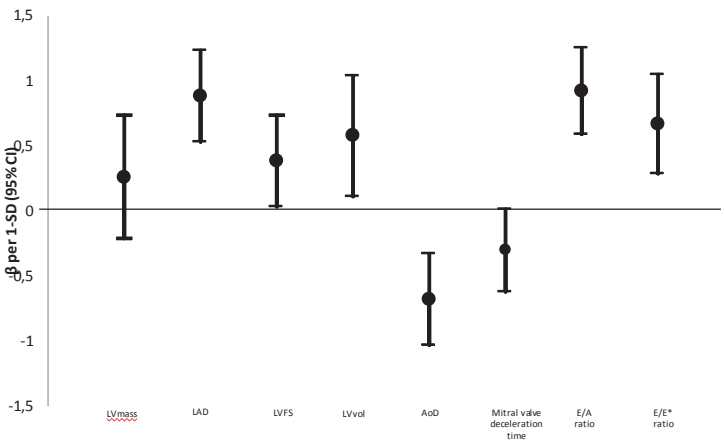

Figure 3b. Large multivariable model: Associations of left heart echocardiographic parameters with ePASP in the Rotterdam Study, restricted to participants without shortness of breath, n = 1604[a,b]

[a] Number of participants for which we had at least information of one left heart echocardiographic parameter and ePASP

[b] Adjusted for age, sex, heart rate, and body surface area and all other left heart echocardiographic parameters in this figure

LVmass: left ventricular mass; LAD: left atrial diameter; LVFS: left ventricular fractional shortening; LVvol: left ventricular volume; AoD: aortic root diameter; E/A ratio: E wave divided by A wave; E/E' ratio: E wave divided by E'

Discussion

In our study among older community-dwelling adults, structural and functional left heart parameters were associated with ePASP. Most of the associations persisted when we restricted the analysis to participants free of shortness of breath, suggesting that these associations are not driven by those with overt symptoms of heart failure.

Our findings are in line with a study including 6598 male recruits from the Israeli air force. The authors reported significant associations of, LAD, and LVEDD with ePASP [5]. Furthermore, LVmass was not significantly related to ePASP in the Israeli study, which also corresponds with our findings. In contrast to our findings, the authors observed no significant association of LVFS with ePASP. This difference might be explained by the differences in age and health status of the study populations. When we restricted our analysis to participants without shortness of breath, and hence to the healthier part of our population, left ventricular fractional shortening was also no longer significantly related to ePASP in our study.

A recent study in 1480 Italians free of structural heart disease with a mean age of 36 years showed similar results to ours, with a significant association of left ventricular stroke volume and E/E' ratio with ePASP and no significant association with left ventricular mass index[4]. Furthermore, Choudhary and colleagues did a similar study in 3282 African Americans with a mean age of 56 years, evaluating echocardiographic correlates of pulmonary hypertension rather than the entire spectrum of pulmonary artery pressures [6]. The authors reported a significant association of LAD and for a LV ejection fraction below 50% with presence of pulmonary hypertension. These results are overall in line with ours, although the latter association attenuated in our multivariable analysis.

We hypothesized that left-sided echocardiographic parameters across the full spectrum would be associated with ePASP, as early alterations in left heart structure and function can herald manifest heart failure, which is a known important cause of pulmonary hypertension. The effect sizes for most left heart parameters attenuated when studied in the large multivariable model, but overall, the associations remained. Only LVFS showed a change in the direction of effect. This warrants further research to elucidate the underlying mechanisms involved. Also, we observed that a greater AoD was associated with a lower ePASP. A similar association between AoD and ePASP was reported in the Israeli study [5]. Other authors have reported an association between higher arterial systolic blood pressure and aortic root size in a population-based setting [17]. Concomitant stiffening of both the aortic and pulmonary artery walls, reducing the compliance of both, might explain these findings. This reduced compliance would cause a higher pulse pressure in the aortic root and a higher pressure in the pulmonary artery, as well as with a smaller aortic root diameter [17]. However, this hypothesis warrants further fundamental experimental research.

Strengths and Limitations

The unselected nature of the population included in this study and the large sample size enabled us to investigate the associations of the full spectrum of left heart echocardiographic parameters with pulmonary arterial pressures in the general older population. Some limitations need to be addressed. First, right heart catheterization is the gold standard for pulmonary arterial pressure measurements, but due to its invasiveness, heart catheterization is not suitable for population-based studies. However, several studies have shown that ePASP correlates well with invasively measured pulmonary arterial pressure and that echocardiography is appropriate for measurement of PASP in this setting [18, 19]. Also, the largest differences between pulmonary pressure measured by heart catheterization and echocardiography arise in those with high pressures. The vast majority of our participants had pressure estimates in the normal range[20]. Secondly, we had a number of missing measurements for the echocardiographic parameters (Figure 1 and Table 1). Participants with missing echocardiographic variables were on average older (mean age 74.7 (SD 8.2) years), had a higher BMI (mean BMI 32.0 (SD 6.2) kg/m^2), had a slightly worse systolic function (mean fractional shortening 40 (SD 7) %), and were more likely to report shortness of breath (47.5%). Thus, these participants were overall less healthy. In addition, 800 participants had undergone echocardiography, but did had a TRV that was too small to quantify and therefore have a very low ePASP. These missing values will have affected the distribution of the studied echocardiographic parameters in our sample, but are unlikely to have affected the ß estimates since we found no indications for nonlinearity in the data (data not shown). Thirdly, by assigning a conservative RAP of 3 mmHg to all participants with measured TRV values but missing RAP, we may have underestimated ePASP in some participants. This would bias our results towards the null. Fourthly, our population was older (mean age 72.6 years) and predominantly white (96.5%). Whether our results can be directly generalized to other populations remains unclear. Lastly, ePASP values were measured once, and follow-up is not yet available. Therefore, the temporal relation between left heart abnormalities and ePASP remains to be established, as well as the clinical sequelae. Moreover, it remains to be studied whether progression to PH could be halted or controlled by pharmacological treatment in persons with (subclinical) left heart disease [21-24].

Conclusion

Structural and functional echocardiographic parameters of the left heart are associated with pulmonary arterial pressures in the general ageing population.

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The Giessen Pulmonary Hypertension Registry: Survival in Pulmonary Hypertension Subgroups

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Abstract

Pulmonary hypertension (PH) is a severe progressive disease. Though five subgroups are recognised, reports on survival focus mainly on pulmonary arterial hypertension (PAH). Long-term transplant-free survival, and its determinants, were investigated in patients with different PH subtypes within a prospective registry at a single referral centre.

In total, 2067 patients with PH were enrolled (PAH, n=685 [33.1%]; pulmonary venous hypertension, n=307 [14.9%]; PH due to lung diseases, n=546 [26.4%; mainly interstitial lung disease and chronic obstructive pulmonary disease]; chronic thromboembolic PH, n=459 [22.2%]; PH owing to miscellaneous/unknown causes, n=70 [3.4%]). Differences in transplant-free survival between aetiological groups were highly significant ($p<0.001$), with 1-, 3-, and 5-year survival rates of 88.2%, 72.2%, and 59.4%, respectively, for those with PAH compared with 79.5%, 52.7%, and 38.1%, respectively, for patients with PH caused by lung disease. Patients' age, sex, and 6-minute walk test distance, but not New York Heart Association (NYHA) functional class, associated significantly with survival across all PH subtypes in multivariate Cox regression analyses.

This is the largest reported single-centre PH cohort. Some parameters used in clinical practice do not independently predict survival. Age, sex, and 6-minute walk test distance outperformed NYHA functional class in predicting survival across all aetiologic groups.

Key words: hypertension, pulmonary; registries; survival.

Introduction

Pulmonary hypertension (PH) is a progressive disease of the pulmonary vasculature that is defined by an elevated mean pulmonary artery pressure (PAP) of ≥25 mmHg. PH is associated with increased pulmonary vascular resistance (PVR) which can lead to right heart failure and subsequent death [1]. The World Health Organization (WHO) discriminates five main PH subtypes [2], but most PH survival studies concern one subtype (pulmonary arterial hypertension [PAH], particularly idiopathic PAH [IPAH]); data regarding other subtypes are rare.

In an early registry (started in 1981), 68%, 48%, and 34% of patients with IPAH survived 1, 3, and 5 years, respectively [3, 4]. Survival has since improved: 1-year survival was 83–91% in more recent French and US-based PAH registries [5-7], and median survival was 3.8 and 5.6 years for women and men with IPAH, respectively, in a Scottish registry [8]. The UK-based ASPIRE registry provided 1- and 3-year survival data for several PH groups: PAH (88% and 68%), pulmonary venous hypertension (PVH, 90% and 73%), lung disease-associated PH (LD-PH, 65% and 44%), and CTEPH (89% and 71%) [9].

Several clinical factors predict PAH course and outcome, including exercise tolerance and New York Heart Association (NYHA) functional class [10-13]. Haemodynamic parameters such as mean right atrial pressure (RAP) and cardiac index are also associated with survival [3, 14]. Serum markers such as brain natriuretic peptide and uric acid are independently related to survival, as is hyponatremia [15-17]. However, equivalent information is lacking for other PH aetiologies.

This report presents, for the first time, comprehensive long-term transplant-free survival data from more than 2000 PH patients from a single referral centre (the Giessen Pulmonary Hypertension Registry [Gi-PH-Reg]), including data for different PH subtypes.

Methods

Data collection

The single-centre Gi-PH-Reg started in March 1993 at the University Hospital Giessen. Eligible patients were recruited by 13 October 2011, with PH defined as mean PAP ≥25 mmHg at rest by right heart catheterisation. Patients with isolated exercise-induced PH (mean PAP >30 mmHg at exercise) were excluded. The Dana Point classification [2] and STROBE guidelines [18] were applied. The definition of a prevalent case was both a diagnosis of PH and PH target therapy started before the first visit to our centre; incident cases were those diagnosed initially at our centre or referred after diagnosis without target therapy. Survival status was determined by contacting the patient or their local physician.

The date and cause of deaths were obtained from medical records; if no information was available the patient was classified as lost to follow-up and censored at the date of the last visit. Patients undergoing lung transplantation were considered to have had an event at the transplantation date. Baseline demographics, PH aetiology, medication use, echocardiographic parameters, and exercise testing, lung function and right heart catheterisation data were entered into an electronic database. Date of first visit was taken as the start date, and patients were classified into modified NYHA functional classes I–IV (hereafter referred to as NYHA I–IV) [19]. The study was approved by the University of Giessen institutional review board (#266/11). All patients gave written informed consent.

Right heart catheterisation

At their baseline visit, 1422 patients underwent right heart catheterisation, usually via the internal jugular vein with a 7F Swan–Ganz catheter. Other patients were diagnosed invasively before referral; heart catheterisation data from these patients were excluded unless the catheterisation was repeated at Giessen. Cardiac output (CO) was measured by thermodilution. Pulmonary capillary wedge pressure (PCWP) was registered and PVR calculated as: (mean PAP–PCWP)×80/CO [20]. Arterial partial oxygen pressure (paO_2) was determined from a capillary blood test, while mixed venous oxygen saturation ($venSO_2$) was measured from blood sampled from the Swan–Ganz catheter.

Six-minute walk test

The six-minute walk test (6MWT) was performed in 1290 patients according to the American Thoracic Society guidelines [21]. Other patients received spiroergometry as a baseline cardiopulmonary exercise test, or were not able to walk for different reasons, so data were not available.

Statistical methods

Data were collected, checked, and entered by independent research assistants, and two PH specialists checked medical information independently. Kaplan–Meier curves were constructed and log rank tests performed to compare survival distributions. For survival analysis, patients who underwent a pulmonary endarterectomy (PEA) were considered as withdrawn alive at the date of the PEA. Association of parameters with survival was tested using univariate and multivariate Cox regression. Regression analysis parameters were selected on clinical grounds, own prior analyses, and literature review. Comparisons between groups used the t-test or chi-square test, as appropriate. P-values of <0.05 were considered statistically significant. Bonferroni correction for multiple testing was performed for multivariate Cox regression (cut-off for significance 0.0029). Statistical analyses were performed using SPSS 20.0 (IBM Armonk, NY, USA).

Results

Study population

In total, 2067 patients were enrolled and analysed (table 1). Mean age was 59.6 years, with a female-to-male ratio of 1.24:1. Of the total study population, 33.1% had PAH, 22.2% had CTEPH, 14.9% had PVH, and 26.4% had LD-PH, the latter consisting mainly of interstitial lung disease (ILD) and chronic obstructive pulmonary disease (COPD). Seventy patients (3.4%) had PH due to miscellaneous or unknown causes (not shown in table 1). Incident cases (n=1861) accounted for 90% of the study population; the remaining 10% were prevalent cases (n=206). Of 294 patients with IPAH, 226 were incident cases (76.9%). In the subgroups with CTEPH, PVH, ILD-associated PH, and COPD-associated PH, 93.0%, 94.8%, 95.9%, and 93.6% were incident cases, respectively.

Table 1. Baseline characteristics.

	PAH (n=685)	CTEPH (n=459)	PVH (n=307)	LD-PH (n=546)
Female sex, No. (%) [female:male ratio]	447 (65) [1.9:1]	258 (56) [1.28:1]	184 (60) [1.5:1]	218 (40) [0.66:1]
Age, mean (SD), y	51 (16)	62 (13)	67 (11)	64 (11)
NYHA FC, No. (%)				
II	106 (19)	52 (15)	41 (18)	39 (12)
III	338 (59)	206 (60)	149 (64)	182 (54)
IV	126 (22)	84 (25)	43 (19)	119 (35)
6MWT, mean (SD), m	325 (126)	308 (116)	302 (110)	263 (115)
RAP, mean (SD), mmHg	8 (6)	8 (5)	10 (6)	5 (4)
mPAP, mean (SD), mmHg	51 (16)	44 (13)	34 (12)	34 (11)
PCWP, mean (SD), mmHg	8 (4)	9 (4)	18 (7)	8 (4)
CI, mean (SD), L/min/m^2	2.3 (0.8)	2.2 (0.6)	2.3 (0.6)	2.5 (0.7)
PVR, median (IQR), dyne.s/cm^5	846 (720)	720 (558)	253 (214)	407 (329)
venSO_2, mean (SD), %	61 (10)	60 (9)	63 (8)	65 (8)
paO_2, mean (SD), mmHg	68 (14)	65 (12)	71 (12)	67 (16)

Abbreviations: CI, cardiac index; CTEPH, chronic thromboembolic pulmonary hypertension; IQR, interquartile range; LD-PH, pulmonary hypertension due to lung disease; mPAP, mean pulmonary artery pressure; NYHA FC, New York Heart Association functional class; PAH, pulmonary arterial hypertension; paO_2, arterial oxygen partial pressure; PCWP, pulmonary capillary wedge pressure; PVH, pulmonary hypertension due to left heart disease; PVR, pulmonary vascular resistance; RAP, right atrial pressure; venSO_2, mixed venous oxygen saturation; 6MWT, 6-minute walk test.

* A total of 70 patients (with PH due to miscellaneous or unknown causes) were not included in this table, but formed the remainder of the 2067 patients enrolled and included in the analysis.

Survival analysis

By the end of the observation period, 924 patients (44.7%) had died or had undergone lung or heart and lung transplantation (n=52), and 162 patients (7.8%) were lost to follow-up. Overall survival at 1, 3, and 5 years was 85.5%, 66.7%, and 53.6%, respectively. The risk of death differed significantly between the aetiological groups (log rank p<0.001, fig. 1a).

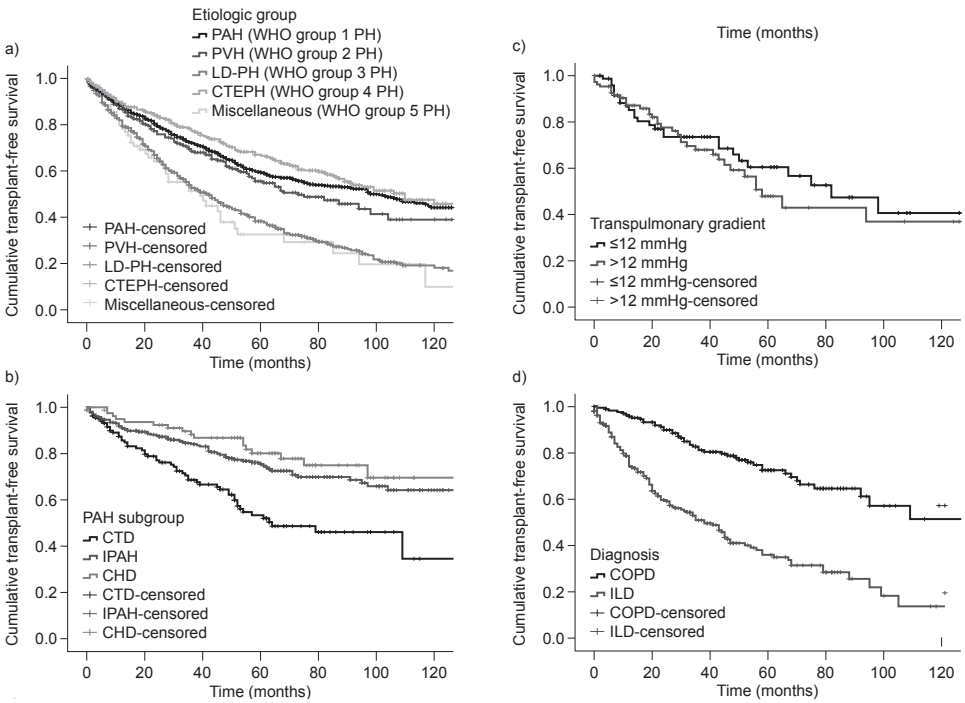
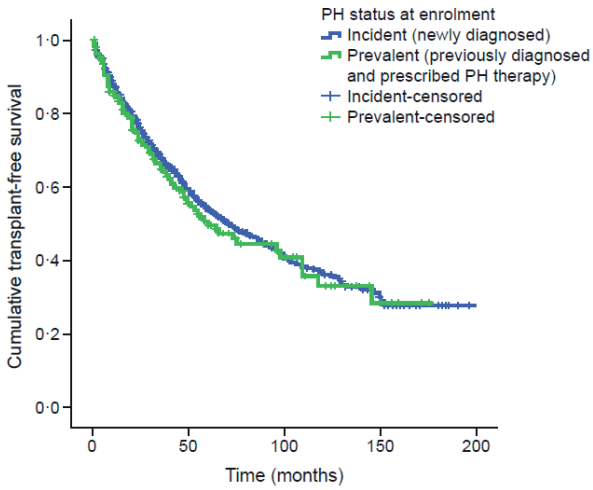


Figure 1. Kaplan–Meier transplant-free survival estimates for a) all PH aetiological groups (significant difference between groups, log rank p<0.001), b) the main PAH subgroups (significant difference between groups, log rank p<0.001), c) patients with PVH categorised by transpulmonary pressure gradient (≤12 mmHg vs >12 mmHg; no significant difference between groups, log rank p=0.516), and d) the main LD-PH subgroups (COPD and ILD; significant difference between groups, log rank p<0.001).
Abbreviations: CHD, congenital heart disease; COPD, chronic obstructive pulmonary disease; CTEPH, chronic thromboembolic pulmonary hypertension; CTD, connective tissue disease; ILD, interstitial lung disease; IPAH, idiopathic pulmonary arterial hypertension; LD-PH, pulmonary hypertension due to lung disease; PAH, pulmonary arterial hypertension; PH, pulmonary hypertension; PVH, pulmonary venous hypertension.

More deaths occurred among men (483/922; 52.4%) than women (441/1145; 38.5%; p<0.001). Baseline NYHA data were available for 1533 patients, and classes I and II were pooled as only 16 patients were class I. Five-year survival was 78.3%, 58.2%, and 39.4% for patients in NYHA I/II, III, and IV, respectively (overall p<0.001). Survival showed no significant difference between incident and prevalent patients across all aetiologies (log rank p=0.447; online supplementary fig. 1).



Supplementary Figure 1. Comparison of transplant-free survival for all pulmonary hypertension aetiologies grouped as incident (n=1861) or prevalent (n=206) case

PAH (WHO group 1 PH)

The subtype distribution among the 685 patients with PAH was: IPAH, 42.9%; connective tissue disease (CTD), 21.2%; congenital heart disease (CHD), 13.3%; porto-PH, 7.4%; pulmonary veno-occlusive disease (PVOD), 4.1%; human immunodeficiency virus (HIV), 3.9%; and PAH from other causes, 7.2% (online supplementary table 1). Women predominated (table 1).

Overall, 295 patients with PAH (43.1%) died within the observation period; this included 180 (40.3%) of the female patients and 115 (48.3%) of the male patients (5-year survival: 63.4% *vs* 51.9%, respectively; p=0.032). Of the patients with PAH, those with CHD had the highest survival rates, followed by IPAH and CTD, whereas patients with PVOD had the worst prognosis (table 2). A comparison between the largest PAH subgroups (IPAH, CTD and CHD) in fig. 1b shows a significant difference in survival (p<0.001).

Supplementary Table 1. Baseline characteristics of PAH patients by main aetiologic sub-type.

	PAH	IPAH	CTD	CHD
Patients, No.	685	294	145	91
Female sex, No. (%)	447 (65)	193 (66)	122 (84)	54 (59)
Age, mean (SD), y	51 (16)	49 (16)	57 (15)	47 (16)
NYHA FC, No. (%)				
II	106 (19)	43 (17)	19 (16)	19 (23)
III	338 (59)	157 (63)	60 (51)	54 (65)
IV	126 (22)	48 (19)	39 (33)	10 (12)
6MWT, mean (SD), m	325 (126)	347 (121)	273 (135)	345 (102)
RAP, mean (SD), mmHg	8 (6)	7 (6)	8 (6)	8 (5)
mPAP, mean (SD), mmHg	51 (16)	53 (16)	46 (14)	58 (25)
PCWP, mean (SD), mmHg	8 (4)	7 (3)	8 (4)	10 (5)
CI, mean (SD), L/min/m^2	2.3 (0.8)	2.1 (0.6)	2.2 (0.7)	2.8 (1.0)
PVR, median (IQR), dyne.s/cm^5	846 (720)	1080 (582)	924 (541)	948 (675)
venSO_2, mean (SD), %	61 (10)	61 (10)	60 (10)	67 (9)
paO_2, mean (SD), mmHg	68 (14)	68 (14)	68 (13)	64 (14)

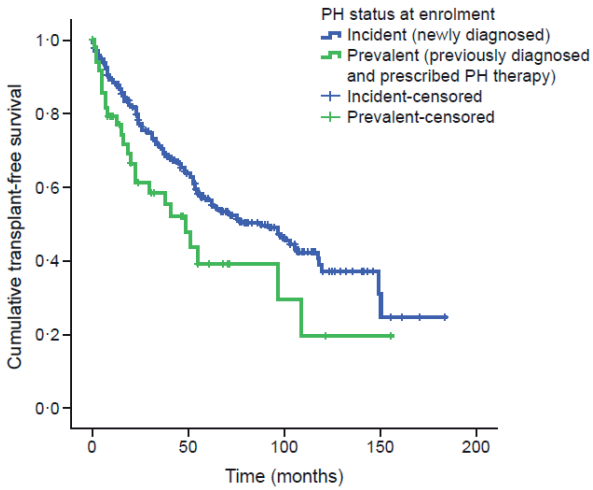
Abbreviations: CHD, pulmonary arterial hypertension associated with congenital heart disease; CI, cardiac index; CTD, pulmonary arterial hypertension associated with connective tissue disease; IPAH, idiopathic pulmonary arterial hypertension; IQR, interquartile range; mPAP, mean pulmonary artery pressure; NYHA FC, New York Heart Association functional class; PAH, pulmonary arterial hypertension; paO_2, arterial oxygen partial pressure; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; RAP, right atrial pressure; venSO_2, mixed venous oxygen saturation; 6MWT, 6-minute walk test.

Table 2. Survival in patients with pulmonary arterial hypertension or pulmonary venous occlusive disease.

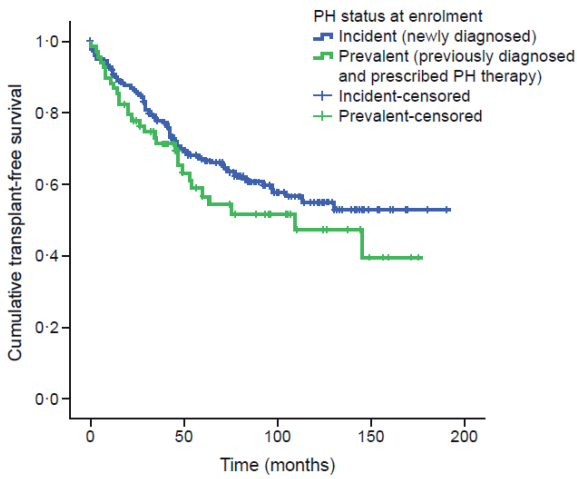
	PAH	IPAH	CTD	CHD	PVOD
Patients, No.	685	294	145	91	28
Survival (%)					
At 1 year	88.2	89.7	85.3	95.4	78.6
At 3 years	72.2	76.2	65.6	84.2	41.2
At 5 years	59.4	65.3	50.9	74.5	18.7

Abbreviations: CHD, pulmonary arterial hypertension associated with congenital heart disease; CTD, pulmonary arterial hypertension associated with connective tissue disease; IPAH, idiopathic pulmonary arterial hypertension; PAH, pulmonary arterial hypertension; PVOD, pulmonary venous occlusive disease.

Survival for incident *vs* prevalent cases of PAH is shown in table 3. A significant difference in survival was detected between incident and prevalent cases for patients with associated PAH (log rank p=0.023, online supplementary fig. 2), but not IPAH (log rank p=0.201; online supplementary fig. 3); however, numbers at risk were low in the prevalent groups (associated PAH: n=49 at first visit, n=8 after 5 years; IPAH: n=68 at first visit, n=24 after 5 years).



Supplementary Figure 2. Comparison of transplant-free survival for patients with associated pulmonary arterial hypertension (APAH) grouped as incident (n=325) or prevalent (n=38) cases.



Supplementary Figure 3. Comparison of transplant-free survival for patients with idiopathic pulmonary arterial hypertension (IPAH) grouped as incident (n=226) or prevalent (n=68) cases.

Table 3. Survival in subgroups with incident or prevalent pulmonary arterial hypertension.

	IPAH		APAH	
	Incident	Prevalent	Incident	Prevalent
Patients, No.	226	68	325	38
Survival (%)				
At 1 year	90.6	86.8	88.2	79.2
At 3 years	77.8	71.3	70.5	61.1
At 5 years	67.1	58.9	56.8	39.2
At 10 years	54.9	47.3	37.2	19.6

Abbreviations: APAH, associated pulmonary arterial hypertension; IPAH, idiopathic pulmonary arterial hypertension.

PVH (WHO group 2 PH)

Of 307 patients with PVH, 111 (36.2%) died within the follow-up period; survival at 1, 3, and 5 years was 86.7%, 68.6%, and 55.6%, respectively. Women predominated (table 1), and 5-year survival was 61.8% for women *vs* 47.1% for men (p=0.004). NYHA data were available for 233 patients with PVH; 5-year survival was worse for patients in NYHA III (59.3%) and NYHA IV (32.5%) than for those in NYHA I/II (85.9%; overall p<0.001).

Categorising PVH patients by transpulmonary pressure gradient (TPG; ≤12 mmHg *vs* >12 mmHg), 78 patients (42%) had low TPG (passive PVH) and 108 (58%) had high TPG (reactive PVH; online supplementary table 2). The haemodynamic measures between these groups differed regarding mean PAP and PVR (p<0.001) with no significant differences in cardiac index, PCWP, or RAP (p>0.20). NYHA functional class also showed no significant difference (p=0.619), though exercise capacity was better in the group with passive rather than reactive PVH (p=0.019). Survival at 1, 3, and 5 years was comparable in both groups (passive PVH: 86.6%, 73.5 %, and 60.4%; reactive PVH: 87.0%, 67.9%, and 47.8%, respectively; log rank p=0.516) (fig. 1c). More patients with reactive than passive PVH were treated with PAH-specific medications (46% *vs* 26%, respectively).

Supplementary Table 2. Baseline characteristics of patients with pulmonary hypertension due to left heart disease (PVH), categorised by transpulmonary gradient.

	"Passive" PVH† (n=78)	"Reactive" PVH‡ (n=108)
Female sex, No. (%)	44 (56)	64 (59)
Age, mean (SD), y	67 (11)	68 (11)
NYHA FC, No. (%)		
II	11 (20)	15 (19)
III	36 (65)	49 (62)
IV	8 (15)	15 (19)
6MWT, mean (SD), m	331 (117)	281 (111)
RAP, mean (SD), mmHg	9 (6)	10 (5)
mPAP, mean (SD), mmHg	26 (6)	40 (11)
PCWP, mean (SD), mmHg	18 (6)	18 (8)
CI, mean (SD), L/min/m^2	2.3 (0.6)	2.4 (0.6)
PVR, median (IQR), dyne.s/cm^5	170 (60)	440 (251)
venSO_2, mean (SD), %	65 (7)	62 (9)
paO_2, mean (SD), mmHg	73 (10)	69 (14)

Abbreviations: CI, cardiac index; IQR, interquartile range; mPAP, mean pulmonary artery pressure; NYHA FC, New York Heart Association functional class; paO_2, arterial oxygen partial pressure; PCWP, pulmonary capillary wedge pressure; PVH, pulmonary hypertension owing to left heart disease; PVR, pulmonary vascular resistance; RAP, right atrial pressure; venSO_2, mixed venous oxygen saturation; 6MWT, 6-minute walk test.
*Of 307 patients with PVH, 186 had transpulmonary gradient data and are presented in this table.
†Transpulmonary gradient equal or below 12.
‡Transpulmonary gradient above 12.

LD-PH (WHO group 3 PH)

Most of the 546 patients with LD-PH had COPD (n=218; 39.9%) or ILD (n=283; 51.8%; online supplementary table 3). All patients with LD-PH were treated with optimised therapy for their lung disorder and received PAH-specific medications if necessary (table 4). Survival was better in patients with COPD than in those with ILD (1-, 3-, and 5-year survival: 87.7%, 66.3%, and 54.0% *vs* 71.9%, 40.3%, and 22.5%; p<0.001) (fig. 1d). Men predominated (table 1), and 5-year survival was 44.0% for women *vs* 34.3% for men (p=0.001). Survival after 1, 3, and 5 years was 90.9%, 74.2%, and 52.4% for patients in NYHA I/II (n=39), 83.2%, 56.2%, and 37.7% for patients in NYHA III (n=182), and 76.6%, 42.5%, and 33.2% for patients in NYHA IV (n=119), respectively (p=0.011).

Supplementary Table 3. Baseline characteristics of patients with pulmonary hypertension owing to lung disease (LD-PH), and its two main aetiologic sub-types.

	LD-PH (n=546)*	COPD (n=218)	ILD (n=283)
Female sex, No. (%)	218 (40)	87 (40)	115 (41)
Age, mean (SD), y	64 (11)	64 (10)	64 (12)
NYHA FC, No. (%)			
II	39 (12)	13 (9)	21 (12)
III	182 (54)	87 (61)	85 (49)
IV	119 (35)	42 (30)	68 (39)
6MWT, m (SD)	263 (115)	259 (105)	263 (123)
RAP, mean (SD), mmHg	5 (4)	5 (4)	5 (4)
mPAP, mean (SD), mmHg	34 (11)	32 (10)	34 (12)
PCWP, mean (SD), mmHg	8 (4)	8 (4)	8 (3)
CI, mean (SD), L/min/m^2	2.5 (0.7)	2.6 (0.7)	2.4 (0.6)
PVR, median (IQR), dyne.s/cm^5	407 (329)	416 (245)	536 (334)
venSO_2, mean (SD), %	65 (8)	66 (7)	64 (8)
paO_2, mean (SD), mmHg	67 (16)	67 (17)	67 (16)

Abbreviations: CI, cardiac index; COPD, chronic obstructive pulmonary disease; ILD, interstitial lung disease; IQR, interquartile range; LD-PH, pulmonary hypertension due to lung disease; mPAP, mean pulmonary artery pressure; NYHA FC, New York Heart Association functional class; paO_2, arterial oxygen partial pressure; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; RAP, right atrial pressure; venSO_2, mixed venous oxygen saturation; 6MWT, 6-minute walk test.
*Most patients with LD-PH (n=546) had COPD (n=218; 39.9%) or ILD (n=283; 51.8%). The remaining patients (n=45) had LD-PH from various other causes.

Table 4. Initial therapy*.

	PAH	CTEPH	PVH	LD-PH
Complete data, No.	510	310	83	357
Monotherapy, No. (%):				
PDE5i	170 (33)	200 (65)	29 (35)	209 (59)
ERA	102 (20)	12 (4)	1 (1)	36 (10)
Prost. inh.	86 (17)	17 (6)	-	11 (3)
Other	8 (2)	2 (1)	-	1 (0)
Combination therapy, No. (%):				
PDE5i+ERA	37 (7)	16 (5)	2 (2)	15 (4)
PDE5i+prost. inh.	21 (4)	7 (2)	1 (1)	4 (1)
Other	16 (3)	-	-	1 (0)
Triple therapy, No. (%)	12 (2)	2 (1)	-	-
No specific therapy, No. (%)	58 (11)	54 (17)	50 (60)	80 (22)

Abbreviations: CTEPH, chronic thromboembolic pulmonary hypertension; ERA, endothelin receptor antagonist; LD-PH, pulmonary hypertension due to lung disease; PAH, pulmonary-arterial hypertension; PDE5i, phosphodiesterase type 5 inhibitor; prost. inh., inhalative prostacyclins; PVH, pulmonary hypertension due to left heart disease.
*Data are presented as absolute numbers and percent of patients with complete data on drug use.

CTEPH (WHO group 4 PH)

Of 459 patients with CTEPH, 138 (30.1%) died and 91 (19.8%) underwent PEA; survival at 1, 3, and 5 years was 89.2%, 77.4%, and 66.7%, respectively. Survival over 5 years was 71.6% for women and 60.1% for men (p=0.012). Survival at 1, 3, and 5 years was 97.8%, 92.2%, and 87.8%, respectively, for patients in NYHA I/II (n=52); the corresponding rates were 93.6%, 85.6%, and 73.5% for those in NYHA III (n=206), and 83.4%, 62.9%, and 45.6% for those in NYHA IV (n=84; overall p<0.001).

PH owing to miscellaneous causes (WHO group 5 PH)

No analyses were performed on this group owing to its small size (n=70) and heterogeneity.

Causes of death

The cause of death was known in 592 patients (from the group of 924 patients who died). Main causes of death were right heart failure related to PH (23.8%), respiratory insufficiency (21.8%), combined left and right heart failure (9.5%), malignancy (9.0%), sepsis (7.6%), pulmonary infection (5.4%), and sudden cardiac death (4.4%).

Factors associated with mortality

The relationship between survival and prognostic factors (NYHA, age, sex, and 6MWT) was assessed by calculating hazard ratios in a univariate model (table 5). All factors were prognostic in the PAH group, and NYHA was predictive in all aetiologic groups except LD-PH. Age was predictive of survival for patients from all aetiologic groups (age <50 *vs* >71 years), for PAH, PVH and LD-PH (age <50 *vs* 63–71 years), and for PAH and PVH (age <50 *vs* 50–63 years). Sex predicted survival in all aetiologic groups, with male sex associated with a worse prognosis. 6MWT was prognostic for all aetiologic groups (except for 311–390m *vs* reference category for PVH and LD-PH). NYHA lost its predictive value, but 6MWT (all etiologic groups), age (PAH), and gender (PAH and CTEPH) were still predictive for mortality when placed in one multivariate model per aetiologic group (online supplementary table 4).

Table 5. Risk factors for survival (all-cause mortality) using univariate Cox regression analysis.

	PAH, n=685 HR (95% CI; p-value)	PVH, n=307 HR (95% CI; p-value)	LD-PH, n=546 HR (95% CI; p-value)	CTEPH, n=459 HR (95% CI; p-value)
NYHA				
Class II	Reference	Reference	Reference	Reference
Class III	1.80 (1.18-2.77; 0.007)	3.04 (1.10-8.40; 0.032)	1.69 (0.99-2.87; 0.054)	3.51 (1.27-9.71; 0.015)
Class IV	3.60 (2.29-5.65; <0.001)	6.35 (2.18-18.52; 0.001)	2.18 (1.27-3.75; 0.005)	7.84 (2.80-21.95; <0.001)
Age (years)*				
<50	Reference	Reference	Reference	Reference
50-63	1.41 (1.06-1.88; 0.20)	6.23 (1.45-26.68; 0.014)	1.38 (0.93-2.03; 0.109)	0.88 (0.50-1.55; 0.654)
63-71	2.11 (1.55-2.86; <0.001)	12.11 (2.92-50.31; 0.001)	1.83 (1.24-2.71; 0.002)	1.30 (0.76-2.24; 0.343)
>71	1.97 (1.34-2.88; 0.001)	12.94 (3.13-53.59; <0.001)	2.47 (1.65-3.70; <0.001)	2.43 (1.46-4.02; 0.001)
Sex				
Male (female as reference)	1.29 (1.02-1.63; 0.033)	1.70 (1.17-2.47; 0.005)	1.43 (1.15-1.80; 0.002)	1.53 (1.10-2.14; 0.013)
6MWT (metres)†				
>390	Reference	Reference	Reference	Reference
311-390	1.99 (1.31-3.02; 0.001)	2.65 (0.56-12.51; 0.217)	1.50 (0.87-2.61; 0.147)	3.98 (1.69-9.40; 0.002)
216-311	2.82 (1.84-4.33; <0.001)	7.94 (1.83-34.52; 0.006)	2.00 (1.18-3.37; 0.010)	3.82 (1.67-8.75; 0.002)
<216	5.78 (3.87-8.63; <0.001)	12.91 (2.96-56.31; 0.001)	2.95 (1.75-4.96; <0.001)	6.56 (2.82-15.26; <0.001)

Abbreviations: CI, confidence interval; CTEPH, chronic thromboembolic pulmonary hypertension; HR, hazard ratio; LD-PH, pulmonary hypertension due to lung disease; NYHA New York Heart Association functional class; PAH, pulmonary arterial hypertension; PVH, pulmonary hypertension due to left heart disease; 6MWT, 6-minute walk test. *Age groups represent quartiles. †6MWT groups represent quartiles of the full population.

Supplementary Table 4. Risk factors for survival (all-cause mortality) using a multivariate Cox regression model.

	PAH, n=685 HR (95% CI; p-value)	PVH, n=307 HR (95% CI; p-value)	LD-PH, n=546 HR (95% CI; p-value)	CTEPH, n=459 HR (95% CI; p-value)
NYHA				
Class II	Reference	Reference	Reference	Reference
Class III	1.06 (0.62-1.81; 0.838)	1.31 (0.34-5.06; 0.695)	1.26 (0.66-2.42; 0.486)	1.65 (0.37-7.43; 0.514)
Class IV	1.08 (0.59-1.98; 0.804)	1.41 (0.33-5.94; 0.641)	1.14 (0.56-2.35; 0.716)	2.45 (0.53-11.34; 0.251)
Age (years)*				
<50	Reference	Reference	Reference	Reference
50-63	1.24 (0.85-1.80; 0.265)	1.36 (0.14-13.58; 0.795)	0.78 (0.40-1.55; 0.484)	0.82 (0.24-2.75; 0.745)
63-71	1.83 (1.23-2.72; 0.003)	3.17 (0.39-25.58; 0.2.78)	0.86 (0.42-1.76; 0.672)	1.41 (0.45-4.45; 0.558)
>71	1.32 (0.81-2.14; 0.266)	2.61 (0.32-21.15; 0.369)	1.28 (0.63-2.59; 0.500)	2.80 (0.93-8.48; 0.068)
Sex				
Male (female as reference)	1.81 (1.34-2.45;<0.001)	1.77 (0.85-3.66; 0.125)	1.14 (0.79-1.65; 0.488)	2.72 (1.59-4.67;<0.001)
6MWT (metres)†				
>390	Reference	Reference	Reference	Reference
311-390	2.17 (1.34-3.51; 0.002)	1.89 (0.36-9.97; 0.454)	1.85 (0.76-4.50; 0.174)	3.23 (0.89-11.68; 0.074)
216-311	2.56 (1.54-4.26; <0.001)	5.03 (0.95-26.72; 0.058)	2.63 (1.10-6.30; 0.029)	3.65 (1.02-13.11; 0.047)
<216	5.87 (3.53-9,78; <0.001)	10.69 (2.05-55.74; 0.005)	3.73 (1.52-9.13; 0.004)	4.53 (1.23-16.76; 0.023)

Abbreviations: CI, confidence interval; CTEPH, chronic thromboembolic pulmonary hypertension; HR, hazard ratio; LD-PH, pulmonary hypertension due to lung disease; NYHA New York Heart Association functional class; PAH, pulmonary arterial hypertension; PVH, pulmonary hypertension due to left heart disease; 6MWT, 6-minute walk test. *Age groups represent quartiles.†6MWT groups represent quartiles of the full population.

Comparison with other key registries

A systematic literature search (described in online supplementary material) identified 11 key registries which are summarised alongside the Gi-PH-Reg in table 6 (PAH populations) and table 7 (CTEPH populations).

Table 6. Comparison of patients with pulmonary arterial hypertension in the Giessen Pulmonary Hypertension Registry and other key registries.

Recruitment period, y	1993-2011	2001-2010	2002-2003	1981-1985	1998-2008	2006-	2005-2007	1982-2006	1986-2001	2001-2009
Registry	Gi-PH Reg	ASPIRE [9]	French [7]	NIH-PPH [3]	REHAP [22]	REVEAL[5]	PAH-QuERI [23]	PHC [24]	SMR [8]	UK & Ireland [25]
PAH population, n	685	600	674	194 (PPH)	866	2716	791	578	374	482
Type of PAH, %										
Idiopathic	43	29	39	-	36	47	35	48*	47	93
CTD	21	31	15	-	18	24	29	30	30	0
CHD	13	33	11	-	19	12	7	11	24	0
Porto-PH	7	4	10	-	7	5	4	7	0	0
HIV	4	1	6	-	6	2	4	1	0	0
PVOD	4	<1†	-	-	2	-	<1	-	0	0
Female sex, %	65	70	65	-	71	79	77	77	70	70
Mean age, y	51	54	50	-	45	50	55‡	48	50-52	50
NYHA FC, %										
II	19	-	-	-	31 (I-II)	38	39	-	-	16 (I-II)
III	59	64	}75	-	58	48	48	}80	-	67
IV	22	14		-	11	5	5		-	18
Mean 6MWT, m	325	-	329	-	363	370	-		-	292
Mean RAP, mmHg	8	10	8	-	9	9	-	11	-	10
Mean mPAP, mmHg	51	48	55	-	54	50	-	52	-	54
Mean PCWP, mmHg	8	9	8	-	-	10	-	10	-	9
Mean CI, L/min/m^2	2.3	2.7	2.5	-	2.6	2.6	-	2.3	-	2.1
Mean PVR, dyne.s/cm^5 (or WU where specified)	846†	780	-	-	12 WU	11 WU	-	13 WU	-	13 WU
Survival, %										
at 1 year	88	88	88	68	86	91	-	84	-	93
at 3 years	72	68	-	48	75	-	71	67	-	73
at 5 years	59	-	-	34	-	-	-	58	-	61

Abbreviations: ASPIRE, Assessing the Spectrum of Pulmonary Hypertension Identified at a Referral Centre; CI, cardiac index; CHD, congenital heart disease; CTD, connective tissue disease; Gi-PH-Reg, Giessen Pulmonary Hypertension Registry; HIV, human immunodeficiency virus; mPAP, mean pulmonary artery pressure; NIH-PPH, National Institutes of Health Patient Registry for the Characterisation of Primary Pulmonary Hypertension; NYHA FC, New York Heart Association functional class; PAH, pulmonary arterial hypertension; PAH-QuERI, Pulmonary Arterial Hypertension-Quality Enhancement Research Initiative; PCWP, pulmonary capillary wedge pressure; porto-PH, porto-pulmonary hypertension; PHC, Pulmonary Hypertension Connection; PPH, primary pulmonary hypertension; PVOD, pulmonary venous occlusive disease; PVR, pulmonary vascular resistance; RAP, right atrial pressure; REHAP, Spanish Registry of Pulmonary Arterial Hypertension; REVEAL, Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management; SMR, Scottish Morbidity Record; WU, Wood Units; 6MWT, 6-minute walk test.

*Idiopathic/familial PAH.

†Patients with PVOD (n=2) were not included in the analysis of patient characteristics and outcomes in the ASPIRE PAH group.

‡Median.

Table 7. Comparison of patients with chronic thromboembolic pulmonary hypertension in the Giessen Pulmonary Hypertension Registry and other key registries.

Registry	Gi-PH Reg	ASPIRE [9]	International CTEPH [26]	REHAP [22]	UK PH Service [27]	
			Surgically treated CTEPH		Surgically accessible CTEPH	Non-surgical CTEPH
Recruitment period, y	1993–2011	2001–2010	2007–2009	1998–2008	2001–2006	2001–2006
CTEPH population, n	459	242	386	162	321	148
Female sex, %	56	54	46	60	47	56
Mean age, y	62	61	60*	61	58	60
NYHA FC, %						
II	15	–	–	23	12	16
III	60	70	68	68	73	68
IV	25	17	13	9	15	16
Mean 6MWT, m	308	–	341*	317	243	239
Mean RAP, mmHg	8	11		8	9*	10*
Mean mPAP, mmHg	44	48	48*	47	48	49
Mean PCWP, mmHg	9	11	–	–	–	–
Mean CI, L/min/m^2	2.2	2.5	2.2*	2.3	2.1	2.1
Mean PVR, dyne.s/cm^5 (or WU where specified)	720*	735	728*	10 WU	1091	1098
Survival, %						
at 1 year	89.2	89	93	93	88†	82
at 3 years	77.4	71	–	75	76†	70
at 5 years	66.7	–	–	65	–	–

Abbreviations: ASPIRE, Assessing the Spectrum of Pulmonary Hypertension Identified at a Referral Centre; CI, cardiac index; CTEPH, chronic thromboembolic pulmonary hypertension; Gi-PH-Reg, Giessen Pulmonary Hypertension Registry; mPAP, mean pulmonary artery pressure; NYHA FC, New York Heart Association functional class; PCWP, pulmonary capillary wedge pressure; PH, pulmonary hypertension; PVR, pulmonary vascular resistance; RAP, right atrial pressure; REHAP, Spanish Registry of Pulmonary Arterial Hypertension; WU, Wood Units; 6MWT, 6-minute walk test.
*Median.
†Survival was reported for 236 patients who underwent pulmonary endarterectomy.

Online supplementary material

Comparison with other key registries – systematic literature search

In order to discuss the results of our study within the context of other published studies, we performed a systematic literature search to identify registry studies of patients with pulmonary hypertension (PH) or pulmonary arterial hypertension (PAH) that reported survival data. This was performed on 8 April 2014 in PubMed and used the following search string: ("pulmonary hypertension" OR "pulmonary arterial hypertension") AND (registry OR cohort) AND (survival OR survived OR surviving OR mortality OR died OR dying OR death) [no limits]. This strategy retrieved 715 publications. Studies were considered for inclusion by manual screening of article titles (and subsequently, of full articles) to identify registry studies in relevant populations, published in English, which also included mortality data. The search identified a range of national and international registry studies [1–19]. Results from registries of particular historical relevance [1] or of a reasonable size (i.e. more than 250 patients with PH) have been reported in the main manuscript [2–13]. Some additional references fulfilled the systematic search criteria but were too small to draw meaningful comparisons concerning overall mortality rates, and thus are not discussed [14–18]. Nevertheless, the mortality rates described in most of these smaller studies broadly agree with those from larger studies of the same era. Another study, whilst large (i.e. a cohort of 1283 patients with incident PAH) concerned the effect of anticoagulant use on patients' survival and thus was not included as it focused exclusively on areas beyond the scope of this article [19].

Discussion

This study encompasses the largest single-centre PH patient cohort reported to date. This single-centre approach has inherent advantages, namely homogeneity of data quality and consistency of standards and procedures. Only two other single-centre registries of any size have been reported, one based in the UK including 1344 incident PH cases [9] and a US registry encompassing 587 patients with PAH [28]. The overall survival rates we report here are similar to results published from other registries covering a similar era. We found significant variation between survival rates for PH subtypes, though these subgroup survival rates were similar to those reported in the literature for PAH and CTEPH, slightly better for LD-PH [9] and slightly worse for PVH [9] (owing at least in part to baseline differences).

Patients with PAH in the Gi-PH-Reg were comparable with those in other national registries in terms of mean age, 6MWT, NYHA distribution, female-to-male ratio, and main haemodynamic parameters [7, 22, 29, 30]. This concordance underlines the appropriate allocation of patients to group 1. We found that patients with IPAH were younger on average than patients with other PAH subtypes, and had more severely impaired haemodynamics. Nevertheless, the NYHA distribution and 6MWT were more favourable in patients with IPAH than in those with CTD-PAH. Patients with CHD had the best survival in the PAH group; this agrees with previous publications, showing good long-term survival in patients with CHD/Eisenmenger syndrome [9, 30]. The survival of patients with IPAH (table 2) compares favourably with outcomes from registries in France (82.9% and 58.2% at 1 and 3 years), UK/Ireland (93%, 73%, and 61% at 1, 3, and 5 years), and the US (77% at 3 years) [25, 30, 31]. However, when comparing patients with incident and prevalent IPAH, no clinically relevant or statistically significant difference was observed within our registry. This is in contrast to the French PAH registry which showed greater survival in prevalent versus incident cohorts, suggesting "immortal time bias" in the prevalent cohort [30]. The discrepancy may be at least partly due to differences in definitions: in the French registry, prevalent cases were patients diagnosed before the start of the study, whereas prevalent cases in the Gi-PH-Reg were patients who were diagnosed and had started PH therapy elsewhere before referral to our centre. It can also be speculated that environmental and/or socio-economic differences between different regions may affect "immortal time bias".

Patients with CTEPH were the third largest group in our database, and had slightly less severe haemodynamic impairment and slightly better overall survival than the PAH group. The phenotype of our CTEPH group was very similar to that of other CTEPH registries [9, 22, 26]. Moreover, the 1- and 3-year survival rates reported in the current study were similar to those reported in a large UK CTEPH registry (table 7 [27]).

Our patients with PVH had poorer survival than those in the ASPIRE registry [9]. In ASPIRE, patients with PVH had better survival than those with PAH, whereas the opposite was observed in the Gi-PH-Reg, despite the PVH group having less compromised haemodynamics than the PAH group. The Gi-PH-Reg PVH group showed classical features of an elderly population with more severely impaired exercise ability than the younger PAH group, which could at least partly explain the reduced survival in the PVH group.

The 2009 European PH guidelines distinguished reactive and passive PVH on the basis of TPG (>12 mmHg and ≤12 mmHg, respectively) [32], and some studies have shown worse survival in patients with reactive vs passive PVH using this definition [33, 34]. However, the TPG is sensitive to changes in cardiac output and pulmonary vascular recruitment and distension; a fixed TPG threshold may therefore not be a reliable indicator of reactive PVH [35]. A study of patients with heart failure with reduced ejection fraction found no difference in mortality between reactive and passive PVH defined on the basis of TPG [36], which is consistent with our findings. The diastolic pulmonary gradient has been suggested as a more suitable measure than TPG to distinguish reactive and passive PVH [34, 35].

The LD-PH group was the only subgroup in which men outnumbered women. The outcome of this group overall was worse than for patients with PAH or CTEPH. Comparing COPD and ILD patients, the latter had a considerably worse outcome, consistent with epidemiological data for these populations [9]. The ASPIRE registry included 178 LD-PH patients with 1- and 3-year survival of 65% and 44%, respectively [9]; our patients lived slightly longer, but this may be because of different proportions of COPD and ILD.

Basic clinical parameters known to be of prognostic value for patients with PAH (particularly IPAH), have been examined thoroughly in our study for other forms of PH: the 6MWT remains the strongest predictor across all groups of PH. Following current guidelines, patients are classified according to PH aetiology. This specific diagnosis should be considered when judging which prognostic factor is relevant.

NYHA functional class has been previously highlighted as an important prognostic factor in PAH [32, 37]. However, in our PAH subgroup, NYHA was identified as a predictor of mortality only by univariate and not multivariate analysis. This is consistent with the findings of several previous studies [38], though a large study of 2716 patients with PAH did identify functional class as an independent prognostic factor [5]. Prognosis may be better assessed by considering a combination of factors rather than one factor in isolation [39].

Limitations

We studied a single-centre cohort, but our reference centre is one of the largest in Germany and therefore data may be representative of the PH population – although milder cases may not be referred to us. We did not have complete data on right heart catheterisation at baseline: some patients came with clinically acceptable right heart catheterisation values from secondary centres but these data were not entered in the database.

Conclusions

This is the largest single-centre PH patient cohort reported to date. We have presented transplant-free survival data from a large number of patients with PH, including all different subtypes of PH, and examined survival determinants in these populations. Overall survival at 1, 3, and 5 years was 85.5%, 66.7%, and 53.6%, respectively, and survival differences were significant between PH subtypes. Although NYHA functional class is used commonly to predict the likelihood of survival, it was a less powerful predictor across all aetiologic groups than patients' age, sex, and 6MWT.

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3.2 |

New Potential Diagnostic Biomarkers for Pulmonary Hypertension

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Abstract

This study aimed to determine whether the VEGF family members soluble VEGF receptor 1 (sVEGFR1/soluble fms-like tyrosine kinase 1, sFlt-1) and placental growth factor (PlGF) could be used as biomarkers for PH.

Consecutive patients undergoing right heart catheterization were enrolled (those with mPAP ≥25 mmHg were classed as having PH; those with mPAP <25 mmHg acted as non-PH controls). Plasma from the time of PH diagnosis was analyzed for PlGF and sFlt-1 using enzyme immunoassays.

In total, 247 patients with PH were enrolled: 62 with idiopathic pulmonary arterial hypertension (IPAH), 14 with associated pulmonary arterial hypertension (APAH), 21 with collagen vascular disease (CVD), 26 with pulmonary venous hypertension, 67 with lung disease-associated PH, and 57 with chronic thromboembolic PH. The non-PH control group consisted of 40 patients. sFlt-1 plasma levels were significantly higher in patients with IPAH, APAH, CVD and LD-PH *vs* controls; PlGF levels were significantly higher in all PH groups *vs* controls. PlGF and sFlt-1 combined had a sensitivity of 62.4% and a specificity of 100% to detect any PH etiology. There was no association between sFlt-1 or PlGF and hemodynamic parameters, 6-minute walking distance, or survival.

In summary, PlGF and sFlt-1 are promising diagnostic biomarkers for PH.

Introduction

Pulmonary hypertension (PH) is defined as resting mean pulmonary arterial pressure (mPAP) ≥25 mm Hg (1). Different PH etiologies have resulted in a classification of PH into five groups, but regardless of etiology, PH involves vasoconstriction, media hypertrophy, and in situ thrombosis, leading to an increase in pulmonary arterial pressure, ultimately resulting in right heart failure (2).

Early diagnosis and monitoring of disease progression are critical for therapy decisions. Right heart catheterization remains the gold standard for diagnosing PH and managing those patients who are receiving PH therapy (1, 3). Brain natriuretic peptide (BNP) is an established prognostic biomarker to monitor right heart failure in PH (4), and reflects myocardial stress. There is, nevertheless, still a need for other non-invasive PH biomarkers that mirror pathological alterations that occur in the pulmonary vasculature, and which also help to diagnose PH.

Vascular endothelial growth factor (VEGF) signaling is known to be associated with PH pathogenesis, particularly in vascular remodeling (5). VEGF expression can be induced by hypoxia through upregulation of hypoxia-inducible factor-1α (HIF-1α) (6). Moreover, VEGF and VEGF receptor 2 (VEGFR-2) are overexpressed in plexiform lesions of patients with PH (7) and plasma VEGF levels are elevated in patients with idiopathic pulmonary arterial hypertension (IPAH) (8).

VEGF receptors are membrane-bound receptor tyrosine kinases. The soluble form of the VEGF receptor 1 (sVEGFR-1/soluble fms-like tyrosine kinase 1, sFlt-1) results from alternative splicing or cleavage of the full-length receptor, Flt-1 (9). The VEGF family member placental growth factor (PlGF) binds exclusively to Flt-1 (10), which leads to pro-angiogenic signaling through several mechanisms, including direct intracellular activation of Flt-1 and downstream target genes, and transphosphorylation of VEGFR-2 by activated Flt-1, thereby increasing the response to VEGF (11). Moreover, the PlGF/VEGF-A heterodimer can bind and activate Flt-1, and induce Flt-1/VEGFR-2 dimerization (12).

PlGF and sFlt-1 have shown diagnostic and prognostic potential in hypoxia-associated preeclampsia (13, 14), in sickle cell disease-associated PH (8, 15) and peripheral and coronary artery disease (16). In a recent study Malhotra et al. showed significantly upregulated sFlt-1 serum levels in PAH patients. Serum sFlt-1 levels were associated with increased New York Heart Association (NYHA) functional class and predict survival (17).
We hypothesize that Flt-1 and PlGF represent important factors in PH and can be used as biomarkers.

Methods

Patients and biomarker study design

Plasma levels of PlGF and sFlt-1 were measured in patients with five different subtypes of PH and compared with a non-PH control group, all undergoing right heart catheterization. Furthermore, plasma levels were correlated with hemodynamics, NYHA functional class, and survival.

For this case control study consecutive patients with suspicion of PH, undergoing right heart catheterization at the Giessen PH referral center, were enrolled in the study. Blood samples were taken at the baseline visit during right heart catheterization. The baseline visit was the first right heart catheter and the diagnosis or exclusion of PH for all patients. Patients with mPAP ≥25 mmHg were defined as having PH. Patients undergoing right heart catheterization with mPAP <25 mmHg were defined as non-PH controls. The non-PH controls underwent right heart catheterization because symptoms led to suspicion of PH, but by right heart catheterization PH was excluded. PH Patients were classified into etiological groups according to current guidelines (3). Plasma was collected using EDTA as an anticoagulant followed by centrifugation at 3000 g for 10 minutes and samples were then frozen at –80°C until analysis. The approval of the Local Research Ethics Committee at the University Hospital of Giessen was obtained. All patients gave written, informed consent for use and storage of plasma and future biomarker analyses on the day the samples were obtained.

Biomarker measurements

PlGF and sFlt-1 were measured with commercially available enzyme immunoassays (Quantikine, R&D Systems, Minneapolis, USA). Samples were assayed in duplicate according to manufacturer's instructions.

Statistical analysis

Baseline characteristics are presented as mean +/- standard deviation or median and interquartile range where appropriate. Kaplan–Meier curves were constructed and log-rank tests performed to compare survival distributions. The association of biomarker concentrations with survival was tested using univariate and multivariate Cox regression analyses. PlGF and sFlt-1 plasma levels were expressed as means (± SEM). One-way ANOVA, Tukey post-hoc test, chi-square test, or Kruskal–Wallis test were used for comparisons between groups, as appropriate. In order to evaluate the performance of sFlt-1 and PlGF as predictors for mortality rates, the area under the curve (AUC) of the receiver operating characteristics (ROC) curve was calculated. Correlation analyses were done with Pearson's or Spearman's co-efficient, as appropriate. A p value <0.05 was considered

statistically significant. Statistical analyses were performed using IBM SPSS Statistics 21.0 (IBM, Armonk, New York, USA).

Results

Demographic characteristics

The demographic characteristics of all patients enrolled in the main biomarker study are shown in Table 1. In total, 247 patients with PH were enrolled: 62 patients with IPAH, 14 with associated PAH (APAH, i.e. Eisenmenger's syndrome, HIV, portopulmonary hypertension), 21 with collagen vascular disease (CVD), 26 with pulmonary venous hypertension (PVH), 67 with lung disease associated pulmonary hypertension (LD-PH), and 57 with chronic thromboembolic PH (CTEPH). Non-PH controls consisted of 40 patients with invasive exclusion of PH due to similar symptoms as PH patients.

Circulating sFlt-1 and PlGF in patients with PH

Blood samples were taken from all patients at the baseline visit, and sFlt-1 and PlGF levels measured. Fig. 1 shows sFlt-1 (Fig. 1A) and PlGF (Fig. 1B) plasma levels from patients with PH and the non-PH control group. Mean levels of sFlt-1 were markedly elevated in all PAH subgroups (IPAH, APAH and CVD). Furthermore, sFlt-1 was also significantly elevated in patients with LD-PH. No statistically significant difference was detected between either the CTEPH or the PVH groups and the non-PH control group. Actual mean plasma sFlt-1 concentrations and *p*-values for PH groups in comparison with the non-PH control group (mean sFlt-1 plasma concentration 3091.6±246.5 pg/mL) were as follows: IPAH, 5049.2±460.3 pg/mL ($p = 0.045$); APAH, 6906.1±1022.1 pg/mL ($p = 0.003$); CVD, 7174.4±1056.1 pg/mL ($p < 0.001$); PVH, 3945.1±628.9 pg/mL ($p = 0.940$); LD-PH, 5338.2±390 pg/mL ($p = 0.009$); CTEPH, 3701.4±353 pg/mL ($p = 0.969$). sFlt-1 plasma levels were significantly higher in patients with APAH ($p = 0.016$) or CVD ($p = 0.001$) compared with CTEPH patients, as well as in patients with CVD *vs* those with PVH ($p = 0.012$).

Table 1. Demographics for enrolled patients with a diagnosis of pulmonary hypertension.

	IPAH	APAH	CVD	PVH	LD-PH	CTEPH
n	62	14	21	26	67	57
Age (years)	48.6±16.1	50.4±11	58.7±14.2	64.3±14	65.6±9.3	64.6±14
Sex (female %)	67.7	57.1	76.2	57.7	35.8	52.6
NYHA II/III/IV (%)	11.7/71.7/16.7	28.6/42.9/28.6	14.3/33.3/52.4	15.4/61.5/23.1	4.5/56.7/38.8	12.1/60.3/27.6
6MWD (m)	381.4±103.1	395.6±113.8	265.1±147.6	306.1±134.4	233.9±109.1	290.4±137.4
BNP (pg/mL)	102.5±220.5	99±162	131±250.5	150±137.8	92±279	116.5±306
Creatinine (mg/dL)	1.2±0.3	1.2±0.2	1.4±0.7	1.3±0.5	1.2±0.3	1.3±0.4
mPAP (mmHg)	56.6±17.6	50.7±14.3	43±10.3	40.7±15.1	36.8±13.4	42.7±14.4
PCWP (mmHg)	7.8±2.9	7.9±2.9	8.3±3.5	19.5±7.7	8.0±3.3	9.8±4.5
CI ($L/min/m^2$)	2.2±0.6	2.7±1.0	2.3±0.8	2.6±0.6	2.4±0.6	2.2±0.6
PVR (dyn x sec/cm^{-5})	939±1865	650±904	677±614	282±257	478±402	626±606
SvO_2 (%)	61.1±9.1	65.9±11.2	59.7±11.3	63.8±8.5	64.8±7.1	61.4±9.7

All values are expressed as mean ± standard deviation, except sex and NYHA functional class (%), and BNP and PVR (expressed as median ± interquartile range). APAH = associated pulmonary arterial hypertension; BNP = brain natriuretic peptide;CI = cardiac index; CTEPH = chronic thromboembolic pulmonary hypertension; CVD = collagen vascular disease; IPAH = idiopathic pulmonary arterial hypertension; LD-PH = lung disease associated pulmonary hypertension; mPAP = mean pulmonary arterial pressure; NYHA = New York Heart Association; PCWP = pulmonary capillary wedge pressure; PVR = pulmonary vascular resistance; SvO_2 = mixed venous oxygen saturation; 6MWD = 6-minute walking distance.

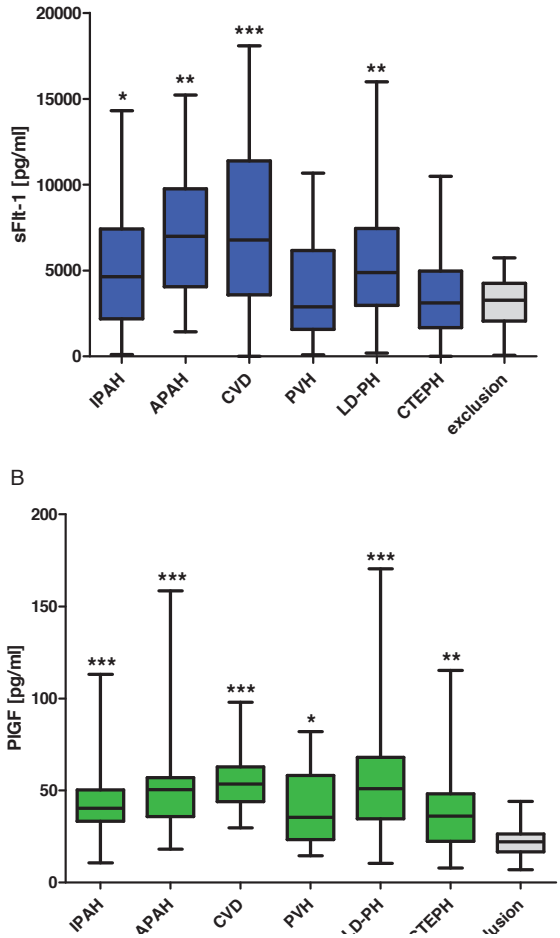

Figure 1. Levels of (A) sFlt-1 (soluble fms-like tyrosine kinase 1) and (B) PlGF (placental growth factor) in patients with pulmonary hypertension (PH) and controls without PH (exclusion), expressed as mean±SEM.

Results for all PH groups were compared with the control group (exclusion) using one-way ANOVA with Tukey post-hoc test: *p < 0.05, **p < 0.01, ***p < 0.001.

APAH = associated pulmonary arterial hypertension; CTEPH = chronic thromboembolic pulmonary hypertension; CVD = collagen vascular disease; IPAH = idiopathic pulmonary arterial hypertension; LD-PH = lung disease associated pulmonary hypertension; PVH = pulmonary venous hypertension.

PlGF levels were elevated significantly in all PH subgroups compared with the non-PH control group (Fig. 1B). Actual mean plasma PlGF concentrations and *p*-values for PH groups in comparison with the non-PH control group (mean PlGF plasma concentration 21.8±1.5 pg/mL) were as follows: IPAH, 45.6±3.6 pg/mL (p < 0.001); APAH, 54.3±9.1 pg/mL (p < 0.001); CVD, 56.3±4 pg/mL (p < 0.001); PVH, 40±3.7 pg/mL (p = 0.030); LD-PH, 53.4±3.4 pg/mL (p < 0.001); CTEPH, 39.3±3.1 pg/mL (p = 0.005).

No significant differences in sFlt-1 and PlGF levels were detected between men and women. Mean plasma concentrations across all PH groups for men and women were as follows: sFlt-1, 5071 and 4916 pg/mL, respectively (p = 0.733); PlGF, 48.6 and 45.8 pg/mL, respectively (p = 0.393). Mean plasma concentrations across PAH groups (i.e. IPAH, APAH and CVD) for men and women were as follows: sFlt-1, 6389 and 5491 pg/mL, respectively (p = 0.307); PlGF, 53.3 and 48.1 pg/mL, respectively (p = 0.376).

sFlt-1 and PlGF as diagnostic markers for PH

ROC analysis was performed and the best cut-off values chosen for sFlt-1 (5753 pg/mL) with an AUC of 0.662 (95% CI 0.592–0.733, p = 0.001; Fig. 2) and for PlGF (29.2 pg/mL) with an AUC of 0.847 (95% CI 0.797–0.898, p < 0.001; Fig. 2). Specificity was 100% for sFlt-1 and 85% for PlGF when using the aforementioned cut-off-values. Likewise, sensitivity values were 36% for sFlt-1 and 77% for PlGF. The combination of sFlt-1 and PlGF resulted in a sensitivity of 62.4% and specificity of 100% (chi-square p < 0.001 for both). For combination analysis only patients with both biomarker values above or both below the cut-off were included.

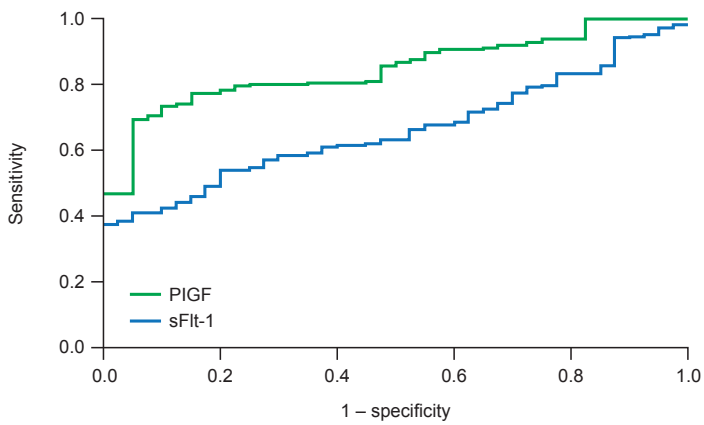

Figure 2. s-Flt (soluble fms-like tyrosine kinase 1) and PlGF (placental growth factor) as diagnostic markers for all pulmonary hypertension (PH) etiologic groups.

Receiver operating characteristic (ROC) curves of sFlt-1 (blue) and PlGF (green) to predict a diagnosis of PH. Area under the ROC curve (AUC) for sFlt-1 is 0.765 (95% CI 0.681–0.848; p < 0.001) and for PlGF is 0.905 (95%CI 0.849–0.960; p < 0.001).

If ROC analysis was performed only for the PAH group (i.e. IPAH, APAH and CVD), the best cut-off value for sFlt-1 was 5753 pg/mL with an AUC of 0.765 (95% CI 0.681–0.848; p < 0.001) and the best cut-off value for PlGF was 29.2 pg/mL with an AUC of 0.905 (95% CI 0.849–0.960; p < 0.001). For the PAH group the specificity was 100% for sFlt-1, and 85%

for PlGF, whilst the sensitivity was 43.3% for sFlt-1 and 83.7% for PlGF. The combination of sFlt-1 and PlGF resulted in a sensitivity of 83.7% with specificity of 100% (chi-square $p < 0.001$ for both).

BNP was added to the analysis for diagnostic accuracy. ROC analysis reveals an AUC of 0.574 with p=0.18 for BNP alone, and an AUC of 0.577 with p=0.16 when combined with PlGF and sFlt-1.

sFlt-1 and PlGF to estimate severity of disease

Neither sFlt-1 nor PlGF correlated with mPAP, pulmonary vascular resistance (PVR), 6-minute walking distance (6MWD) or BNP. Across all PH etiologies the plasma concentration of sFlt-1 was significantly different between NYHA classes ($p = 0.015$; Kruskal–Wallis), and PlGF borderline non-significant ($p = 0.064$, Kruskal–Wallis).
No correlations for PlGF or sFlt-1 with age and creatinine plasma levels were found.

sFlt-1 and PlGF as prognostic markers

Mean survival time was 91 month, in total 130 of 247 patients died. Survival analysis showed no statistically significant difference between patients below and above cut-off concentrations or median concentrations of sFlt-1 or PlGF. These results were confirmed by Cox regression analysis. This was true for both the whole cohort and each etiological group in isolation (results not shown).

Discussion

VEGF signaling is known to be involved in vascular remodeling in PH (18).VEGF family members are overexpressed in lungs from PH patients and circulating VEGF is elevated in plasma of PH patients (8). Inhibition of the VEGFR1 and VEGFR2 with the tyrosine kinase inhibitor SU5416 in combination with chronic hypoxia causes severe PAH in rats (19). The role of VEGF signaling in PH development is not fully understood, but PlGF is thought to act pro-angiogenic by binding to Flt-1, whereas sFlt-1 can bind VEGF and thereby decrease its activity (11, 20).

In this study, sFlt-1 and PlGF plasma levels were measured in a population of 247 patients with PH of various etiologies, and in 40 patients without PH who served as controls. Consecutive patients with suspicion of PH coming to the Giessen PH referral center were included in the study. Right heart catheterization was performed in every patient at baseline visit, in parallel with blood sample collection.

Plasma levels of sFlt-1 were significantly increased in patients with IPAH, APAH, CVD, and LD-PH compared with the non-PH controls, whereas PlGF levels were significantly increased in all PH groups. Use of PlGF for diagnosing PH resulted in a higher sensitivity than for sFlt-1, but sFlt-1 had an associated specificity of 100% for diagnosing PH in our patient cohort. When both parameters are combined the specificity still reaches 100% and sensitivity levels reach 62.4%. These data show a high potential for PlGF and sFlt-1 as diagnostic biomarkers for PH.

The natriuretic peptides, BNP and its N-terminal fragment NT-proBNP, are established biomarkers for PH and are in routine use for the clinical management of patients with PH. BNP levels are elevated in different groups of PH (4, 21-23) and correlate with hemodynamic parameters (24), exercise capacity, WHO functional class (25) and are strong predictors of survival in PH (26, 27). Also in direct comparison BNP is a poor diagnostic tool in this study and should therefore be used differently than PlGF and sFlt-1. Whilst NT-proBNP and BNP can be used as prognostic biomarkers for patients with PH, and can also mirror patients' therapeutic response, their plasma levels can also be normal in severely ill patients with PH, and thus cannot be used to exclude a PH diagnosis.

It is important to emphasize that this is in contrast with the results of the current study, where PlGF and sFlt-1 levels show no correlation with PH disease severity – and thus PlGF and sFlt-1 have no prognostic potential. Nevertheless, PlGF and sFlt-1 seem likely to be useful diagnostic biomarkers for PH.
With their moderate sensitivity and their high specificity for PH (and PAH respectively), sFlt-1 and PlGF are reasonably suitable screening tools, however their main strength lies in their almost unfailing specificity. Therefore, in the decision making tree of diagnostic workup of patients with suspected PH, in cases of a negative test, confirmation by means of invasive hemodynamic assessment remains obligatory provided other non-invasive tests (e.g. echocardiography) remain suggestive of PH. In case of a positive test, sFlt-1 and PlGF are novel tools that confirm PH with high certainty already at an early stage of diagnostic evaluation. It must be kept in mind; however that right heart catheterization remains mandatory for purposes of diagnostic classification and assessment of current hemodynamic severity of the disease. In addition, if the majority of non-invasive diagnostic tools are suggestive for a low probability of having PH during early work-up of symptomatic patients, but the novel biomarkers are positive, this in itself may become a novel means by which the likelihood to oversee PH in symptomatic patients is substantially reduced.
Malhotra et al. measured sFlt-1 serum levels in PAH patients, first degree IPAH and HPAH relatives, and healthy controls (17). They find significantly increased sFlt-1 levels in PAH patients, with an AUC for diagnosing PAH that is comparable to this study. Furthermore

they find an association with increased NYHA and improved survival in patients below the median sFlt-1 level. We could confirm the association of sFlt-1 plasma levels with NYHA class in our study population, but we find no difference in survival time. Like in our study no correlation with hemodynamic parameters could be found.
In summary, we could confirm the diagnostic potential of sFlt-1 and furthermore combined sFlt-1 plasma levels with PlGF and which results in a sensitivity of 83.7% with a specificity of 100% to diagnose PAH.

The missing correlation of sFlt-1 and PlGF with mPAP, PVR, 6MWD and BNP suggests a pathological role that is not directly linked to the increased pressure in the pulmonary vasculature and the resulting changes of the right ventricle. Immunohistochemical stains of lungs from patients with PAH and from healthy donor lungs showed that the VEGF family members Flt-1 and PlGF are expressed in pulmonary arterial smooth muscle cells (see online supplement). As vascular remodeling in PH is mainly driven by smooth muscle cell proliferation, PlGF and Flt-1 may play a role in remodeling processes in PH. Moreover, as smooth muscle cells seem to be the main source of Flt-1 and PlGF in the PH lung, increased plasma levels of these molecules may mirror these changes in smooth muscle cells.

It is important to consider what pathophysiological mechanism might underlie the presence of PlGF and sFlt-1 as biomarkers for PH, firstly by examining the documented role of these molecules in other conditions. Elevated sFlt-1 levels cause endothelial dysfunction during preeclampsia by binding circulating VEGF and PlGF, thereby preventing their interaction with endothelial cell-surface receptors. Levels of sFlt-1 are also elevated in sickle cell disease-PH compared with patients who have sickle cell disease without PH, and a correlation with urinary albumin secretion was reported (15). PlGF plasma levels are raised in sickle cell disease and higher levels associate with an increased incidence of vascular occlusive events (28)
Furthermore, in patients with peripheral and coronary artery disease, sFlt-1 plasma levels were lower than in healthy controls (16). It is also notable that PlGF can be induced by HIF-1α after exposure to hypoxia, both in vitro and in vivo (29-31).

In conclusion to this section, the relevance of sFlt-1 and PlGF plasma levels to specific pathophysiological mechanisms is unclear, but certainly warrants further research.
The control group in this study consists of patients visiting our clinic with symptoms similar to that in PH patients, and therefore underwent right heart catheterization. This is an adequate control group to evaluate potential diagnostic biomarkers as a physician wants to differentiate patients with and without PH presenting with similar symptoms

rather than to differentiate PH patients from healthy people. We included patients from different groups of the World PH classification to test the diagnostic potential of PlGF and sFlt-1 for PH *vs* non-PH in patients showing the same symptoms. In the literature plasma levels in healthy controls are reported to be 94.8 pg/ml for sFlt-1 and 13.7 pg/ml for PlGF (32). Our control group shows higher levels for both biomarker, but still significantly lower levels than PH patients.

Moreover, as plasma was only tested at the time of PH diagnosis, this may represent an early window into a mechanism that, conceivably, may only be present at this early stage. As such, this study showed a high potential for PlGF and sFlt-1 as diagnostic biomarkers for PH. Both proteins were expressed in remodeled vessels of patients with PH, but increased plasma levels did not correlate with hemodynamic parameters, 6MWD and BNP.
This study focused on the diagnostic potential of sFlt-1 and PlGF in PH patients, although the sensitivity was higher in PAH than for unselected PH patients. The decision, whether a patient should undergo invasive right heart catheterization, is based on a variety of non-invasive measurements like echocardiography, imaging, clinical symptoms, and circulating biomarkers, respectively. sFlt-1 and PlGF may become useful additional parameters which help to decide whether or not invasive assessment by means of right heart catheterization is required.

Undoubtedly, further studies are necessary to understand the role of PlGF and sFlt-1 in PH, and greater numbers of patients may be needed to investigate correlations with disease parameters. We find comparable results to the study of Malhotra et al. (17), but confirmations in other patient cohorts are definitely needed. Furthermore, the investigation of patient cohorts over time is needed to reveal whether PlGF and sFlt-1 are only increased in the early phase of disease, and whether their plasma levels change in response to specific therapy.

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3.3 |

HbA1c in Pulmonary Arterial Hypertension
A Marker of Prognostic Relevance?

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Abstract

Background: Patients with pulmonary arterial hypertension (PAH) exhibit impaired glucose metabolism and increased insulin resistance. The clinical consequences of these metabolic changes are not known.

Patients and methods: We assessed HbA1c levels in 115 patients newly diagnosed with PAH (79 female, 36 male; mean age 49.2 years; idiopathic n=67, collagen vascular disease n=16, congenital heart defect n=19, pulmonary veno-occlusive disease n=8, porto-pulmonary n=5). No patients suffered from diabetes, or received antidiabetic medication or systemic steroids. After initiation of pulmonary vasoactive treatment, patients remained in long-term follow-up.

Results: Initially, patients were in an advanced stage of disease (mean pulmonary arterial pressure 53±18 mmHg, cardiac index 2.3±0.8 $l.min^{-1}.m^{-2}$) with a six-minute walking distance of 337±123 m, and in NYHA functional class 3.0±0.7. The HbA1c was 5.73±0.75%. A moderate but statistically significant positive correlation was observed between HbA1c levels and BNP (r_p=0.41, p=0.014) but no correlation was found with hemodynamics or six-minute walk distance.
The 5-year survival rate for the entire group was 68%. Kaplan-Meier analysis and multivariate Cox proportional hazard models correcting for demographic and clinical covariates revealed that patients with HbA1c <5.7% had a significantly better 5-year survival compared to patients with higher initial values (85.1% *versus* 55.9%; log-rank p=0.002). HbA1c was a predictor of all-cause mortality with a hazard ratio of 2.23 (95% CI, 1.06 to 4.70; p=0.034) per 1 Unit increase of HbA1c.

Conclusion: In patients with pulmonary arterial hypertension, the HbA1c level at time of diagnosis is an independent predictor of long-term prognosis.

Introduction

Pulmonary arterial hypertension (PAH) is a chronic pulmonary vascular disease characterized by progressive pulmonary vascular remodelling leading to right ventricular dysfunction and right heart failure. PAH encompasses several forms of the disease, including idiopathic PAH and PAH due to collagen vascular disease, congenital heart disease and portopulmonary hypertension (1). All of these forms of PAH are characterized by similar histological features, including intimal and medial hypertrophy and plexiform lesions. In severe pulmonary hypertension, atherosclerotic lesions might also be present (2). In recent years, PAH has been recognized as a disorder with pronounced systemic and metabolic consequences, including systemic hypotension, renal impairment, hyperuricemia, hyponatremia and hypocapnia (3-6).

Impaired glucose metabolism has been noted in pulmonary hypertension. The loss-of-function mutations in bone morphogenetic protein receptor II (BMPR2) associated with the development of PAH may affect downstream targets of BMPR2 signalling, such as peroxisome proliferator-activated receptor gamma (PPARγ) and apolipoprotein E (apoE). Both PPARγ and apoE are involved in glucose metabolism. Animal studies have demonstrated that insulin resistance in apoE-deficient mice and deficiency of PPARγ in smooth muscle cells of transgenic mice lead to the development of a mouse pulmonary hypertension phenotype (7-11).

In female PAH patients, insulin resistance appears to be more common than in the general population and has been associated with worse short-term survival (12).

Increased glucose intolerance - assessed by the concentration of glycosylated hemoglobin A1c (HbA1c) - has been noted in patients with PAH, but without effect on 6 months event free survival (13).

In the present study, we assessed HbA1c concentrations in patients newly diagnosed with PAH prior to commencement of therapy and correlated HbA1c values with the long-term survival of these patients.

Patients and Methods

In the Giessen Pulmonary Hypertension Center (Department of Internal Medicine, University Hospital Giessen, Germany), a specialized referral centre, patients with pulmonary hypertension (PH) were diagnosed and evaluated with respect to the underlying disease. Diagnosis of PAH was made according to current recommendations (1) by means of clinical examination, transthoracic echocardiography, pulmonary function tests, cardiopulmonary exercise testing, blood gas analyses, screening for HIV and collagen vascular diseases, coronary angiography or sleep studies, as clinically indicated. The presence of chronic

thromboembolic pulmonary hypertension or parenchymal lung disease was excluded using thoracic computed tomography, computed tomographic pulmonary angiography and pulmonary perfusion scintigraphy. The diagnosis of precapillary PH was confirmed on initial right heart catheter investigation by a mean pulmonary arterial pressure (mPAP) of >25 mmHg, a pulmonary arterial wedge pressure (PAWP) of <15 mmHg and a pulmonary vascular resistance (PVR) of > 240 dyn.s.cm-5. The presence of right-to-left shunting had been excluded by transthoracic contrast echocardiography and standardised contrast enhanced transcranial Doppler sonography (14).

In addition to routine laboratory values, brain natriuretic peptide (BNP), fasting blood glucose levels and HbA1c levels (Dimension Vista® HbA1c Kit, Siemens Healthcare, Erlangen, Germany) were assessed. After diagnosis of PAH, patients were started on pulmonary vasoactive treatment with either endothelin receptor blockers, phosphodiesterase 5 inhibitors or prostanoids. When indicated, treatment with calcium channel blockers was initiated. Additionally, patients were started on oral anticoagulation if not contraindicated.

Patients were further assessed by World Health Organization (WHO) functional classification, six-minute walking distance (6MWD), and if indicated, pulmonary hemodynamics, according to current guidelines (1).

The HbA1c levels were assessed with respect to clinical, functional and hemodynamic parameters at the time of presentation. Furthermore, long-term follow-up was undertaken, paying particular attention to survival while on pulmonary vasoactive treatment with respect to initial HbA1c levels.

Clinical stabilization on initial treatment was assessed by measuring time to clinical worsening (TTCW), which has been defined by including time from start of specific medication to the beginning of an additional pulmonary vasoactive treatment compound or all-cause-death. The decision of initiation of combination therapy has been based on criteria as outlined in the current PAH guideline (1).

Follow-up data were retrieved from the local PAH database, review of patient histories and correspondence, or via telephone contact to the patient or family doctor, respectively.

Statistical analysis: Since the data exhibited a normal distribution, parameters are displayed as mean±standard deviation, otherwise median±interquartile range was used. To test for significant differences between groups, the two-tailed Student's *t*- test was used. Correlations between two variables were analysed using the Pearson correlation coefficient. ANOVA was used to identify relevant covariates for survival.

Kaplan-Meier analysis with log-rank test and multivariate Cox proportional hazard models correcting for demographic and clinical covariates were used to assess the difference in survival depending on the level of HbA1c at time of initial assessment.

Univariate and multivariate Cox regression models were used to calculate hazard ratios and a multivariate survival analysis (also using Cox's regression mode) was used to eliminate significant covariates and to assess independent predictors for long-term-survival. A p-value of <0.05 was considered statistically significant. Statistical analysis was performed with SPSS 17.0 (SPSS, Chicago, IL, USA).
Patients gave written informed consent for entering the study. The study has been approved by the Ethics Committee of the Medical Division of the Justus Liebig University of Giessen (approval number 113/11).

Results

Characteristics of PAH Patients

Between June 1996 and July 2007, 529 patients were diagnosed with PAH in our centre. For the present study we excluded patients on treatment with corticosteroids (n=42), diagnosis of diabetes mellitus according to the new guidelines (n=53) (15), previous treatment with pulmonary vasoactive compounds (n=27), lack of available HbA1c measurement at time of initial presentation (n=194) or lack of follow up data (n=98). Survival data were collected until May 2010.
Subsequently, 115 therapy-naïve patients newly diagnosed with PAH were included in the analysis, among them 79 women and 36 male with a mean age of 49.2 years and a mean HbA1c of 5.73±0.75% prior to commencement of pulmonary vasoactive treatment. The demographic, hemodynamic, clinical and laboratory characteristics of the study subjects are summarized in Table 1. Baseline criteria of the patients excluded from analysis were similar and are also shown in this table.

A moderate but statistically significant positive correlation was observed between HbA1clevels and BNP (r_p=0.41, p=0.014) and NYHA functional class (r_p=0.32, p=0.001), respectively. No correlation was found between hemodynamic parameters, 6MWD, body mass index, age, uric acid, creatinine, pO2 or pCO2.
For further analysis the patient population was separated by initial HbA1c and dichotomized at a value equal to or greater than 5.7%, selecting a cutoff with a clinical basis according to current guidelines (15). Dose-related effects on survival have been shown by also stratifying into quartiles and by using glycosylated hemoglobin as a continuous variable. Baseline data are shown in table 2.

Table 1. Baseline characteristics and hemodynamics, dichotomized for HbA1c values.

HbA1c (%)	total	< 5.7	≥ 5.7 %	p	excluded	p
Subjects (n)	115	50	65		414	
Male / female	36 / 79	17 / 33	19 / 46		132 / 282	
Age (yrs)	49.2 ± 15.9	46.6 ± 15.5	51.1 ± 16.0	0.13	51.3 ± 16.0	0.27
BMI (kg/m^2)	25.6 ± 5.2	25.1 ± 5.0	25.9 ± 5.3	0.46	25.9 ± 4.3	0.63
BNP (pg/ml)	342 ± 461	101 ± 87	467 ± 527	0.02	275 ± 342	0.24
Blood glucose (non-fasting, mg%)	103 ± 26	101 ± 28	105 ± 23	0.48	109 ± 37	0.43
Hemoglobin (g/dl)	15.5 ± 2.0	15.4 ± 1.8	15.6 ± 2.1	0.66	14.7 ± 2.2	0.17
NYHA	3.0 ± 0.7	2.8 ± 0.8	3.1 ± 0.6	0.06	3.0 ± 0.6	0.78
mPAP (mmHg)	53 ± 18	50 ± 16	55 ± 19	0.25	50 ± 16	0.14
PVR (dyn.s.cm^{-5})	1014 ± 657	843 ± 672	1101 ± 639	0.12	967 ± 572	0.32
CI (l.min^{-1}.m^{-2})	2.3 ± 0.8	2.5 ± 0.7	2.2 ± 0.9	0.17	2.3 ± 0.8	0.58
Blood pressure (mmHg)						
Systolic	127 ± 23	139 ± 25	120 ± 18	0.01	131 ± 23	0.34
Diastolic	77 ± 15	80 ± 17	75 ± 13	0.11	75 ± 14	0.17
6MWD (metres)	337 ± 123	346 ± 124	330 ± 123	0.61	325 ± 126	0.55

For comparison, characteristics of the excluded patients without HbA1c value are presented. Data are presented as mean ± SD or n (%), unless otherwise stated. For abbreviations, see text.

Table 2. Baseline characteristics, hemodynamics and etiology, divided into quartiles according to the HbA1c value. Data are presented as mean ± SD or n (%), unless otherwise stated. For abbreviations, see text.

HbA1c (%)	≤ 5.3	5.31-5.79	5.8-6.19	≥ 6.2	p
Subjects (n)	30	29	31	25	
Male / female	8 / 22	13 / 16	9 / 22	6 / 19	
Age (yrs)	46.9 ± 15.6	48.2 ± 15.4	50.2 ± 16.1	51.6 ± 16.8	0.70
BNP (pg/ml)	78 ± 53	280 ± 519	430 ± 562	533 ± 348	0.26
NYHA	2.7 ± 0.9	3.0 ± 0.6	3.0 ± 0.6	3.2 ± 0.7	0.14
mPAP (mmHg)	47 ± 14	52 ± 20	53 ± 19	59 ± 18	0.30
PVR (dyn.s.cm^{-5})	671 ± 436	1030 ± 770	1062 ± 712	1224 ± 566	0.11
CI (l.min^{-1}.m^{-2})	2.7 ± 0.7	2.2 ± 0.5	2.3 ± 1.1	2.1 ± 0.6	0.17
Blood pressure (mmHg)					
Systolic	137 ± 18	128 ± 34	125 ± 20	119 ± 15	0.12
Diastolic	82 ± 15	73 ± 20	78 ± 13	73 ± 11	0.22
6MWD (metres)	341 ± 123	369 ± 126	351 ± 93	279 ± 146	0.23
Etiology of disease (n)					
idiopathic					
collagen vascular	12	20	21	14	
congenital heart defect	4	5	4	3	
pulmonary veno-	8	2	4	5	
occlusive	2	1	2	3	
porto-pulmonary	4	1	0	0	

Clinical, functional and hemodynamic parameters did not differ between both patient groups at the time of initial assessment, apart from BNP and systolic blood pressure (Table 1). There was no statistical significance difference in HbA1c, brain natriuretic peptide, clinical, functional and hemodynamic parameters between male and female patients at the time of initial assessment (data not shown).

Survival rates and factors affecting survival

The overall 1-, 3-, and 5-year survival rates were 94.6%, 80.6% and 68.2%, respectively. (Table 3). Four patients developed diabetes mellitus requiring antidiabetic medication during the follow up. Seven patients suffered from acute myocardial infarction and 3 patients suffered from stroke.

A total of 40 deaths occurred during follow-up, in 25 patients due to right heart failure in progressive PAH (62.5%).

Patients presenting with a baseline HbA1c <5.7% had a significantly better 5 year survival compared to patients with an initial HbA1c ≥5.7% (85.1% vs. 55.9%, log rank p=0.002). (Figure 1 and Table 3). This statistical significant difference was consistent after correction for PH related death (log rank p=0.03).

Table 3. Comparison of survival rate and number of deaths in pulmonary arterial hypertension patients based on initial HbA1c levels and dichotomized at a level of 5.7%.

HbA1c (%)	total	< 5,7%	≥ 5,7%	log-rank p
Subjects (n)	115	50	65	
Survival rate (%)				
1 year	94.6	97.8	92.3	0.20
3 years	80.6	97.8	68.2	< 0.001
5 years	68.2	85.1	55.9	0.002
Deaths	40 (34.8%)	8 (16.0%)	32 (49.2%)	
PH related	25 (62.5%)	3 (37.5%)	22 (68.8%)	
non-PH related	15 (37.5%)	5 (62.5%)	10 (31.2%)	

Data are presented as n (%), unless otherwise stated. For abbreviations, see text.

A Cox regression model was built for evaluation of predictive value of HbA1c. After adjustment for the plausible covariates age, gender, 6MWD, NYHA functional class and PVR, HbA1c was a predictor of all-cause mortality with a hazard ratio (HR) of 2.23 (95% CI, 1.06 to 4.70; p= 0.034) per 1 Unit increase of HbA1c.

For the patient group with HbA1c ≥5.7% the adjusted HR for all-cause mortality was 3.91 (95% CI, 0.97-12.03; p=0.056, unadjusted p <0.05).

The adjusted all-cause mortality increased per quartile increase in HbA1c, with a HR ratio of 1,21 (95% CI 0,39-3,78) between quartile 1 and 2, a HR of 1,27 (95% CI 0,41-3,93)

between quartile 2 and 3, and a HR of 2,52 (95% CI 0,87-7,30) between quartile 3 and 4 (overall p = 0.04) (Figure 2 and table 4). After correction for PH related death this misses statistical significance (log rank p = 0.11).

There was, however, no difference in time to clinical worsening between HbA1c quartiles using Kaplan-Meier analysis and a Cox regression model (data not shown).

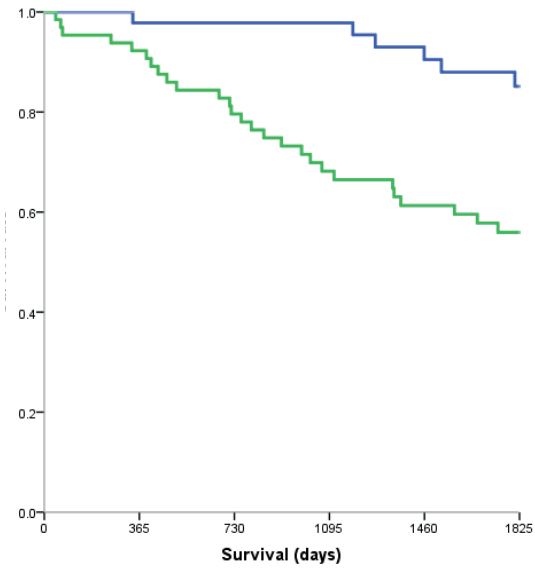

Figure 1. Kaplan-Meier 5-year survival curve in pulmonary arterial hypertension patients. Patients presenting with HbA_{1c} levels below 5.7% (---) had a significantly better survival rate compared with their counterparts with an HbA_{1c} of greater than or equal 5.7% (---).
(85.1% *versus* 55.9%; overall log-rank p=0.002; n=115)

Table 4. Comparison of survival rate and number of deaths in pulmonary arterial hypertension patients based on initial HbA1c levels and divided into four groups (quartiles).

HbA1c (%)	≤ 5.3	5.31-5.79	5.8-6.19	≥ 6.2	log-rank p
Subjects (n)	30	29	31	25	
Survival rate (%)					
1 year 3 years 5 years	96.2 96.2 83.6	100 82.5 73.3	93.4 75.9 68.5	88.0 68.0 50.6	0.26 0.07 0.04
Deaths	5 (16.6%)	9 (31.0%)	11 (35.4%)	15 (60.0%)	

Data are presented as n (%), unless otherwise stated. For abbreviations, see text.

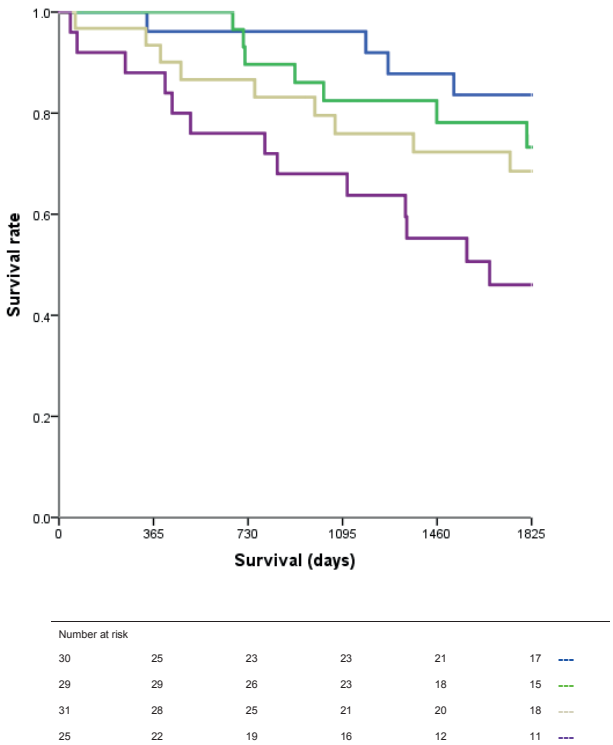

Figure 2. Cumulative 5-year mortality from all causes stratified in quartiles of HbA_{1c}.
First: HbA_{1c} < 5.3% ➡; Second: HbA_{1c} 5.31-5.79% ➡; Third: HbA_{1c} 5.8-6.19% ➡; Fourth: HbA_{1c} ≥ 6.2 ➡ (83.6% *versus* 73.3% *versus* 68.5% *versus* 50.6%; overall log-rank p=0.04; n=115)

Discussion

Impaired glucose metabolism has been noted in PAH, and animal and clinical studies describe a higher rate of insulin resistance associated with this disease.

In the present study, we measured HbA1c levels in non-diabetic PAH patients prior to initiation of pulmonary vasoactive treatment. In the whole study population, the mean HbA1c level was 5.73±0.75% at a mean age of 49.2±15.9 years. This is higher than the normal HbA1c in non-diabetic subjects within this age-group (between 4.9-5.5% at age of 40 to 70 years) according to large epidemiological studies (16). We excluded patients with manifest diabetes mellitus according to recent, revised guidelines (15) and indeed, all patients included in the study exhibited HbA1c levels of <6.5% or a non-fasting random blood glucose concentration <200mg%.

In patients with heart failure, impaired glucose metabolism has been associated with worse clinical parameters and outcome (17, 18). In PAH, a previous study connected

insulin resistance with short term event-free survival (12). In contrary, HbA1c level has not been connected to short term event free survival in PAH (13).

In our PAH patient group, no association between HbA1c level and anthromorphometric parameters, gender, hemodynamic or functional parameters was observed. This is in accordance with the recent findings of Pugh et al. (13). Importantly, we could show that PAH patients with a HbA1c levels <5.7% had a significantly better prognosis in long term follow up at 1, 3 and 5 years compared to patients presenting with initial HbA1c levels of ≥5.7% (Figure 1) even after correction for PH related death. The long-term outcome was associated with HbA1c levels in a dose-dependent manner. We calculated a 2.2-fold increase in hazard of all-cause mortality in PAH patients per 1 Unit increase in HbA1c levels after adjustment for age, gender, 6MWD, NYHA functional class and PVR. These data identify HbA1c as an indicator of impaired glucose metabolism, and connect HbA1c levels with long-term prognosis of patients with PAH. We suggest that HbA1c concentration might serve as a novel biomarker of long-term prognosis of patients classified as having PAH according to recently described criteria (19).

What could be the causes of impaired glucose metabolism in PAH? Possible explanations involve inactivity due to exercise impairment with a subsequent change in metabolism, impaired PPARγ activity due to impaired BMP signalling, but also an impairment of pancreatic-hepatic interaction due to venous congestion (11, 12). Furthermore, impaired gas exchange with hypoxaemia might impact glucose metabolism (20). However, in the present study, there was no demonstrable association between pO2, pCO2 and HbA1c levels. Studies in animal models of PAH indicate that insulin resistance is involved in structural changes to the pulmonary vasculature (9-11). Our retrospective study points to a long-term consequence of altered glucose metabolism in PAH which is reflected in the impact on survival. Certainly, a longterm prospective study on HbA1c in PAH is warranted, the importance and usefulness of such a study is underlined by our data.

In our study population there was a moderate correlation between HbA1c levels and BNP levels. Previous studies in diabetic heart failure patients connect insuline resistance to an increase in neurohormones such as BNP, however a causative impact of BNP on HbA1c concentration is not reported (21, 22). In our study the mid- and long-term survival was better than recently reported from the French and the US registry for PAH. We consider this as a result of the inclusion criteria. In our study we intentionally excluded patients with significant comorbidities, such as diabetes mellitus or steroid treatment. Furthermore there were less patients included with PAH due to connective tissue disease, a subset of PAH with a known worse prognosis (23, 24).

Our study has several limitations. Firstly, it is the result of a retrospective data analysis. Secondly, we cannot exclude a selection bias, as HbA1c was missing at baseline in many patients. Thirdly, we did not perform serial HbA1c measurements during active

pharmacological treatment or other interventions such as exercise training. Prospective evaluation of HbA1c levels with respect to treatment in a larger cohort of PAH patients is required for further validation of HbA1c as a prognostic parameter in PAH.
In conclusion, non-diabetic therapy-naïve PAH patients have higher HbA1c levels compared to the normal population. Levels of ≥5.7% are associated with a worse long-term outcome. HbA1c levels in patients with PAH might serve as an independent prognostic factor of long term prognosis with a hazard ratio of 2.2 per Unit increase in HbA1c.

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4.1 |

Assessment and Prognostic Relevance of Right Ventricular Contractile Reserve in Patients with Severe Pulmonary Hypertension

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Abstract

Background: This study sought to analyze a new approach to assess exercise-induced systolic pulmonary artery pressure (PASP) increase by means of stress-Doppler echocardiography as a possible measure of right ventricular (RV) contractile reserve in patients with severe pulmonary hypertension (PH) and right heart failure.

Methods: In this prospective study, patients with invasively diagnosed pulmonary arterial hypertension or inoperable chronic thromboembolic PH and impaired right ventricular pump function despite a stable targeted PAH medication underwent a broad panel of non-invasive assessments including stress echocardiography and cardiopulmonary exercise testing. Based on the assumption that exercise-induced PASP is a measure of right ventricular contractile reserve patients were classified into 2 groups according to an exercise-induced PASP increase above or below median. Patients were followed for 3.0±1.8 years. Uni- and multivariate analysis were employed for analysis of factors predicting survival.

Results: Of 124 patients, 66 were below the median exercise-induced PASP increase of 30 mmHg (low PASP), and 58 patients were above (high PASP). These groups were not significantly different concerning medication and resting hemodynamics. Low PASP was associated with a significantly lower 6-minute walking distance, peak VO_2/kg and 1-, 3-, and 4-year survival rate (92%, 69% and 48% respectively vs. 96%, 92% and 89%). In the stepwise forward selection multivariate analysis, only peak VO_2/kg and exercise-induced PASP-increase remained independent prognostic markers (hazard ratio 2.51 for peak VO_2/kg and 2.99 for PASP increase).

Conclusion: Exercise-induced PASP-increase is of high clinical and prognostic relevance in PH-patients and may indicate right ventricular contractile reserve. Stress Doppler echocardiography may be a useful tool for prognostic assessment in PH-patients.

Introduction

Right ventricular (RV) pump function is of essential clinical and prognostic importance in a variety of heart and lung diseases, and in pulmonary arterial hypertension (PAH).[1-5] The survival in PH patients depends on the capability of the RV to adapt to chronically elevated pulmonary artery pressures.[6] Therefore, an accurate evaluation of RV pump function would be crucial for screening, diagnosis and follow-up assessment in PH.[7] However, it is difficult to assess RV function due to its complex geometry and load-dependence and due to an inadequate standardization of the assessment.[8] This is true for both non-invasive and invasive techniques. Evaluation of RV performance in PH patients has been recommended to be obtained at rest.[9] It is unknown if assessment of RV function during exercise may be of additional benefit or even preferable. According to clinical experience, some PH patients with severely enlarged right heart and impaired RV pump function at rest do much better in their exercise capacity, WHO functional class and quality of life than others. They might differ in their RV reserve defined as the ability of the ventricle to increase ejection fraction and stroke volume during exercise or pharmacological stress.[10,11] RV and left ventricular contractile reserve refers to changes in systolic function[11] and has not been assessed in patients with pulmonary arterial hypertension (PAH) or inoperable chronic thromboembolic PH (CTEPH). In small studies, RV contractile reserve has been estimated during dobutamine stress echocardiography and had a prognostic impact in patients with mitral valve disease using RV tissue Doppler indices (isovolumic contraction)[12] and in left heart failure using plots with RV pressure-area relations[1] but there are no established methods to assess RV contractile reserve.[11] Exercise-induced changes in radionuclide RV ejection fraction seemed to be inversely related to pulmonary artery pressure changes in patients with valvular left heart disease.[3]

The objective of this study was to prospectively assess exercise-induced increase of systolic pulmonary arterial pressure (PASP) as a possible measure of the RV contractile reserve using exercise stress echocardiography in patients with invasively confirmed manifest PH and to analyze the prognostic impact of exercise-induced PASP increase in context with other prognostically relevant parameters.

Methods

Study Population and Design

This prospective study investigated patients with severe chronic PAH or inoperable CTEPH and right heart failure, WHO functional class II-IV. For safety reasons only patients between 18 and 80 years, and those who received an optimized medical PAH-targeted therapy (as endothelin-antagonists, inhaled or parenteral prostanoids, phosphodiesterase-inhibitors, anticoagulants, diuretics, and supplemental oxygen) for at least 2 months before entering the study were included. Patients had to be able to participate to standard echocardiography and cardiopulmonary exercise testing (CPET). The maximal PASP-value during exercise was obtained as mean value of at least 3 measurements. To avoid the inclusion of patients with only mild impairment of RV pump function and to make both groups comparable, patients with a workload of >75 Watt during stress Doppler echocardiography were excluded, Thus, PASP-increase has been compared in low workloads only. Patients were then assigned to a "high" or "low PASP increase" group according to the median of the obtained maximal increase of PASP reached during exercise. The diagnosis was established at the participating PH centers according to current guidelines.[7,9]

The patients were referred to Heidelberg from several PH centers. All patients underwent a detailed clinical work-up including medical history, physical examination, electrocardiogram, 2-D-echocardiography at rest and during exercise, lung function test, arterial blood gases, CPET, 6-minute walking distance (6MWD) under standardized conditions,[13] quality of life questionnaire (SF-36), laboratory testing including NTpro-BNP levels, was performed in Heidelberg and set as baseline assessment. Left heart catheterization and/or computed tomography of the lungs were performed in all patients with suspected left heart or respiratory diseases and when clinically indicated. Exercise-induced PASP and peak VO_2 were assessed by an experienced team at the Thoraxclinic in Heidelberg in all patients. All patients gave written informed consent for this study, which was approved by the Ethics Committee of the University of Heidelberg.

Echocardiography: Two-dimensional and colour-flow guided Continuous-wave-Doppler-echocardiographic recordings were obtained by experienced cardiac sonographers (EG, CN) using 2.5 MHz Duplex probes and conventional equipment (Vivid 7, GE Healthcare, Milwaukee, Wisconsin) on two occasions: 1) at rest; 2) during stress Doppler echocardiography as described previously.[14] PASP was estimated from peak tricuspid regurgitation jet velocities (TRV) according to the equation: $PASP = 4 (V)^2$ + right atrial pressure, where V is the peak velocity (in m/s) of tricuspid regurgitation jet (TRV).[15] For all calculations the mean value of at least 3 TRV measurements was used. Right atrial pressure was estimated from characteristics of the inferior vena cava.[16] If it was

< 20 mm in diameter and decreased during inspiration we added 5 mmHg, ≥20 mm we added 10mmHg and 15 mmHg if no decrease of diameter during inspiration occurred. Echocardiographic assessments were performed according to current guidelines.[8]

Stress-Doppler-Echocardiography and cardiopulmonary exercise testing: Patients were examined on a variable load supine bicycle ergometer (model 8420; KHL Corp., Kirkland, Washington) in Heidelberg as described previously.[14] Workload was increased by 25 Watt every 2 minutes to an exercise capacity or symptom limited maximum. TRV, heart rate, oxygen saturation and systemic blood pressure were analyzed at each stage. Echocardiographic assessment was stored in DICOM format. The anaerobic threshold was determined using the V-Slope method.[17] Peak VO_2 was defined as the highest 30-second average value of oxygen uptake during the last minute of the exercise test. Borg dyspnea index (with 6 representing no exertion and 20 maximal exertion)[18] was inquired immediately after the test.

Follow-up Assessment

In 2012 all participating patients were interviewed either by phone or at a control visit in the Thoraxclinic Heidelberg evaluating symptoms, WHO functional classification, current medication, any cardio-pulmonary events that might have occurred since last observation. Furthermore, medical records were reviewed to document among others potential worsening events and current medication. In the case where a patient deceased, date of death was recorded and treating physicians and/or relatives were asked for the cause and circumstances of death. The PASP-increase during exercise as well as cardiopulmonary exercise parameters, echocardiographic parameters and 6-minute walking distance were analyzed for their predictive value on survival.

Statistical Methods

Statistical analyses were conducted by a statistician (CF). Data are desribed as means ± standard deviations. For follow-up assessments baseline was defined as the day when the patient underwent the cardiopulmonary exercise test. Survival time was estimated from baseline until June 2012 (end of follow-up in this study). All quantitative characteristics between the two groups at baseline and during follow up were compared by t-tests. For comparison of categorical variables between groups chi-square test was used. In case of sparse cell counts with less than 5 rows and columns Craddock-Flood tests and Haldane-Dawson test for larger sparse tables were used.

For analysis of survival, death was the endpoint and one case of lung transplantation was added to the deaths.

No patient died of non-cardiopulmonary causes. Three patients were lost to follow-up and described but not included in the survival analysis.

For analysis of predictive parameters for probability of death we performed a univariate analysis and a multivariate analysis. In all quantitative variables we used the median value to divide the cohort into two groups and used the dichotomous variable for analysis of their influence on survival. The univariate analyses were performed using log-rank tests for comparing Kaplan-Meier survival curves.[19]

All variables identified within the univariate analysis as being significantly associated with survival ($p<0.05$) and being measured in more than 95% of the patients were selected for the multivariate analysis. For the multivariate analyses, variables were chosen for the model by stepwise forward selection within the Cox model based on likelihood ratio tests with $p<0.05$ for inclusion and $p<0.10$ for exclusion. Effect sizes are given as hazard ratios point estimates with 95% confidence intervals (CI) within Cox proportional hazard model.[20] For uni- and multivariate analysis we performed a sensitivity analysis including only patients with PAH. All analyses were performed using IBM SPSS 20 (SPSS Statistics V20, IBM Corporation, Somers, New York).

Results

Study population (Table 1)

We prospectively included 153 patients diagnosed with severe PH and right heart failure, 7 patients were excluded because TRV could not be obtained during exercise, 21 because they reached higher workloads than 75 Watt during stress Doppler echocardiography, suggesting an only mild impaired RV pump function. Thus, the final study group consisted of 124 patients with invasively confirmed manifest PH and severely impaired RV pump function (37 males and 87 females, mean age 54±16 years; 104 with pulmonary arterial hypertension (PAH) and 20 with inoperable CTEPH (Table 1, figure 1)). The exclusion of the 21 patients who reached higher workloads made the assessment of PASP-increase during exercise better comparable between groups and did not significantly change the results of univariate and multivariate analysis or the estimated survival rates.

Subgroups with different exercise-induced PASP-increase suggesting different RV contractile reserve: According to the median exercise-induced PASP increase of 30mmHg we defined two subgroups: Group A “low PASP” (PASP ≤30mmHg, suggesting reduced or low RV contractile reserve) included 66 patients (24 males, 42 females, mean age 57±15 years) and Group B “high PASP” (PASP >30mmHg, suggesting preserved RV contractile reserve) included 58 patients (13 males, 45 females, mean age 50±17 years) (Table 1).

Table 1.

		Increase of systolic pulmonary arterial pressure		
		A	B	
	All patients	≤ 30mmHg	> 30mmHg	p-Value
Patients, n	124	66	58	
Gender male/female	37 / 87	24 / 42	13 / 45	0.09
Age, years	54 ± 16	57 ± 15	50 ± 17	0.018
Height, cm	167 ± 7	168 ± 8	166 ± 7	0.071
Weight, kg	74 ± 17	77 ± 16	71 ± 18	0.012
WHO Functional Class - No. (%)				
II	10 (8%)	5 (8%)	5 (9%)	0.052*
III	104 (84%)	52 (79%)	52 (90%)	
IV	10 (8%)	9 (14%)	1 (2%)	
Diagnosis				
Pulmonary artery hypertension	(79.0%)	51 (81.8%)	47 (81%)	0.89*
Chronic thromboembolic pulmonary hypertension	(21.0%)	12 (18.2%)	8 (19%)	
NTproBNP, pg/ml				
	1578 ± 2192	1973 ± 2419	1156 ± 1849	0.006
Cardiac Catherization:				
Pulmonary artery pressure [mmHg]	50 ± 15	47 ± 15	52 ± 14	0.070
Pulmonary Vascular Resistance [dyn×sec×cm-5]	861 ± 430	800 ± 423	935 ± 430	0.079
Right Atrium Pressure [mmHg]	8 ± 4	8 ± 3	8 ± 4	0.88
Pulmonary Artery Oxygen Saturation [%]	92 ± 6	91 ± 6	92 ± 5	0.63
Pulmonary capillary wedge pressure [mmHg]	9 ± 4	9 ± 5	9 ± 4	0.83
Cardiac Index [l×min×m^{-2}]	2.4 ± 0.7	2,3 ± 0,7	2,4 ± 0,7	0.64
PAH-targeted medication				
Endothelin Receptor Antagonists	86 (69.4%)	48 (72.7%)	38 (65.5%)	0.29**
Phosphodiesterase-5-Inhibitors	88 (71%)	49 (74.2%)	39 (67.2%)	
Prostanoids inhaled	10 (8.1%)	4 (6.1%)	6 (10.3%)	
Prostanoids intravenous	4 (3.2%)	3 (4.5%)	1 (1.7%)	
Calcium Channel Blockers	18 (14.5%)	8 (12.1%)	10 (17.2%)	
Soluble guanyl cyclase-Stimulator	3 (2.4%)	0 (0.0%)	3 (5.2%)	
Combination Therapy				
Monotherapy	47 (37.9%)	24 (36.4%)	23 (39.7%)	0.71***
Dualtherapy	60 (48.4%)	33 (50%)	27 (46.6%)	
Tripletherapy	16 (12.9%)	9 (13.6%)	7 (12.1%)	
Quadrupletherapy	1 (0.8%)	0 (0%)	1 (1.7%)	
Oxygen Therapy y/n	68 / 56	39 / 27	29 / 29	0.31

y = yes, n = no
Values are mean ± standard deviation; p-values refer to two-sided tests, t-tests incase of quantitative variables, Chi square tests* for 2by2 contingency tables
Haldane-Dawson** and Craddock-Flood test*** in tables; monotherapy compared to combination therapy

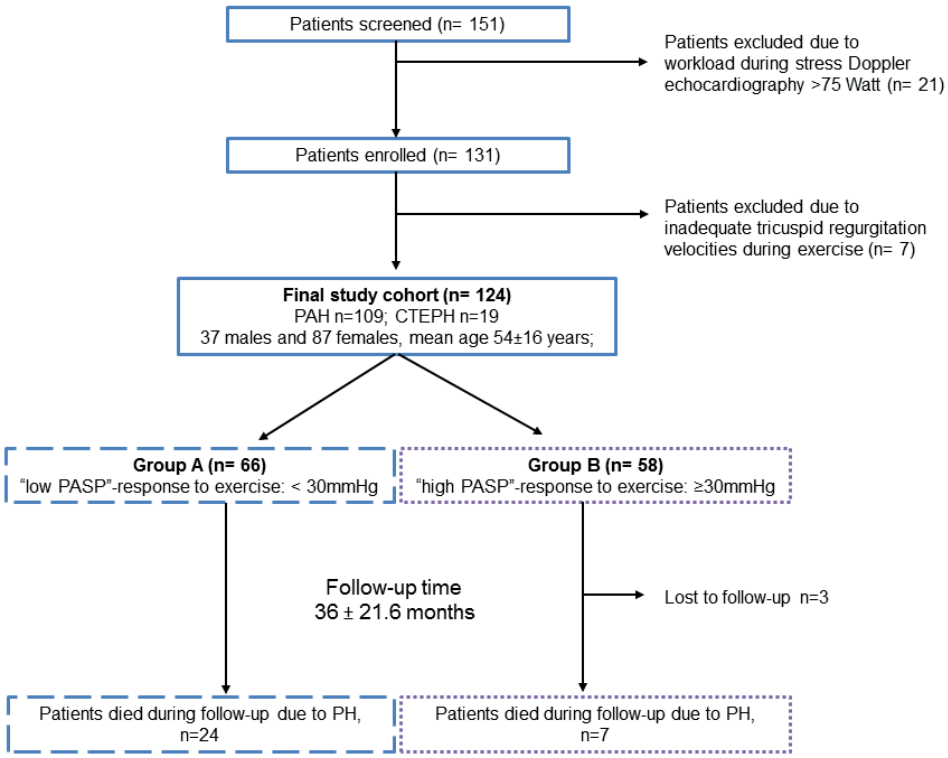


Figure 1: Study flow-chart
Illustration of the entire study from the enrollment process until the end of follow-up.

Both groups did not significantly differ concerning their hemodynamic values measured by right heart catheterization or targeted PAH medication at baseline (Tables 1 and 2). According to right heart catheterization, at rest both groups had markedly reduced cardiac output and cardiac index, elevated pulmonary vascular resistance and mean pulmonary arterial pressures (Table 1). Echocardiography showed markedly enlarged RV area, reduced tricuspid annular plane systolic excursion (TAPSE), severely elevated left ventricular excentricity index (LVEI) due to impressed LV by the enlarged RV (Table 2). LVEF did not significantly differ between groups (PASP increase < 30mmHg 82.6±16.8 vs. PASP increase > 30mmHg 85.8±17.7; p=0.356). Patients with impaired left ventricular function/ PH due to left heart disease were not included into the study. In Subgroup A (PASP increase <30mmHg) were 5 patients with atrial fibrillation, in subgroup B 3 patients, respectively. In group A, patients were older (p=0.018), had higher mean NTproBNP-levels (p=0.006), mean heart rate at rest (p=0.033) and mean right atrial area (p=0.011) compared to group B. Apart from these resting parameters, the patients of both subgroups did not significantly differ concerning right heart catheterization and echocardiography measures

(table 1). Patients with high PASP-response (group B) had similar echo findings at rest with severely enlarged RV- compared with patients in group A (figure 2). Right atrial area significantly differed between groups (26.0±10.2 group A vs. 20.0±5.7 group B, p=0.005).

Table 2

		Increase of systolic pulmonary arterial pressure		
		A	B	
Characteristic	**Total**	**≤30mmHg**	**>30mmHg**	**p-value**
Mean 6-Minute-Walking Distance, meter				
	420 ± 83	400 ± 89	443 ± 70	0.011
Echocardiography				
Right Ventricular Area, cm^2	25.4 ± 7.4	26.9 ± 7.4	24.3 ± 7.2	0.13
Right Atrial Area, cm^2	23.1 ± 8.8	26.0 ± 10.2	20.0 ± 5.7	0.005
LVEI	1.5 ± 0.4	1.5 ± 0.4	1.4 ± 0.3	0.18
TAPSE	1.86 ± 0.42	1.85 ± 0.36	1.87 ± 0.49	0.89
TDI s	10.6 ± 3.3	9.9 ± 2.6	11.5 ± 3.8	0.14
Cardiopulmonary exercise testing				
peak VO_2/kg, mL/Min/kg	11.5 ± 2.52	11.0 ± 2.4	12.0 ± 2.6	0.025
peak VO_2, ml/min	832 ± 192	829 ± 202	835 ± 184	0.39
$EqCO_2$ at AT, ml/min	48.4 ± 10.0	47.8 ± 9.8	49.1 ± 10.2	0.67
VO_2 at AT, ml/min	603 ± 165	635 ± 174	564 ± 147	0.11
Oxygen pulse, (mL/min)/min-1	6.9 ± 1.7	7.1 ± 1.9	6.7 ± 1.6	0.29
HR rest, min-1	79 ± 12	81 ± 11	77 ± 13	0.033
HR max, min-1	123 ± 18	120 ± 19	126 ± 16	0.047
RR sys rest, mmHg	116 ± 18	114 ± 19	117 ± 17	0.19
RR dia rest, mmHg	76 ± 16	77 ± 20	76 ± 11	0.64
RR sys max, mmHg	144 ± 25	141 ± 24	148 ± 27	0.12
RR dia max, mmHg	84 ± 14	82 ± 15	86 ± 13	0.072
Oxygen saturation rest, %	94 ± 5	94 ± 4	94 ± 6	0.41
Oxygen saturation max, %	87 ± 10	87 ± 11	87 ± 8	0.84
sPAP rest, mmHg	64 ± 17	65 ± 18	63 ± 17	0.63
sPAP max, mmHg	98 ± 25	85 ± 20	111 ± 23	<0.001
Workload max, W	60 ± 12	58 ± 12	62 ± 13	0.12
Respiratory quotient	0.97 ± 0.12	0.99 ± 0.12	0.96 ± 0.10	0.19
Left ventricular ejection fraction, %	84.1 ± 17.2	82.7 ± 16.8	85.8 ± 17.7	0.36
Increase in sPAP, mmHg	34 ± 19	21 ± 8	49 ± 17	

Values are mean ± standard deviation; two-sidel t-test pvalues are given
6MWD=6-minute walking distance, VO2/kg=max. oxygen consumption/kg, HR=heart rate,
RR=Blood pressure, sys=systolic, dia=diastolic, sPAP=systolic Pulmonary arterial pressure, W=Watt.
LVEI=left ventricular ejection index, TAPSE=tricuspid annular plane systolic excursion, TDI_s=Strain tissue doppler imaging

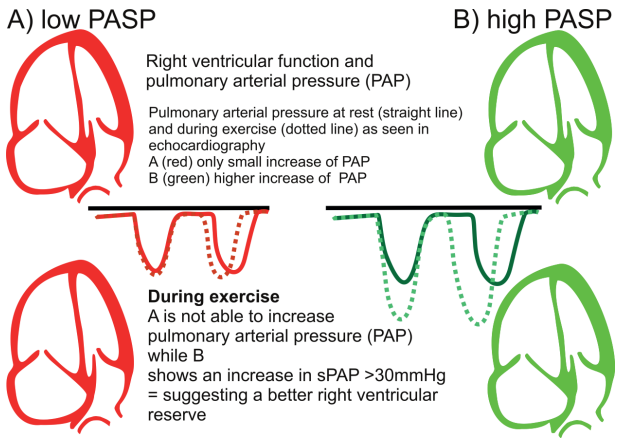


Figure 2: Illustration of two groups with low (group A) and high (group B) PASP-increase during exercise.

PASP changes that occur during cardiopulmonary exercise testing among PH patients with medium to severe impairment of right ventricular pump function. In some of the patients a significant increase in PASP can be observed during exercise whilst in others the change is minimal, though echocardiographic parameters as right heart size were not significantly different.

Clinical-technical observations during exercise stress echocardiography

In almost all patients TRV could be assessed by echocardiography at rest and during exercise due to the presence of tricuspid regurgitation in the enlarged right hearts. Stress echocardiography and CPET was safe and could be performed without complications in all patients.

Correlation of peak VO_2/kg and 6MWD in groups with low and high exercise-induced PASP-increase: There was a significant, but weak correlation of the PASP-increase during exercise with 6MWD and peak VO_2/kg (Figure 3a and 3b). Patients with high exercise-induced PASP-increase also showed higher values for peak VO_2/kg and 6MWD than patients with a low PASP-increase.

Follow-up and survival analyses (Figure 4a, 4b)

During a mean follow-up period of 3.0±1.8 years (median 2.9 years; interquartile range 2.0-3.9 years) 31 patients died due to PH and/or right heart failure, and 3 patients were lost to follow-up. Within follow-up, significantly more patients died in group A compared to group B (24 vs. 7 patients). Patients in group A revealed significantly lower 1-, 3-, and 4-year survival rates of 92%, 69% and 48% than patients of group 2 with 96%, 92% and 89% (p=0.002, figure 4a). The 1-, 3-, and 4-year survival rates for all patients were 94%, 80% and 67%, respectively (Figure 4a). As a further prognostic factor, the median of peak VO_2/

kg (11.4ml/min/kg) was used to split the patients into two groups. Patients with a peak VO_2/kg >11.4ml/min/kg had significantly higher survival rates (98%, 93% and 80%) than patients with peak VO_2/kg ≤11.4ml/min/kg (90%, 67% and 56%) for the 1-, 3- and 4-year survival rates (figure 4b).

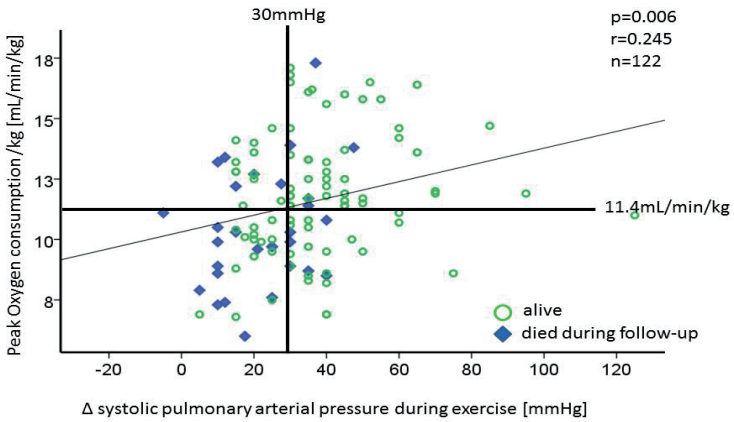


Figure 3a: Correlation of peak VO_2/kg and PASP-increase during exercise

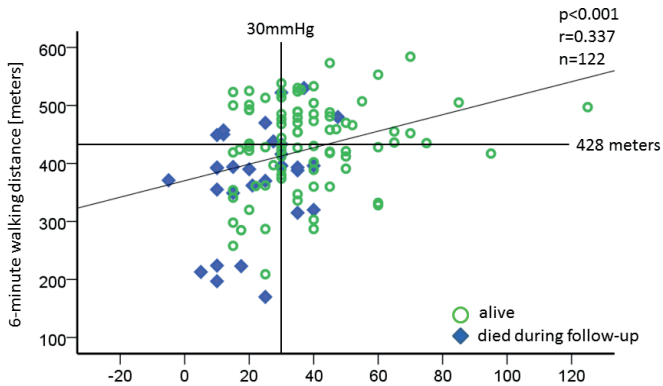
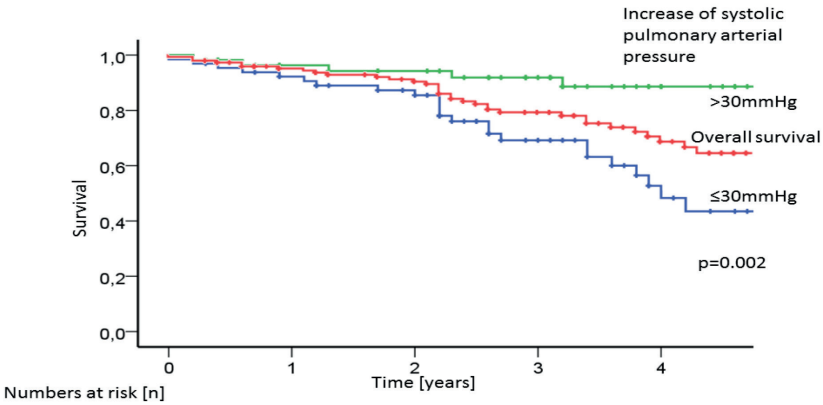


Figure 3b: Correlation of 6MWD and PASP-increase during exercise

The correlation between PASP-increase and peak VO_2/kg (r=0.245) and 6MWD (r=0.337) was significant. There was a significant difference (p=0.006 for peak VO_2/kg and p<0.001 for 6MWD) but weak correlation between the two subgroups in relation to the median peak oxygen consumption of 11.4mL/min/kg and walking distance of 428m.

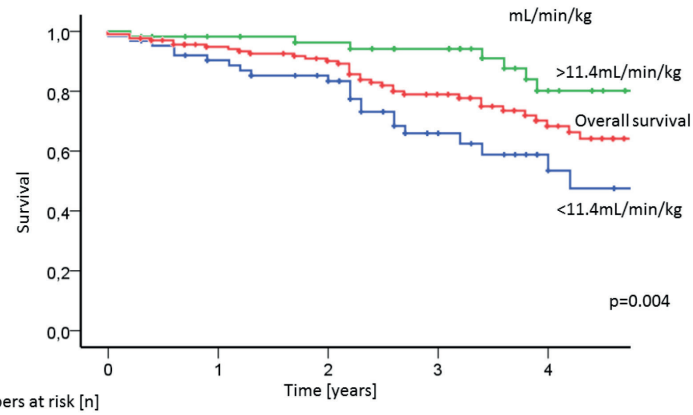


Numbers at risk [n]

Time [years]	0	1	2	3	4
>30mmHg	56	47	42	31	16
≤30mmHg	65	57	47	26	11

Figure 4a: Survival rate of PH patients in relation to right ventricular contractile reserve obtained by PASP-increase during exercise

The 1-, 3-, and 4-year survival rate amongst the patients with PASP-increase ≤30mmHg were 92%, 69% and 48% respectively and 96%, 92% and 89% amongst group B with high PASP >30mmHg.



Numbers at risk [n]

Time [years]	0	1	2	3	4
>11.4mL/min/kg	58	51	45	38	19
≤11.4mL/min/kg	63	53	46	21	11

Figure 4b: Survival amongst patients in relation to peak VO_2/kg.

The median of peak VO_2/kg (11.4ml/min/kg) was used to split the patients into two groups. Patients with a peak VO_2/kg >11.4ml/min/kg had significantly higher survival (98%, 93% and 80%) rates than patients with peak VO_2/kg ≤ 11.4ml/min/kg (90%, 67% and 56%) for the 1-, 3- and 4-year survival rates.

Prognostic factors in the univariate and multivariate analysis (Table 3)

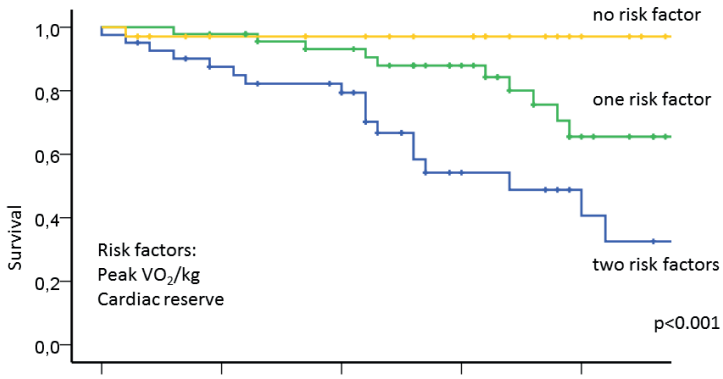
Clinical parameters were analysed using univariate and multivariate methods. Table 3 shows the results of the univariate log-rank test. Baseline parameters predictive of survival in univariate analysis (log-rank test) were gender (p=0.032), WHO functional class (p=0.004), trough oxygen saturation (p=0.047), NTproBNP (p=0.043), 6MWD (p=0.023), heart rate at rest (p=0.038), peak VO_2 (p=0.046), peak VO_2/kg (p=0.004, figure 4b), peak PASP (p=0.005), right ventricular area (p=0.005), TAPSE (p=0.015) and right atrial area (p=0.006). Hazard ratios define the ratio of death probability. The value for increased death probability is given in table three. In patients with pericardial effusion (PE) and in patients without PE, the total amount of deaths was 25%. Thus, in the univariate analysis pericardial effusion was no significant prognostic factor (p=0.745). Distribution of patients with pericardial effusion in the two subgroups (PASP increase above and below 30mmHg) was similar with 8 patients in each group (12.1 vs. 13.8%).

Table 3

Variable (cut-off for higher probability of death)	Parameters Predictive of Survival					
	N	Median	p-value	HR	95% CI	
Univariate Analysis						
Sex (male)	121		0.032	2.15	1.05 -	4.41
Who Functional Class (III/IV)	121		0.004	5.75 (III:II)	1.23 -	29.41
				3.91 (IV:II)	1.56 -	9.80
Peak Oxygen saturation, % (≤ 89)	111	89	0.047	2.14	0.99 -	4.64
NTproBNP, pg/ml (> 619)	117	2192	0.043	2.15	1.00 -	4.63
6MWD, meter (≤ 428)	119	428	0.023	2.33	1.10 -	4.96
HR rest, bpm (> 77)	121	77	0.038	2.15	1.02 -	4.53
Workload, W (≤ 50)	121	50	0.021	2.60	1.11 -	6.06
Peak VO_2, mL/min (≤ 812)	121	812	0.046	2.08	0.99 -	4.26
Peak VO_2/kg, mL/min/kg (≤ 11.4)	121	11.40	0.004	2.98	1.36 -	6.49
Peak PASP, mmHg (≤ 95)	121	95	0.005	2.95	1.34 -	6.50
Δ Systolic pulmonary arterial pressure, mmHg (≤ 30)	121	30	0.002	3.49	1.50 -	8.12
Right ventricular Area, cm^2 (> 24)	64	24	0.005	10.75	1.36 -	83.33
TAPSE, cm (≤ 1.88)	95	1.88	0.015	2.83	1.17 -	6.85
Right atrial Area, cm^2 (> 20.5)	65	20.5	0.006	10.20	1.30 -	76.92
Multivariate Analysis						
Peak VO_2/kg, mL/min/kg (≤ 11.4)	121	11.40	0.022	2.51	1.14	5.52
Δ Systolic pulmonary arterial pressure, mmHg (≤ 30)	121	30	0.012	2.99	1.28	7.04

logrank test P values, continuous variables were classified in low ≤ median, and high > median
Hazard ratios and their 95% confidence intervals (95% CI) were estimated with Cox regression models, showing probability of death. The value with higher probability of death is defined in brackets after the variable name.

The ability to increase PASP >30mmHg during exercise, was predictive for survival and had the highest hazard ratio (3.48) of all variables. In the multivariate analysis only peak VO_2/kg and PASP-increase during exercise remained as independent predictors of survival (Table 3, figure 5). Patients who were positive for both independent risk factors at baseline (peak VO_2/kg ≤11.4ml/min/kg and exercise-induced PASP-increase ≤30mmHg) had the worst survival rate of 84%, 54% and 42% for the 1-, 3- and 4-year survival rate, respectively (figure 5). In contrast, patients with high exercise-induced PASP-increase and peak VO_2/kg >11.4ml/min/kg had a 3-year survival rate of 97% (figure 5).



Numbers at risk [n]

Time [years]	0	1	2	3	4
No risk factor	34	27	25	22	12
One risk factor	46	44	36	25	10
Two risk factors	41	33	27	10	5

Figure 5: Kaplan Meier survival curves of patients with up to two risk factors (low PASP increase and low peak VO_2/kg).

The number at risk increased with the number of risk factors present. The survival rates when both risk factors were present were 84%, 54% and 42% for the 1-, 3- and 4-year survival.

Sensitivity analysis excluding patients with CTEPH led to similar results with a median value of PASP increase of 30mmHg and significant results for uni- and multivariate analysis. The multivariate analysis for the cohort excluding CTEPH patients defines peak VO2 below 11.45mL/min/kg (HR 4.63), peak oxygen saturation below 89% (HR 3.66) and PASP increase during exercise below 30mmHg (HR 3.31) to be of higher probability of death.

Discussion

This is the first study to evaluate exercise-induced PASP-increase using stress echocardiography in a large cohort of patients with severe PAH or inoperable CTEPH and right heart failure. The group of patients who revealed a high exercise-induced PASP increase i.e. a response above the median value of 30mmHg, had a significantly higher mean 6MWD, mean peak VO_2/kg, lower mean heart rate, NTproBNP, right atrial area and

a better 1-, 3,- and 4-year survival rate. The study revealed that PASP-increase during low workload exercise assessed by echocardiography is an independent prognostic factor. Exercise-induced PASP-increase could be an estimate of right ventricular contractile reserve. Therefore, stress echocardiography may be instrumental for follow-up assessment especially to identify PH-patients at high mortality risk.

Measurements of contractile reserve in heart failure

Contractile reserve of the right and left ventricle (LV) have been defined by the change in ejection fraction (EF) or stroke volume during exercise or dopamine/norepinephrine-infusion.[10,11] An increase of LV and RV radionuclide ejection fraction ≥5% during exercise was found in healthy volunteers with significantly higher levels of LVEF-increase in males vs. females (+10.5% vs. +5.3).[21]

LV contractile reserve has been shown to be a strong prognostic predictor in patients with left heart failure due to idiopathic dilated cardiomyopathy,[22-26] valvular heart disease[12,27] and coronary artery disease.[28,29] There are only few data on RV contractile reserve and no standardized methods how to measure it. LV contractile reserve has been estimated by invasively[28,30] or non-invasively measured changes in LV- ejection fraction,[31] radionuclide stroke volume,[24] cardiac power (aortic pressure x aortic flow)[23] or by echocardiographic parameters of LV function including wall thickness.[25,26] There have been no studies on RV contractile reserve in patients with right heart failure due to PAH or CTEPH. The concept to estimate RV contractile reserve by PASP-increase during exercise has not been used in PH-patients previously. Patients with severe tricuspid regurgitation have not been included in this patients cohort. However, it has to be noted, that measurement of PASP-increase during exercise is limited in patients with severe TR as estimates of PASP (and RV cardiac output) are less accurate.

Rationale for choosing exercise-induced PASP-increase for estimation of RV contractile reserve

Exercise-induced PASP increase has been shown to be closely related to RV function. The ability of the RV to increase pressure depends on the maintenance of RV arterial coupling[32] and the ability to maintain or increase stroke volume during exercise without dilating.[33] Janicki et al. have shown that the exercise-induced pulmonary arterial pressure-flow relationship in PH is very steep.[34] Thus, a large increase in PASP implies the capacity of the RV to eject a large stroke volume despite high pressures reflecting a preserved RV contractile reserve. In our study patients of group A and B had similar workloads and no pulmonary stenosis. The slightly lower heart rate at rest in group B with better PASP-increase reflects most likely the better contractile reserve. The lower peak heart rate during exercise in the group with inadequate PASP-increase may reflect a slightly lower

level of stress. It might also reflect a higher proportion of patients with chronotropic insufficiency, which is a common symptom in patients with pulmonary hypertension. Both patient groups did not differ in their respiratory quotient which also indicates a similar exercise capacity. Most patients with severely impaired RV pump function reached in the symptom limited supine exercise test workloads below 75 Watt. We excluded patients with higher work load in order to obtain comparable results between both groups (A and B) and to avoid that different PASP-response to exercise is due to different levels of stress. Thus, differences in PASP increase during exercise most likely represented different abilities of the RV to increase cardiac output and could therefore be used to assess the contractile reserve of the RV. For further hemodynamic assessment/prove of RV contractile reserve it would be useful to perform follow-up assessments using right heart catheterization not only at rest but also during exercise. However, this will be not suitable in every patient and even more dangerous than the non-invasive parameters.

Apart from the physiologic rationale for choosing exercise-induced PASP-increase for estimation of RV contractile reserve there are technical advantages. Estimation of PASP by echocardiography is more easy and precise than the echocardiographic assessments of RV ejection fraction or cardiac output during exercise. Both parameters can be estimated by pulsed Doppler velocity-time integral measurements but are difficult to measure even at rest and not very reliable.[35] The assessment becomes even more demanding during exercise, especially in PH-patients with enlarged right ventricle and complex geometry. Due to a high intra- and inter-observer variability, it would be difficult to establish these methods for routine follow-up in PH patients. In contrast, the measurement of TRV by means of stress Doppler echocardiography and CPET, is by far easier and accurate in patients with manifest PH and enlarged RV although this technique had many pitfalls in the screening for PH.[36] Nearly all patients with PH and enlarged RV have at least a mild tricuspid regurgitation. Therefore, we were able to obtain the PASP-increase in ≈ 95% of our patients with clear-shaped Doppler-profiles.

Stress echocardiography in manifest PH

The assessment during exercise with low workloads (50-75 Watt) remained without complications although all patients had a severely impaired RV pump function and severe PAH/CTEPH. As cut-off value for a high or low PASP-increase we used the median value of 30mmHg. In healthy subjects normal PASP increase during low-dose exercise (within 75 Watt on supine bicycle ergometer test) was about 10 mmHg (from 20.4±5.3 to 29.8±5. mmHg ≈ 46% increase).[14] In the PH-patient cohort mean PASP at baseline was 70 mmHg, and an increase >30mmHg corresponds to a 45% increase. However, the true cut-off value can obviously not be obtained in healthy controls. It may even be more useful performing echocardiography together with cardiopulmonary exercise testing. Peak VO_2/

kg and PASP-increase were the only independent prognostic predictors and indicated a very poor prognosis if both parameters were reduced. Other signs of a severely reduced RV contractile reserve during stress Doppler echocardiography as the insufficient increase or even drop of systemic pressures should also be regarded.[37]

Today, the only indication listed in international guidelines for evaluating PASP during exercise by stress echocardiography is in symptomatic patients with mild mitral valve stenosis or insufficiency. In these patients percutaneous valvotomy or valve surgery may be performed if the PASP during exercise exceeds 60 mmHg.[38] In patients with left heart failure or with significant mitral regurgitation, PASP-increase during exercise has a different pathophysiology and also reflects an increase of left atrial pressure and impaired LV diastolic reserve and is a predictor of poorer prognosis.[39]

Prognostic parameters

So far, hemodynamic variables at baseline (such as elevated mean right atrial pressure, mean pulmonary artery pressure and reduced cardiac index) have shown to be prognostic predictors.[4,37,40-41] Based on these parameters, an equation to predict a patient's likelihood of survival has been created.[4] However, this equation is based on patients with idiopathic and heritable PAH before PAH-targeted medication was available and therefore it may not reflect the wider PAH population in the modern treatment era.[42] In addition to baseline hemodynamic parameters, the New York Heart Association functional class,[43] heart rate,[44] 6MWD,[45,46] echocardiographic predictors[47] such as pericardial effusion, NTproBNP[48] and platelet level[49] have been found to correlate with the prognosis in PH patients.[41,42] Peak oxygen uptake and peak systolic blood pressure (SBP) during cardiopulmonary exercise testing were also shown to be of major prognostic value in patients with IPAH.[50] Recently, Nickel et al. described changes in World Health Organization functional class, cardiac index, mixed-venous oxygen saturation and NT-proBNP during follow-up visits as significant predictors of outcome.[51] The here described non-invasive estimation of PASP-increase during exercise has been obtained in patients who were under optimized PAH-targeted treatment and may therefore also be useful in follow-up assessments of patients. Most recent findings confirmed that the RV pump function is a stronger predictor for prognosis than baseline PVR and may decrease despite a reduced PVR by PAH-targeted medical therapies.[33] The results of our study may add to this finding that the assessment of PASP-increase during exercise as estimate for right ventricular contractile reserve may be even more important than the resting hemodynamics for the follow-up and therapeutic management of PH-patients. Patients with high PASP-increase and peak oxygen consumption >11.4mL/min/kg, had a good long term survival and do probably not need an increase of treatment although they might have severely impaired resting hemodynamics. In contrast, patients with reduced PASP-increase during exercise and peak oxygen consumption

might need escalation of PAH-medication or even be listed for lung transplantation. Both patient-groups could be hardly distinguished by resting hemodynamics and clinical parameters alone. Estimation of right ventricular contractile reserve may be even more important than resting hemodynamics measured by echocardiography for the follow-up and therapeutical management of PH-patients. In contrast, patients with reduced PASP-increase during exercise and peak oxygen consumption might be further evaluated if an escalation of PAH-medication is necessary.

In this study patients with moderate to severe impairment of right ventricular function were included, mostly WHO functional class III-IV. PASP increase during exercise might also be a feasible parameter in patients with less severe pulmonary hypertension; however the cut-off value for PASP increase might be different. Further studies are needed to investigate the effect on patients with less severe disease and in pre-defined workloads e.g. 50 Watts or workloads adjusted to the patients' body weight.

Clinical Implications and Conclusion

Assessment of PASP-increase during exercise by stress echocardiography at low workloads may reflect right ventricular contractile reserve and can contribute to follow-up assessment and risk-stratification of PAH/CTEPH patients. The combination of stress Doppler echocardiography and cardiopulmonary exercise test revealed the most important independent prognostic factors as peak VO_2 and low PASP increase and may be useful for therapeutic decision making by identifying patients of especially high risk and inadequate therapy. Estimation of right ventricular contractile reserve may be even more important than resting hemodynamics measured by echocardiography for the follow-up and therapeutical management of PH-patients.

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4.2 |

Sildenafil vs Nitric Oxide for Acute Vasodilator Testing in Pulmonary Arterial Hypertension

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Abstract

Vasoreactivity testing with inhaled nitric oxide (NO) is recommended for pulmonary arterial hypertension (PAH) because of its therapeutic and prognostic value. Sildenafil has acute pulmonary vasodilating properties, but its diagnostic and prognostic impact in PAH is unknown. Our objective was to compare acute vasodilating responses to sildenafil vs. NO during right heart catheterisation and their prognostic values in patients with PAH.

99 patients with idiopathic PAH and 99 with associated PAH underwent vasoreactivity testing with NO and sildenafil. Only mild adverse effects of sildenafil in the form of hypotension were observed at a rate of 4.5%. The acute responder rate was 8.1% for NO and 11.6% for sildenafil. The NO-induced response in mean pulmonary arterial pressure and cardiac output correlated with the response to sildenafil. 13 patients were long-term responders to CCBs and three of them were correctly identified by acute vasoreactivity test with both drugs. The specificity of the vasoreactivity test for identifying long-term CCB responders was 88.9% for NO and 85.1% for sildenafil testing. A trend towards better survival was found in sildenafil and NO responders compared with non-responders.

Use of sildenafil for vasoreactivity testing is safe. Sildenafil may be useful as alternative vasoreactivity testing agent identifying the same number of long-term CCB responders as NO. However, NO seems to be a more ideal testing drug due to it's pharmacologic properties. Moreover, sildenafil vasoreactivity testing might contribute to an improved estimate of prognosis among patients with PAH.

Introduction

Pulmonary arterial hypertension (PAH) is characterized by an increase in pulmonary arterial pressure leading to progressive right ventricular dysfunction and failure. Invasive hemodynamic measurement by right heart catheterization (RHC) is necessary to establish the diagnosis. In order to choose appropriate therapy, acute vasodilator testing during RHC is recommended in patients with idiopathic PAH. A 'positive acute vasodilator response' predicts the long-term response to high-dose oral calcium channel blockers (CCBs) [1,2] and is currently defined as a reduction of mean pulmonary arterial pressure (mPAP) > 10 mm Hg leading to mPAP < 40 mm Hg and normal cardiac output (CO) upon acute pulmonary vasodilator challenge generally performed with inhaled nitric oxide (NO).[3] A formerly used definition of vasoreactivity is decrease in mPAP and pulmonary vascular resistance (PVR) by > 20% .[4]

IPAH patients with a significant acute response during vasoreactivity test are recommended for treatment with high-dose CCBs. Furthermore, they have a better prognosis than non-responders, for whom treatment with CCB is not advisable.[2,4]high doses of oral calcium-channel blockers (CCB However, despite an initial positive response, only one third of these patients show a long-term benefit from CCB, rendering re-evaluation of responders compulsory.[1]

Sildenafil is approved and widely used for the treatment of PAH.[5-7] It targets the cyclic guanosine monophosphate (cGMP) pathway downstream of NO. Administration of sildenafil leads to an acute pulmonary vasodilation that can be tracked during hemodynamic measurements.[8-11] It may thus be tempting to use sildenafil for acute vasoreactivity testing as it is cheaper, more stable and easier to handle than inhaled NO and intravenous epoprostenol. However, it is not yet known how similar the acute responses to sildenafil and NO and the long-term therapeutic and prognostic implications are.

Hence, we aimed to compare acute vasoreactivity in response to oral sildenafil vs inhaled NO in patients with PAH and to determine their prognostic values for long-term treatment.

Methods

Patients

In this retrospective, open-label, single-center study, we included consecutive patients who were admitted to our adult pulmonary hypertension unit from 2002 to 2011 and met the following criteria:

1. Patients had PAH defined as mPAP > 25 mm Hg at rest and pulmonary capillary wedge pressure < 15 mm Hg with PVR > 240 dyn•sec/cm. [5, 12]the practical implementation of the European Guidelines in Germany requires the consideration of several country-specific issues and already existing novel data. This requires a detailed commentary to the guidelines, and in some aspects an update already appears necessary. In June 2010, a Consensus Conference organized by the PH working groups of the German Society of Cardiology (DGK In case of congenital heart disease (CHD) only patients with repaired CHD and absence of shunts were included.
2. Patients were treatment naïve.
3. Patients had undergone vasoreactivity testing with both, NO and sildenafil during right heart catheterisation at initial assessment.

Right heart catheterization and vasoreactivity testings were performed on clinical grounds solely. Patients' written informed consent was obtained. Compassionate treatment of patients with Sildenafil in patients who received sildenafil during periods when the drug was not yet approved was done in agreement with the local IRB and in accordance with national legal requirement.

The decision to apply sildenafil was made by the physician performing the right heart catheterization. Sildenafil was applied if the drug was considered as a treatment for the patient at hand, the systolic SAP was >90mmHg and the general condition of the patient during the catheterization allowed for this longer testing procedure.

Assessment of Pulmonary Hemodynamics and Vasoreactivity Testing

A pulmonary artery catheter (Edwards Swan-Ganz, 93A-754H 7.5-F; Baxter Healthcare, Irvine, California) was used to measure pulmonary hemodynamics. Cardiac output was measured by the Fick method. For patients receiving oxygen therapy, oxygen uptake was calculated using the formulas of LaFarge and Bergstra. [13] For the other patients oxygen uptake was measured using indirect calorimetry using the Fitmate device (COSMED, Rome, Italy). Patients received continuous nasal oxygen throughout the test procedure if initial arterial oxygen saturation was <90%.

After evaluation of baseline parameters, acute vasoreactivity testing was performed using inhaled NO at a concentration of 20 ppm for 5 to 10 min (INO therapeutics AB, Lidingo, Sweden). After 30 min of washout, all patients received a single dose of 25 mg of oral

sildenafil. Pulmonary hemodynamics were registered every 15 min for 60 min after the administration of sildenafil. The values at the timepoint with the greatest decrease in mPAP were used for further analysis. A positive vasoreactivity test was defined as a drop in mPAP > 10 mm Hg leading to an absolute value of < 40 mm Hg and a normalization of cardiac index (CI) to a value of ≥ 2.4 L/min/m^2.

Assessments and Long-Term Response to Calcium Channel Blockers

Patients underwent clinical examination, a 6-minute walk test, and measurement of brain natriuretic peptide at baseline and at each follow-up visit. The 6-minute walk test was performed according to the American Thoracic Society guidelines.[14] Long-term responders to CCBs were defined as patients whose condition improved with CCB therapy according to clinical assessment of non-invasive parameters (functional class, 6minute walk test, BNP) and were still on CCB monotherapy after 1 year.

Statistical Analysis

Groups were compared using *t*test or analysis of variance. The correlation coefficient *r* was computed using univariate linear regression, graphs were plotted with IBM® SPSS® Statistics 20 graph builder (IBM Corp, Armonk, NY, USA). Differences in survival between groups were analyzed by log-rank test. A 2-tailed *P*-value of <0.05 was considered significant.

Results

Baseline Characteristics

A total of 198 treatment-naïve, adult patients with PAH (group 1 of the Nizza classification)[15] underwent right heart catheterization and vasoreactivity testing with NO and oral sildenafil at the time of diagnosis. The patients had a mean age of 52.1 years and 64% were women (Table 1). Half of the patients (n = 99) were diagnosed with idiopathic PAH (IPAH). At the time of diagnosis, mean 6-minute walk distance was 320 m and the majority of patients were in functional class III. Patients with IPAH showed a severe haemodynamic compromise (mPAP: 51.6 ± 13.9 mm Hg, PVR: 969 ± 458 dyn•sec/cm^5, and CI: 2.1 ± 0.6 L/min/m^2). On average, those with APAH had slightly less severe hemodynamic impairment with elevated mPAP (49.3 ± 15.3 mm Hg) and PVR (830 ± 483 dyn•sec/cm^5), while the CI (2.5 ± 0.8 L/min/m^2) was well preserved (Table 2).

Table 1. Clinical characteristics of patients with pulmonary arterial hypertension

Characteristic	Mean (SD) or n (%)
Age at RHC, yr*	52.1 (15.7)
Female sex	126 (63.6)
6-minute walk test, m*	320 (127)
NYHA functional class	
II	32 (16.2)
III	107 (54.0)
IV	46 (23.2)
Unknown	13 (6.6)
Diagnosis subgroup	
IPAH	99 (50.0)
APAH	99 (50.0)
CTD	46 (23.2)
CHD	27 (13.6)
HIV	8 (4.0)
PCH	1 (0.5)
PoPH	17 (8.6)

* Data are presented as mean (SD).
APAH = associated pulmonary arterial hypertension; CHD = congenital heart defect; CTD = connective tissue disease; HIV = human immunodeficiency virus; IPAH = idiopathic pulmonary arterial hypertension; NYHA = New York Heart Association; PCH = pulmonary capillary hemangiomatosis; PoPH = portopulmonary hypertension; RHC = right heart catheterization; SD = standard deviation.

Safety of vasoreactivity testing

No severe adverse events such as pulmonary edema were noted during vasoreactitvity testing with both drugs in the studied patients. Mild adverse effects were observed in response to sildenafil in form of systemic hypotension that could be managed by fluid infusion. We defined symptomatic systemic hypotension if systolic SAP fell <90mmHg and fluid infusion was applied and found this in 9 patients (4.5%).

Vasoreactivity

Both patient groups showed a significant drop in mPAP and PVR in response to administration of each vasodilator compared with baseline (Table 2, $P < 0.001$, P values in comparison to baseline not shown in the table). The mean systemic arterial pressure (SAP) did not change in response to iNO, but a mild decrease was observed in response to sildenafil. In patients with IPAH, the mean response to sildenafil was significantly stronger than to NO in terms of right atrial pressure (RAP), CI, and PVR, whereas the changes in mPAP and heart rate did not show significant differences (Table 2). In the APAH group, the mean response to sildenafil was significantly stronger than that to NO for all hemodynamic parameters.

Table 2. Hemodynamic variables at baseline and in response to inhaled nitric oxide and oral sildenafil

Variable	IPAH				APAH			
	Baseline Mean (SD)	NO Mean (SD)	Sildenafil Mean (SD)	*P* value*	Baseline Mean (SD)	NO Mean (SD)	Sildenafil Mean (SD)	*P* value*
Heart rate, beats/min	78.5 (13.7)	76.7 (14.1)	75.6 (14.7)	0.24	77.1 (14.7)	77.0 (13.6)	75.4 (13.5)	0.022
mSAP, mm Hg	90.1 (15.1)	90.1 (16.8)	87.7 (15.2)	0.06	90.0 (16.6)	91.9 (16.0)	85.0 (15.2)	< 0.001
mPAP, mm Hg	51.6 (13.9)	44.1 (15.4)	44.6 (13.7)	0.49	49.3 (15.3)	45.0 (16.1)	42.8 (15.2)	< 0.001
RAP, mm Hg	7.6 (5.0)	6.5 (5.5)	5.5 (5.0)	< 0.001	8.5 (5.7)	7.6 (5.8)	6.4 (5.3)	< 0.001
PVR, dyn•sec/cm^5	969 (458)	789 (463)	751 (403)	< 0.001	830 (483)	713 (446)	640 (408)	< 0.001
CI, L/min/m^2	2.1 (0.6)	2.2 (0.6)	2.4 (0.6)	0.047	2.5 (0.8)	2.6 (0.8)	2.7 (0.9)	< 0.001

**P* values were calculated using *t*-test between the values obtained after NO and those obtained after sildenafil administration in each diagnosis group.
APAH = associated pulmonary arterial hypertension; CI = cardiac index; IPAH = idiopathic pulmonary arterial hypertension; mPAP = mean pulmonary arterial pressure; NO = nitric oxide; RAP = right atrial pressure; PVR = pulmonary vascular resistance; SD = standard deviation.

8.1% of the patients met the responder criteria for NO and 11.6% for sildenafil (Table 3). It was noted that, of the 16 NO responders, 13 were also sildenafil responders. The highest frequency of NO responders (10.1%) was found among patients with IPAH (Table 4). In patients with APAH, a positive NO response was found less often (6.1%). Responder rates for sildenafil were comparable in IPAH (11.1%) and APAH (12.1%); the highest frequency (18.5%) was found in patients with congenital heart defects.

Table 3. Response to nitric oxide and sildenafil

		Sildenafil response		Total
		Negative, n	Positive, n	
Nitric oxide response	**Negative, n**	172	10	182
	Positive, n	3	13	16
Total		175	23	198

A positive response to each drug was defined as a drop in mean pulmonary arterial pressure of ≥ 10 mm Hg from an absolute value of ≥ 40 mm Hg and normalization of the cardiac index to ≥ 2.4 L/min/m^2.

Table 4. Response to nitric oxide and sildenafil by diagnosis

Diagnosis	Nitric oxide Number of responders/ total patients (%)	Sildenafil Number of responders/ total patients (%)	LT CCB Number of responders/patients treated with LT CCB (%)
IPAH	10/99 (10.1)	11/99 (11.1)	11/27 (40.7)
APAH	6/99 (6.1)	12/99 (12.1)	2/13 (15.4)
CTD	3/46 (6.5)	5/46 (10.9)	2/9 (22.2)
CHD	2/27 (7.4)	5/27 (18.5)	0/3 (0)
HIV	0/18 (0)	0/18 (0)	0/0 (0)
PCH	0/1 (0)	0/1 (0)	0/0 (0)
PoPH	1/17 (5.9)	2/17 (11.8)	0/1 (0)

APAH = associated pulmonary arterial hypertension; CHD = congenital heart defect; CTD = connective tissue disease; HIV = human immunodeficiency virus; IPAH = idiopathic pulmonary arterial hypertension; LT CCB = long-term calcium channel blocker; PCH = pulmonary capillary hemangiomatosis; PoPH = portopulmonary hypertension.

Comparing the intra-individual responses to both drugs, we found that the NO-induced decrease in mPAP correlated with the decrease in mPAP after sildenafil administration (Figure 1A; $r = 0.516$, $P < 0.001$). The same was true for the increase in CO in response to both drugs (Figure 1B; $r = 0.521$, $P < 0.001$).

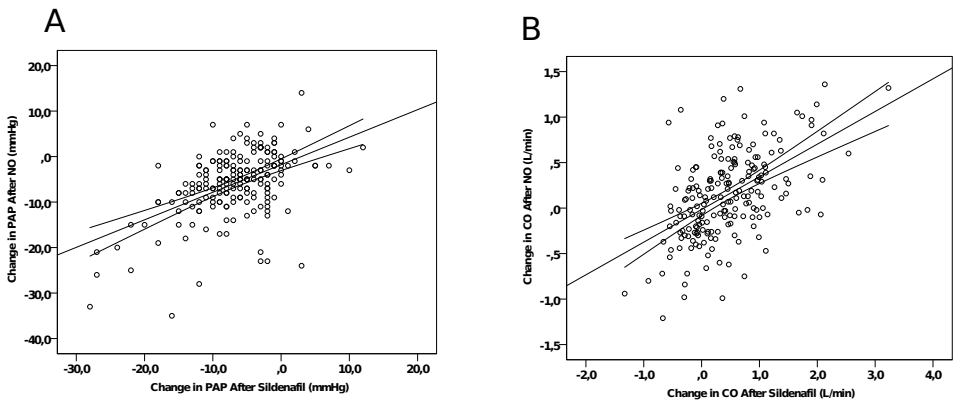


Figure 1. Correlation of hemodynamic responses induced by sildenafil and nitric oxide administration. (*A*) For each patient, the change in mean pulmonary arterial pressure (mPAP) in response to sildenafil administration is plotted against the change in mPAP after nitric oxide (NO) inhalation. The correlation coefficient was $r = 0.516$ with $P < 0.001$. (*B*) For each patient, the change in cardiac output (CO) in response to sildenafil is plotted against the change in CO in response to NO. The correlation coefficient was $r = 0.521$ with $P < 0.001$.

Nitric Oxide and Sildenafil Vasoreactivity and Long-term Calcium Channel Blocker Response

Therapy with CCBs was started if the patient met the acute vasoreactivity response criteria for NO valid at that time (until 2004: decrease in mPAP and PVR > 20%; from 2005 onwards: current criteria).[16] Therefore, the number of patients starting CCB therapy was greater than the number of patients who met the current NO responder criteria.

Of the 40 patients who received CCBs, 13 (32.5%) were long-term responders (Table 4), the majority of whom (n = 11) had IPAH. Two patients with APAH with a positive long-term response were noted, both of whom had connective tissue disease as the underlying disease.

We then looked at the initial response to vasoreactivity testing in those patients who received long-term CCB therapy, and differentiated between long-term responders or non-responders (according to the pre-defined criteria of treatment success described in the methods). Three of the 13 long-term responders to CCBs were correctly identified by acute vasoreactivity test with both drugs (sensitivity 23 % Table 5). The number of false positives – that is, patients who were responders in acute testing but non-responders to long-term CCBs – was 3 for NO and 4 for sildenafil testing, resulting in a specificity of 88.9% for NO and 85.1% for sildenafil testing.

Table 5. Cross-tabular comparison between long-term response to calcium channel blockers and acute response to nitric oxide and sildenafil

		Nitric oxide response			Sildenafil response		
		Negative	Positive	Total	Negative	Positive	Total
Full group							
Long-term CCB	Negative	24	3	27	23	4	27
	Positive	10	3	13	10	3	13
Total		34	6	40	33	7	40
IPAH							
Long-term CCB	Negative	13	3	16	14	2	16
	Positive	8	3	11	8	3	11
Total		21	6	27	22	5	27
APAH							
Long-term CCB	Negative	11	0	11	9	2	11
	Positive	2	0	2	2	0	2
Total		13	0	13	11	2	13

CCB = calcium channel blocker.
APAH, IPAH

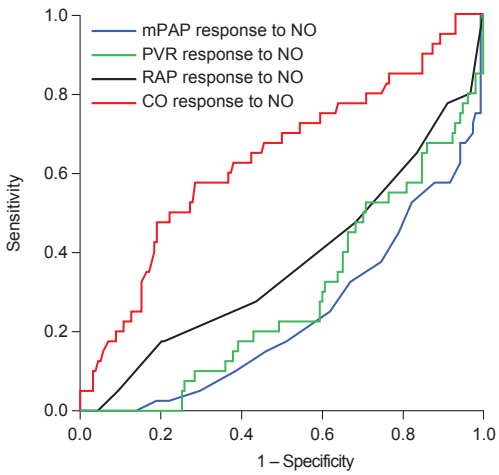
Table 6. Comparison of acute hemodynamic responses to nitric oxide and sildenafil of long-term responders and non-responders to calcium channel blockers

	CCB LT responders (n = 13)	CCB LT non-responders (n = 27)	*P* value CCB LT responders vs. CCB LT non-responders	NO - non-responders (CCB non-exposed)	*P* value CCB LT responders vs. CCB non-exposed
Acute NO response					
ΔPAP	−19.8 (7.7)	−8.0 (5.8)	< 0.001	-4.4 (5.9)	<0.001
ΔPVR	−460 (285)	−200 (178)	0.001	-115 (163)	<0.001
ΔRAP	−2.3 (2.4)	−1.7 (2.2)	0.412	-0.8 (1.9)	0.009
ΔCO	0.5 (0.4)	0.3 (0.5)	0.158	0.1 (0.5)	0.004
Acute sildenafil response					
ΔPAP	−10.4 (9.7)	−8.3 (7.0)	0.451	-6.2 (5.4)	0.014
ΔPVR	−321 (232)	−226 (184)	0.170	-191 (172)	0.012
ΔRAP	−2.3 (3.4)	−2.7 (3.3)	0.753	-2.0 (2.6)	0.719
ΔCO	1.0 (0.9)	0.6 (0.7)	0.142	0.4 (0.6)	0.002

The results of the vasoreactivity tests are shown as change from baseline, all values mean (SD). PAP and RAP in mmHg, PVR in dynes, CO in L/min.
P value was calculated by *t*-test.
CCB = calcium channel blocker; CI = cardiac index; NO = nitric oxide; PAP = pulmonary arterial pressure; PVR = pulmonary vascular resistance; RAP = right atrial pressure; SD = standard deviation, LT = long-term

We next looked at the potential of NO and sildenafil administration to predict long-term CCB response by the absolute changes in hemodynamic parameters (Table 6). Responders to long-term CCBs exhibited a significantly stronger decrease in mPAP and PVR after NO administration than non-responders to long-term CCBs ($P_{mPAP} < 0.001$, $P_{PVR} = 0.001$). In sildenafil testing this difference was smaller and non-significant.

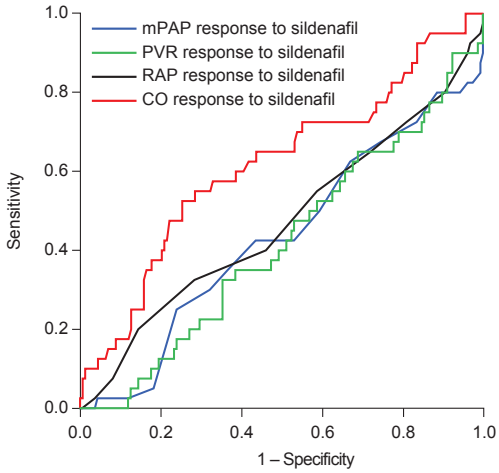
The receiver operating characteristic curves in supplemental Figures S1 and S2 confirm that the changes in mPAP and PVR in response to NO are suitable parameters to identify long-term responders to CCBs (AUC 0.763 and 0.713 for mPAP and PVR, respectively). Logistic regression analysis showed that the decrease of mPAP and the increase of CO after sildenafil and NO administration are independent predictors for long-term CCB response (Table S1). After dividing the patient sample according to the diagnosis, a stable logistic regression model could no longer be built due to the low number of patients.



Area Under the Curve

Test Result Variable(s)	Area	Standard Error*	Asymptotic Significance†	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
mPAP response to NO	0.237	0.042	0.000	0.155	0.319
PVR response to NO	0.287	0.044	0.000	0.201	0.372
RAP response to NO	0.371	0.053	0.012	0.268	0.474
CO response to NO	0.636	0.052	0.008	0.534	0.737

Figure S1. Receiver operating characteristic curve for the prediction of the long-term response to calcium-channel blockers by the acute nitric oxide response. To correctly display all the parameters in one Figure, the curves for mean pulmonary arterial pressure (mPAP; blue), pulmonary vascular resistance (PVR; green) and right atrial pressure (RAP; black) are displayed below the diagonal. They are based on negative values since all these parameters decrease upon vasoreactivity testing. The red line shows the curve for cardiac output (CO). Diagonal segments in the figure are produced by ties.



Area Under the Curve

Test Result Variable(s)	Area	Standard Error*	Asymptotic Significance†	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
mPAP response to sildenafil	0.432	0.053	0.186	0.329	0.535
PVR response to sildenafil	0.417	0.050	0.107	0.319	0.516
RAP response to sildenafil	0.469	0.055	0.549	0.361	0.577
CO response to sildenafil	0.618	0.053	0.021	0.515	0.721

Figure S2. Receiver operating characteristic curve for the prediction of the long-term response to calcium-channel blockers by the acute sildenafil response. In order to correctly display all the parameters in one Figure, the curves for mean pulmonary arterial pressure (blue), pulmonary vascular resistance (green) and right atrial pressure (black) are displayed below the diagonal. They are based on negative values since all these parameters decrease upon vasoreactivity testing. The red line shows the curve for cardiac output (CO).

Table s1. Logistic regression analysis for long-term response to calcium channel blockers in the full patient cohort*

	Nitric oxide response		Sildenafil response	
	OR	*P* value	OR	*P* value
ΔmPAP	0.85	0.001	0.912	0.014
ΔPVR	1.001	0.47	1.001	0.385
ΔRAP	0.929	0.479	0.975	0.725
ΔCO	3.086	0.041	2.269	0.01

* After grouping the patients by diagnosis (idiopathic or associated pulmonary arterial hypertension), a stable logistic regression model could no longer be built.
Multivariate logistic regression analysis was performed to evaluate the predictive power of acute hemodyamic response to NO and sildenafil for long-term CCB response using the following parameters: NO_response_PAP, NO_response_PVR, NO_response_RAP, NO_response_CO, SIL_response_PAP, SIL_response_PVR, SIL_response_RAP, SIL_response_CO.CO = cardiac output; PAP = pulmonary arterial pressure; PVR = pulmonary vascular resistance; OR = odds ratio; RAP = right atrial pressure; SD = standard deviation.

Nitric Oxide and Sildenafil Vasoreactivity and Survival

A tendency towards better survival was noted for NO responders and sildenafil responders in comparison with non-responders in the PAH patient sample but the differences were not statistically significant, irrespective of long-term treatment. The five year survival rate was 75% for NO responders versus 63% for non-responders. For sildenafil responders the five year survival rate was 77% while it was 62% for non-responders (Supplemental Figure 3A and 3B).

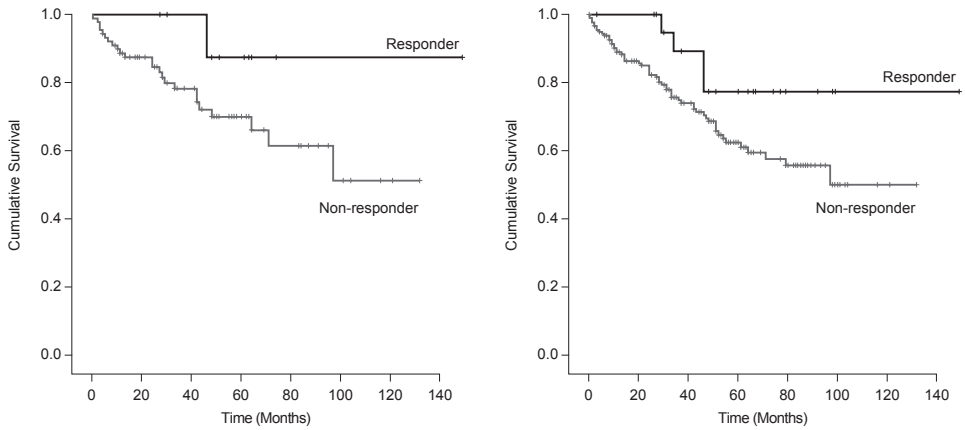


Figure S3. Nitric oxide and sildenafil vasoreactivity and survival in pulmonary arterial hypertension. (*A*) Kaplan-Meier survival curves for patients with pulmonary arterial hypertension stratified by vasoreactivity to nitric oxide (as defined by current guideline criteria, $P = 0.151$). (*B*) Kaplan-Meier survival curves for patients with pulmonary arterial hypertension stratified by vasoreactivity to sildenafil ($P = 0.083$). The vertical ticks indicate censoring, which refers to patients reaching the end of observation period alive.

Discussion

PAH is a severe disease requiring costly and complicated care and treatment. A positive response to acute vasodilator challenge identifies a subgroup of patients in whom mortality is lower compared to the general PAH group and long-term treatment with calcium channel blockers, a relatively cheap drug without significant toxicity, is effective. Performing acute vasodilator challenge involves expensive and difficult to manage drugs such as iNO or epoprostenol and is therefore currently limited to specialized centers in developed countries. With PAH increasingly recognized as a disease that is common in developing countries where resources are scarce, a less complex and costly vasoreactivity testing is needed.

We compared acute vasoresponse to inhaled NO and oral sildenafil in a large cohort of patients with IPAH and APAH, and assessed the related prognostic value for prediction of long-term outcomes in this population.

As sildenafil also causes systemic vasodilation and has a half-life of four hours, it might induce a higher number or increased severity of adverse effects compared to iNO. In the present study, administration of sildenafil was safe as no severe adverse effects were observed using 25mg of sildenafil. The rate of mild adverse events, namely hypotension that was managed by fluid infusion, was moderate. However, sildenafil was not applied if the patients' systolic SAP was already < 90mmHg.

Our patient cohort is comparable to other cohorts of patients with IPAH and APAH in terms of age, female predominance, and severity of elevation of pulmonary arterial pressure.[1,17]

The previous definition of a positive vasoractivity test (relative drop in mPAP and PVR by at least 20%) has been replaced by the current criteria of a drop in mPAP of > 10 mm Hg to an absolute value < 40 mm Hg and a normalization of CI to a value ≥ 2.4 L/min/m^2. Using these criteria, the rate of positive acute vasoreactivity to NO in patients with IPAH in our study was 10.1%, which is slightly less than the rate reported in previous studies.[4]high doses of oral calcium-channel blockers (CCB This could be due, at least in part, to the application of the more recent response criteria as opposed to the less strict criteria used in former studies. As shown previously, and also in our study, positive vasoreactivity to NO was less frequent in patients with APAH than in patients with IPAH.[17]

Our data suggest that sildenafil may also be used for vasoreactivity testing in patients with IPAH identifying the same number of long-term responders to CCBs as the gold-standard iNO when current criteria are used. Moreover, the intra-individual responses to both drugs were highly correlated and the specificity to identify long-term CCB responders by vasoreactivity testing was similar with both drugs.

Recently, the intravenous formulation of sildenafil has become available, with the potential of shortening the vasoreactivity testing procedure due to a more rapid onset of action and more reproducible bioavailability than the oral formulation.

Nevertheless, iNO seems more discriminative, as the difference in the acute vasoresponse between long-term CCB responders and non-responders were larger than with sildenafil (19.8 vs. 8.0 mmHg for iNO, 10.4 vs. 8.3 mmHg for sildenafil).

The rate of positive vasoreactivity was higher for sildenafil than for iNO (11.6% vs. 8.0% for sildenafil vs iNO, respectively). This difference was largely observed in the patients with APAH who exhibited a clinically significant response to sildenafil but not to iNO. Comparing the intra-individual responses to both drugs, we have shown that 25mg of sildenafil exerts a stronger acute effect on all hemodynamic parameters than iNO in patients with APAH. Similar observations with a more pronounced acute response to sildenafil compared to

iNO were made in previous studies using higher doses of iNO and oral sildenafil in patients with PAH [10,11] or intravenous sildenafil in patients with chronic thromboembolic pulmonary hypertension.[18] In contrast to the present study, these studies were conducted on small numbers of patients and did not evaluate the prognostic value of the vasoreactivity testing. In patients with IPAH, the drop in mPAP was equivalent in response to both drugs, but a significantly stronger response to sildenafil was noted for RAP, PVR and CI. The reasons for these differences may be found in the pharmacodynamic profiles of the drugs, but may also reflect a different pathogenesis or state of the underlying disease.

NO is a physiologically produced gas with an extremely short half-life. When inhaled, it is almost 100% pulmonary selective and, through an increase in wedge-pressure, may additionally unmask left heart disease as a cause of pulmonary hypertension. [4]

The phosphodiesterase-5 inhibitor sildenafil targets the NO pathway downstream of NO. Sildenafil exhibits systemic vasodilatory effects and animal studies suggest it might have additional, long-term effects beyond vasodilation that counteract remodeling processes in the heart and vasculature. [19,20] Further, there is evidence that phosphodiesterase type 5 inhibitors may directly enhance right ventricular contractility through cyclic guanosine monophosphate–mediated inhibition of phosphodiesterase type 3. [21,22]

Systemic hypotensive effects of sildenafil might have secondary effects on pulmonary hemodynamics. However, these secondary effects can be recognized by a reflectory increase in heart rate. As heart rate did not increase in response to sildenafil in our study, this does not seem to be a major contributor here.

Even though NO and phosphodiesterase-5 inhibitors target the same pathway, different responses to both substances may be observed within one patient due to the different endogenous activities of NO-synthase and phosphodiesterase-5. It can be speculated, for instance, that in a patient with high basal phosphodiesterase-5 activity and normal endogenous NO-production, a strong response to phosphodiesterase-5 inhibitors can be observed, while exogenous NO-inhalation is hardly effective. On the other hand, patients with low endogenous phosphodiesterase-5 activity may not respond well to phosphodiesterase-5 inhibitors, whereas they may show a good response to inhaled NO. The down-regulation of NO synthase found in some patients with IPAH reported by Giaid et al [23] could provide a clue to this differential responsiveness to agents targeting the same signaling pathways on different levels.

Of note, only one in every three patients receiving CCB therapy was a long-term responder. In accordance with previous studies,[17] we found that long-term responders to CCBs were almost exclusively patients with IPAH, suggesting that CCB therapy should only be started in these patients. Surprisingly, sensitivity for detection of long-term responders to CCBs both by iNO vasoreactivity testing and by sildenafil vasoreactivity testing with the current criteria was low (23 % for both substances). Several of the long-term responders to CCBs

fulfilled the old, but not the new criteria or just barely missed them. The current criteria were defined after retrospective analysis of one large, single-center IPAH cohort,[1] but have not been prospectively evaluated. Hence, the reasons for the low sensitivity of these criteria in our cohort may be diverse. Differences in patients' characteristics compared with the defining cohort could be an explanation for the unsatisfying transferability.

Previous studies have found large differences in the positive predictive value of acute vasoreactivity testing for long-term response to CCB therapy. Jing et al [24] recently found that acute responsiveness to adenosine infusion and iloprost inhalation successfully predicted long-term CCB response in nine young patients with IPAH. On the other hand, Barst et al [25] found that 15 of 31 children who were acute responders did not improve with long-term CCB therapy. From a practical point of view, we suggest that patients with IPAH who fulfill the old or new responder criteria for iNO or sildenafil testing should start CCB therapy with close monitoring. The ability to closely monitor therapeutic response is prerequisite to initiating CCB therapy as, even in patients identified by vasoreactivity testing, the rate of long-term non-responders may be high. Moreover, vasoreactivity testing remains crucial to identifying non-responders and saving them from a potentially dangerous trial of CCB.

It has been suggested that patients with good initial vasoreactivity may identify patients in an earlier disease state or a more benign variant of the disease.[2,26,27] Our data indicate that initial vasoreactivity to both iNO and sildenafil, irrespective of the modern specific treatments later applied in the non-responders, might be of prognostic value for the long-term outcome. However, the difference was not statistically significant for both drugs, possibly due to the low number of patients in the responder group.

Limitations of the Study

The main limitation of this study is its retrospective design. A prospective study to confirm the value of the current vasoreactivity criteria for the prediction of long-term CCB response has not yet been conducted and would be desirable. However, with the retrospective data and other treatment options for this fatal disease available, such a study could not be performed for ethical reasons.

Moreover, selection bias cannot be excluded as only treatment-naïve patients who underwent vasoreactivity testing with both, iNO and sildenafil, were studied. Another limitation of the study, which is due to the low rate of positive vasoreactivity, is the low number of patients in the long-term CCB analyses.

Finally, a systematic bias was inherent to this analysis, as the decision to start long-term CCB treatment was solely based on the hemodynamic criteria achieved with inhaled NO and not with sildenafil. Therefore, we cannot provide data on the long-term CCB response of patients that responded to sildenafil, but not to iNO.

Conclusion

We conclude that sildenafil may serve as a vasoreactivity testing agent in patients with IPAH, even though iNO might be more discriminative and should be preferred due to its pulmonary selectivity and short half-life. Therefore, when sildenafil is used for vasoreactivity testing, the positive predictive value to identify long-term CCB responders should be assessed to provide more evidence for this approach. Given the low number of positive acute responders to NO and the even lower rate of long-term CCB response in patients with APAH, vasoreactivity testing may be dispensable for these patients in terms of CCB trial. Interestingly, our data suggest that acute vasoresponsiveness to sildenafil might be of prognostic value for both IPAH and APAH patients regardless of the type of PAH-specific treatment.

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4.3 |

Survival with Sildenafil and Inhaled Iloprost in a Cohort with Pulmonary Hypertension

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Abstract

Background: Combination therapy is frequently used to treat patients with pulmonary hypertension but few studies have compared treatment regimens. This study examined the long-term effect of different combination regimens of inhaled iloprost and oral sildenafil on survival and disease progression.

Methods: This was a retrospective study of patients in the Giessen Pulmonary Hypertension Registry who received iloprost monotherapy followed by addition of sildenafil (iloprost/sildenafil), sildenafil monotherapy followed by addition of iloprost (sildenafil/iloprost), or upfront combination therapy (iloprost + sildenafil). The primary outcome was transplant-free survival (Kaplan–Meier analysis). When available, haemodynamic parameters and 6-minute-walk distance were evaluated.

Results: Overall, 148 patients were included. Baseline characteristics were similar across treatment groups; however, the iloprost + sildenafil cohort had higher mean pulmonary vascular resistance and pulmonary arterial pressure than the others. Transplant-free survival differed significantly between groups ($P = 0.007$, log-rank test). Cumulative transplant-free survival was highest for patients who received iloprost/sildenafil (1-year survival: iloprost/sildenafil, 95.1%; sildenafil/iloprost, 91.8%; iloprost + sildenafil, %62.9); this group also remained on monotherapy significantly longer than the sildenafil/iloprost group (median 17.0 months vs 7.0 months, respectively; $P = 0.004$). Compared with pre-treatment values, mean 6-minute-walk distance increased significantly for all groups 3 months after beginning combination therapy.

Conclusions: In this observational study of patients with pulmonary hypertension receiving combination therapy with iloprost and sildenafil, cumulative transplant-free survival was highest in those who received iloprost monotherapy initially. However, owing to the size and retrospective design of this study, further research is needed before making firm treatment recommendations.

Keywords: Combination therapy, Iloprost, Sildenafil, Pulmonary hypertension, Giessen Pulmonary Hypertension Registry

Background

Pulmonary hypertension (PH) is a life-threatening disorder with a variety of aetiologies [1]. Because PH is a multifactorial condition, monotherapy focused on a single pathological pathway may be insufficient to halt disease progression. By acting on two or more biological pathways, combination therapies have the potential for increased efficacy over monotherapies. In patients with PH, two main approaches for combining treatments may be followed, with therapies introduced sequentially or concomitantly as 'upfront' combination therapy. Monotherapy is normally used initially, with additional therapy introduced if clinical deterioration occurs. Less frequently, combination treatment is used as first-line therapy to exploit the 'hit hard and early' model, which aims to use early and aggressive treatment to halt disease progression [2].

Treatment guidelines suggest combining established pharmacotherapies for patients with PH who do not respond adequately to monotherapy, but do not recommend particular combinations or regimens [3]. During a 3-year study employing pre-defined treatment goals to guide therapeutic decisions, combination therapy was eventually required by almost half of patients initially prescribed monotherapy [4]. Several studies have examined the combination of the prostanoid iloprost and the phosphodiesterase type 5 (PDE-5) inhibitor sildenafil in the treatment of patients with PH. In acute haemodynamic testing, combining these drugs led to a greater reduction in pulmonary vascular resistance (PVR) than each agent alone [5]. Furthermore, patients with pulmonary arterial hypertension (PAH) showed improved exercise capacity and haemodynamics when given sildenafil as an add-on to existing iloprost therapy [6]. Randomized controlled trials directly comparing the efficacy of iloprost and sildenafil have not been undertaken, although a meta-analysis found no significant difference in efficacy between these therapies [7]. The aim of this study was to examine the long-term effect of different combination regimens of inhaled iloprost and oral sildenafil on the survival and disease progression of patients with PH.

Methods

Study design

This was an observational study [8] of patients in the Giessen Pulmonary Hypertension Registry, a single-centre registry including more than 2500 patients. The first patient was enrolled in March 1993. Adult patients who received a combination of inhaled iloprost and oral sildenafil were eligible for inclusion. Patients who received intravenous iloprost or sildenafil, or who had begun treatment with therapies other than iloprost or sildenafil, were excluded. Each patient gave informed consent to participate. The study was approved by the Institutional Review Board and followed the principles of the Declaration of Helsinki.

Three treatment regimens were studied: iloprost monotherapy followed by addition of sildenafil (iloprost/sildenafil); sildenafil monotherapy followed by addition of iloprost (sildenafil/iloprost); and upfront combination therapy of iloprost and sildenafil (iloprost + sildenafil). No pre-defined protocol was followed; treatment and doses were tailored to the individual patient's needs and optimized by dose-titration.

Outcome measures

The primary outcome measure was transplant-free survival, as calculated by Kaplan-Meier analysis. As this was a retrospective study of patient records, complete information could not be obtained in all cases. When available, 6-minute-walk distance (6MWD), New York Heart Association (NYHA) functional class, pulmonary arterial pressure (PAP), PVR, and cardiac output were analysed. Changes were compared using intra-individual paired analysis (i.e. including only patients with both baseline and post-treatment data available). Values were determined pre-treatment (baseline), 3 months after monotherapy initiation, before combination therapy initiation (post-monotherapy baseline), and 3 months after combination therapy initiation. Patients lost to follow-up were classified as having withdrawn alive on the date of last contact.

Statistical methods

Data are presented as mean (standard deviation) or median (interquartile range), as applicable. The log-rank test was used to analyse differences in cumulative transplant-free survival; analysis of variance was applied to test for differences between groups; and the paired *t*-test (two-tailed) was used to examine changes in response to therapy. Cox regression, defining iloprost as the reference, was applied to control for possible confounders in survival analysis, correcting for NYHA functional class, 6MWD, and cardiac output. The Kruskal–Wallis test was performed to test for differences in parameters with skewed distributions.

Results

Baseline characteristics

In total, out of 685 patients assessed, 148 patients were eligible for the study. Similar numbers of patients initially received iloprost or sildenafil monotherapy (61 patients and 63 patients, respectively), and 24 received upfront combination therapy (Table 1). In the iloprost/sildenafil group, idiopathic PAH and PAH associated with other conditions (Dana Point classification 1.4) [1] were the most frequent aetiologies (35.0% and 33.3%, respectively). Similarly, patients treated with sildenafil/iloprost were mainly those with idiopathic PAH or PAH associated with other conditions (25.0% and 43.3%, respectively).

Table 1. Baseline characteristics of patients who were eligible for the observational study

Characteristic	Treatment regimen[a]		
	Iloprost/sildenafil n = 61	Sildenafil/iloprost n = 63	Iloprost + sildenafil n = 24
Female sex, %	65.0	66.7	78.3
	[n = 60]	[n = 60]	[n = 23]
Mean age at diagnosis, years (SD)	48.7 (14.9)	53.0 (15.2)	43.3 (17.1)
Classification of PH, n (%)			
Idiopathic PAH	21 (35.0)	15 (25.0)	11 (47.8)
PAH associated with other conditions[b]	20 (33.3)	26 (43.3)	6 (26.1)
Associated with lung diseases	4 (6.7)	9 (15.0)	1 (4.3)
CTEPH	14 (23.3)	10 (16.7)	3 (13.0)
Miscellaneous	1 (1.7)	0 (0.0)	2 (8.7)
	[n = 60]	[n = 60]	[n = 23]
NYHA functional class, n (%)			
II	3 (10.3)	4 (8.5)	0 (0)
III	13 (44.8)	18 (38.3)	5 (38.5)
IV	13 (44.8)	25 (53.2)	8 (61.5)
	[n = 29]	[n = 47]	[n = 13]
Mean PAP, mmHg (95% CI)	55 (51–58)	57 (53–61)	73 (65–82)
	[n = 50]	[n = 51]	[n = 21]
Mean cardiac output, L/min (95% CI)	3.4 (3.1–3.7)	3.6 (3.3–3.9)	3.1 (2.5–3.7)
	[n = 49]	[n = 51]	[n = 21]
Mean PVR, dyn.s.cm^{-5} (95% CI)	1287 (1134–1440)	1143 (1016–1270)	1824 (1538–2109)
	[n = 49]	[n = 51]	[n = 21]
Mean 6MWD, m (95% CI)	276 (232–319)	281 (245–317)	222 (179–265)
	[n = 38]	[n = 48]	[n = 16]

[a]The treatment regimens were: iloprost/sildenafil (iloprost followed by addition of sildenafil), sildenafil/iloprost (sildenafil followed by addition of iloprost), or iloprost + sildenafil (combined iloprost and sildenafil as upfront therapy); [b]Dana Point classification 1.4 [1].
6MWD, 6-minute-walk distance; CHD, congenital heart disease; CI, confidence interval; CTD, connective tissue disease; CTEPH, chronic thromboembolic pulmonary hypertension; ILD, interstitial lung disease; NYHA, New York Heart Association; PAH, pulmonary arterial hypertension; PAP, pulmonary arterial pressure; PVR, pulmonary vascular resistance; SD, standard deviation.

The most common classification for patients who received upfront combination therapy was idiopathic PAH (47.8%). Baseline characteristics were broadly similar in the treatment groups (Table 1). The mean age at diagnosis of the sildenafil/iloprost group was significantly higher than that of the iloprost + sildenafil group (53.0 years vs 43.3 years, respectively; $P = 0.029$); otherwise, there were no significant differences between the mean ages of the groups. Patients who initially received iloprost monotherapy were admitted to the study

centre earlier (median date November 2000) than those beginning sildenafil monotherapy or combination therapy (median dates April and August 2003, respectively; $P < 0.001$). The mean baseline 6MWD was lower for patients who received upfront combination therapy than for the other groups (Table 1), but not significantly so ($P = 0.227$).

Patients who received upfront combination therapy had significantly higher mean PAP than patients initially treated with iloprost or sildenafil monotherapy ($P < 0.001$ [Table 1]). Between treatment groups, however, there was no significant difference in cardiac output ($P = 0.264$). Patients treated with upfront combination therapy had higher mean PVR than those who started on iloprost or sildenafil monotherapy ($P < 0.001$). Data for exercise capacity and haemodynamic parameters were not available for all patients. The proportions of patients who went on to receive additional therapy with an endothelin receptor antagonist, an intravenous prostanoid or both were 48.6%, 5.4%, and 13.5%, respectively. Patients were followed up for a mean of 60.9 months.

Duration of monotherapy treatment

Patients initially treated with iloprost remained on monotherapy significantly longer than those starting with sildenafil ($P = 0.004$; Figure 1). Median time on monotherapy was 17.0 months (95% confidence interval: 10.4–23.6 months) with iloprost and 7.0 months (95% confidence interval: 4.2–9.8 months) with sildenafil.

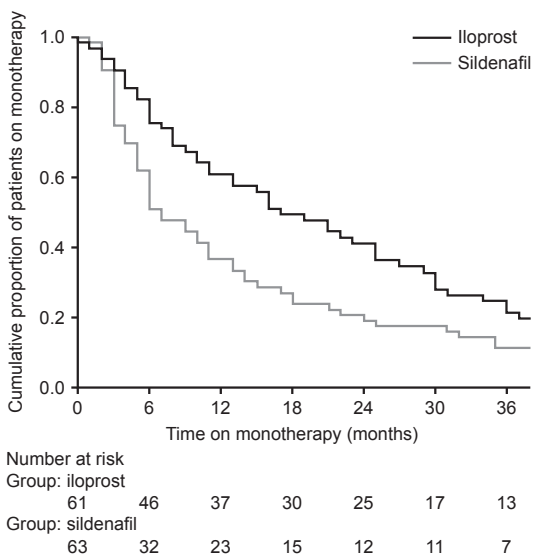
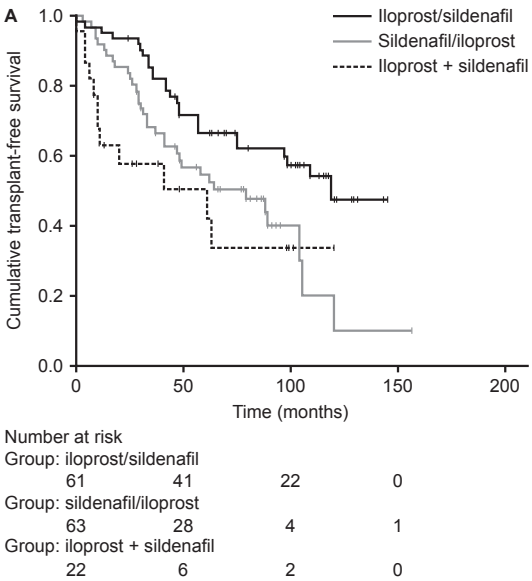


Figure 1 Kaplan–Meier plot of proportions of patients remaining on iloprost or sildenafil monotherapy over time.

Cumulative transplant-free survival

In total, eight patients were lost to follow-up: three in the iloprost/sildenafil group, one in the sildenafil/iloprost group, and four in the iloprost + sildenafil group. There was a significant difference in transplant-free survival among groups (P = 0.007, log-rank test; Figure 2A). Cumulative transplant-free survival was highest in the iloprost/sildenafil group and lowest for those who received upfront combination therapy. In the iloprost/sildenafil group, survival rates were 95.1% at 1 year, 81.8% at 3 years, and 66.4% at 5 years. In the sildenafil/iloprost group, survival rates were 91.8% at 1 year, 68.1% at 3 years, and 54.5% at 5 years. Survival rates were 62.9% at 1 year, 57.7% at 3 years, and 50.5% at 5 years for patients who received upfront combination therapy.



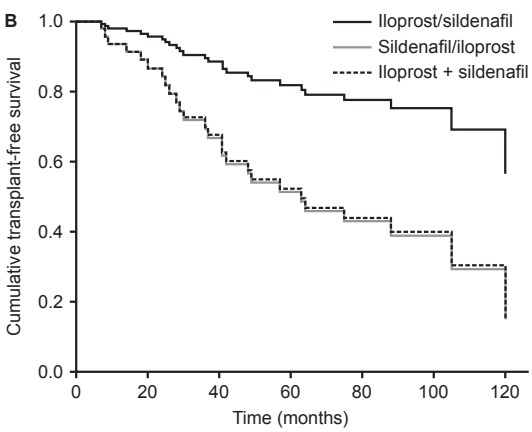


Figure 2 Transplant-free survival. (A) Kaplan–Meier plot of cumulative transplant-free survival and (B) Cox regression estimate of transplant-free survival after correction for possible confounders (New York Heart Association functional class, 6-minute-walk distance, and cardiac output). Patients were treated sequentially with iloprost and sildenafil (either iloprost followed by addition of sildenafil [iloprost/sildenafil] or sildenafil followed by addition of iloprost [sildenafil/iloprost]), or with upfront combination therapy (iloprost + sildenafil).

After Cox regression analysis, cumulative transplant-free survival was significantly higher in the iloprost/sildenafil group than in the sildenafil/iloprost group (P = 0.035; Figure 2B). Survival was also higher for patients treated with iloprost/sildenafil than for those treated with upfront combination therapy, but this difference was not statistically significant (P = 0.120).

Cumulative transplant-free survival based on the aetiology of pulmonary hypertension

For patients with PAH initially treated with iloprost or sildenafil, cumulative transplant-free survival was analysed by PH classification (Additional file 1: Figure S1). For all groups assessed (PAH associated with collagen-vascular disease, idiopathic PAH, and PAH associated with systemic-to-pulmonary shunt), survival was higher in the iloprost/sildenafil group than in the sildenafil/iloprost group. No statistical analyses were conducted because the number of patients in these sub-analyses was small.

Change in functional class

The iloprost/sildenafil group had a lower proportion of patients in NYHA functional class IV at pre-treatment baseline than the sildenafil/iloprost group (Figure 3). The proportion of patients in NYHA functional class IV showed a more pronounced decrease with sildenafil than with iloprost. The lowest proportion of patients in NYHA functional class IV was observed after addition of the second therapy in both groups.

Change in mean pulmonary arterial pressure

There was no significant change in mean PAP measured 3 months after therapy initiation from pre-treatment baseline for patients initially treated with iloprost (Figure 4A). Following combination therapy, mean PAP was significantly reduced compared with post-monotherapy baseline (P = 0.037). However, there was no significant change in mean PAP after 3 months of combination therapy compared with pre-treatment baseline.
Mean PAP was significantly reduced from a pre-treatment baseline of 57 mmHg to 50 mmHg for patients who received sildenafil monotherapy (P = 0.001; Figure 4B). However, mean PAP was unchanged 3 months after beginning combination therapy compared with post-monotherapy baseline. Compared with pre-treatment baseline, there was no significant change in mean PAP after combination therapy (P = 0.148).

For patients who began initial combination therapy, mean PAP was significantly reduced from a pre-treatment value of 79 mmHg to 69 mmHg after 3 months of treatment (P = 0.018; Figure 4C).

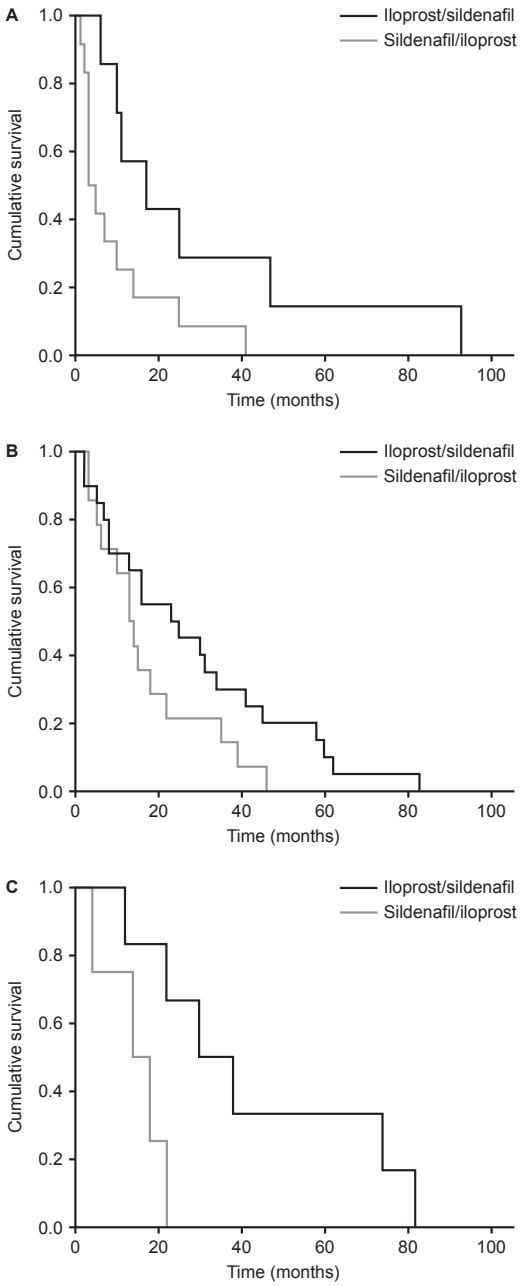


Figure S1. Kaplan-Meier plots of cumulative transplant-free survival in patients with pulmonary arterial hypertension associated with collagen-vascular disease, idiopathic pulmonary arterial hypertension, and pulmonary arterial hypertension associated with systemic-to-pulmonary shunt. Data are shown for patients who were treated with iloprost followed by addition of sildenafil (iloprost/sildenafil) or sildenafil followed by addition of iloprost (sildenafil/iloprost).

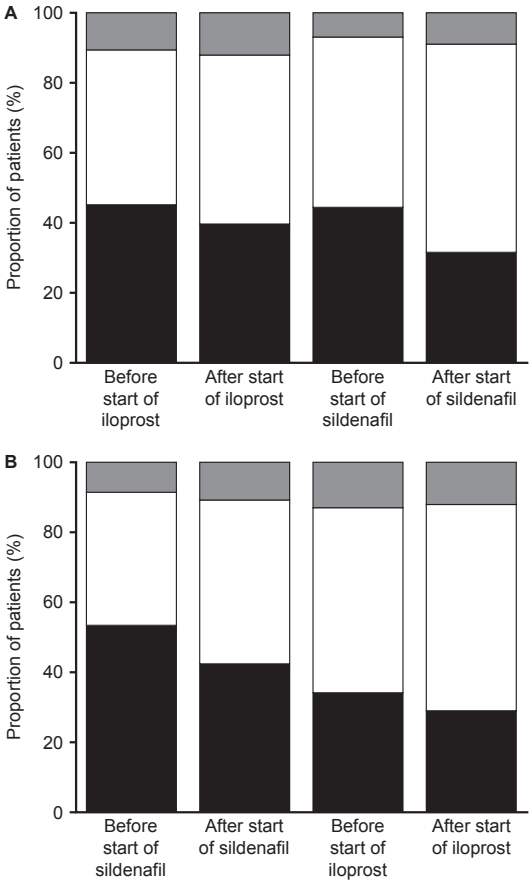


Figure 3 New York Heart Association (NYHA) functional class over the study. (A) Patients received iloprost followed by addition of sildenafil. (B) Patients received sildenafil followed by addition of iloprost.

Change in mean cardiac output

Mean cardiac output was unchanged 3 months after beginning iloprost therapy compared with pre-treatment values (Figure 4D). However, after combination therapy, mean cardiac output was increased compared with post-monotherapy baseline, from 2.9 L/min to 3.4 L/min (P = 0.001). There was no significant difference between mean cardiac output pre-treatment and following combination therapy.

For patients initially treated with sildenafil, mean cardiac output increased from 3.5 L/min at pre-treatment baseline to 4.1 L/min 3 months after beginning treatment (P = 0.001; Figure 4E). Following iloprost addition, there was no significant change in mean cardiac output compared with post-monotherapy or pre-treatment baselines. Similarly, for patients treated with combination therapy initially, there was no significant change in mean cardiac output compared with pre-treatment baseline (Figure 4F).

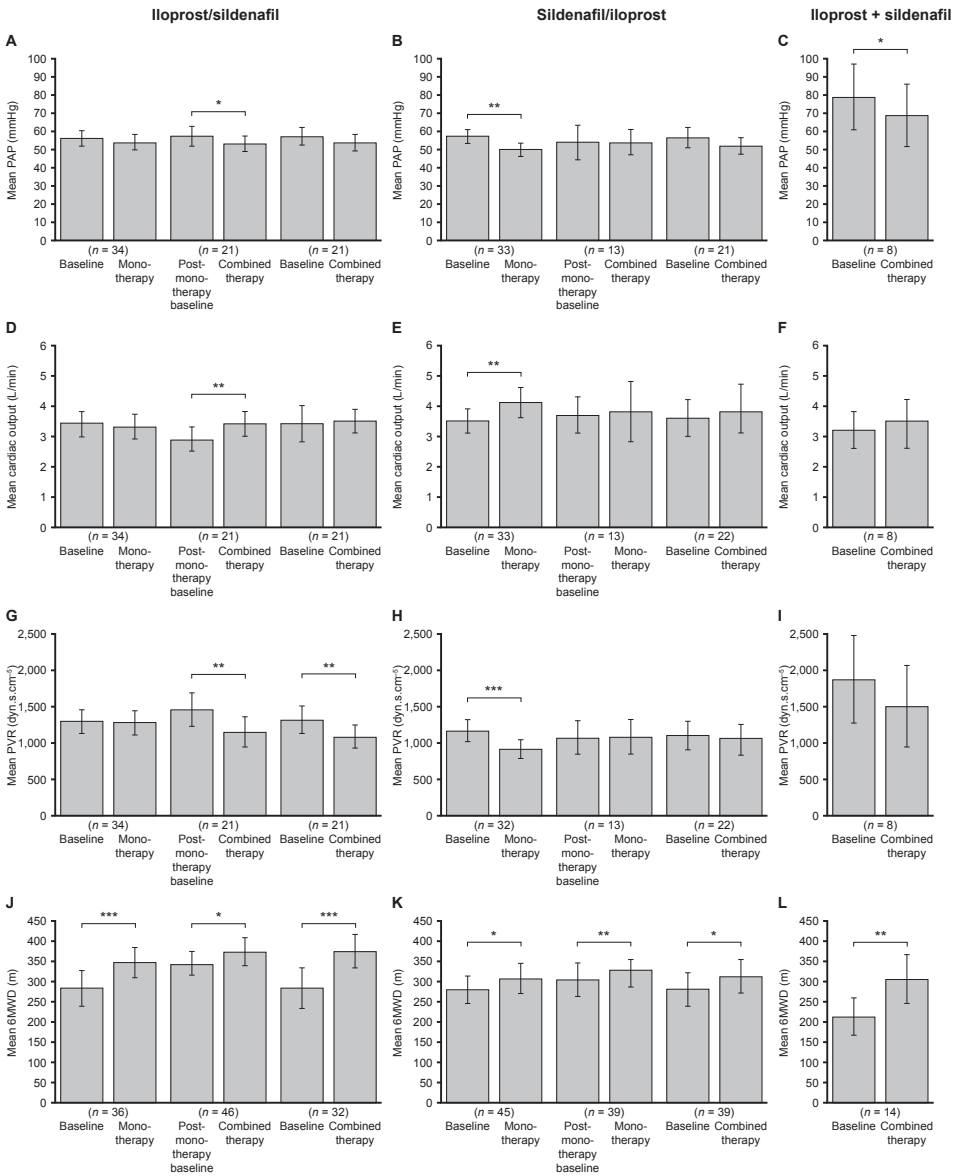


Figure 4 Changes in haemodynamic parameters and 6-minute-walk distance over the study (intra-individual responses). (A–C) Pulmonary arterial pressure (PAP), (D–F) cardiac output, (G–I) pulmonary vascular resistance (PVR), and (J–L) 6-minute-walk distance (6MWD). Data are presented as means ± 95% confidence interval. Patients were treated with iloprost followed by addition of sildenafil (iloprost/sildenafil), sildenafil followed by addition of iloprost (sildenafil/iloprost), or upfront combination therapy with iloprost and sildenafil (iloprost + sildenafil). Values are shown pre-treatment (baseline), 3 months after therapy initiation (monotherapy), before combination therapy (post-monotherapy baseline), and 3 months after starting combination therapy (combined therapy). *P < 0.05; **P < 0.01; ***P < 0.001. Statistical analysis was conducted using the paired sample t-test (two-tailed).

Change in mean pulmonary vascular resistance

After 3 months of iloprost monotherapy, there was no significant change in mean PVR compared with pre-treatment baseline (Figure 4G). However, mean PVR was significantly reduced 3 months after initiating combination treatment compared with post-monotherapy baseline, from 1455 dyn.s.cm^{-5} to 1143 dyn.s.cm^{-5} (P = 0.006). A significant reduction in mean PVR was also seen following combination therapy when compared with pre-treatment values (P = 0.006).

Mean PVR was significantly reduced from a pre-treatment baseline of 1161 dyn.s.cm^{-5} to 909 dyn.s.cm^{-5} 3 months after beginning sildenafil monotherapy (P < 0.001; Figure 4H). However, 3 months after beginning combination therapy there was no change in mean PVR compared with post-monotherapy or pre-treatment baselines.

For patients treated initially with combination therapy, there was no significant change in mean PVR compared with pre-treatment baseline (Figure 4I).

Change in 6-minute-walk distance

Compared with pre-treatment values, patients who received initial iloprost monotherapy showed significantly increased mean 6MWD, from 283 m to 346 m (P < 0.001; Figure 4J). Exercise capacity was also improved following add-on sildenafil therapy: compared with post-monotherapy baseline, mean 6MWD increased from 345 m to 374 m (P = 0.01). Mean 6MWD increased from 283 m at pre-treatment baseline to 374 m 3 months after beginning combination therapy (P < 0.001).

For patients who received initial sildenafil monotherapy, mean 6MWD increased from 278 m at pre-treatment baseline to 307 m 3 months after beginning treatment (P = 0.036; Figure 4K). Subsequently, patients treated with add-on iloprost therapy showed increased exercise capacity compared with post-monotherapy baseline, with mean 6MWD increased from 303 m to 328 m (P = 0.002). Compared with pre-treatment values, mean 6MWD increased from 280 m to 312 m for patients treated with combination therapy (P = 0.038).

6MWD increased from 213 m to 305 m for patients treated with upfront combination therapy compared with pre-treatment baseline (P = 0.001; Figure 4L).

Discussion

In the treatment of patients with PH, clinical studies have evaluated combinations of major pharmacological classes of medical therapies, i.e. endothelin receptor antagonists and prostanoids [9-12], endothelin receptor antagonists and PDE-5 inhibitors [13, 14], and prostanoids and PDE-5 inhibitors [6, 5, 15-19]. However, only one study, of administration of the prostanoid treprostinil for up to 2 years in patients receiving oral background PAH therapy, examined long-term outcomes (survival and clinical worsening [defined as addition of a new PAH therapy, discontinuation due to disease progression, or death]) [20]. A meta-analysis of randomized controlled studies in patients with PAH found that, compared with monotherapy, combination therapy significantly reduced clinical deterioration, increased 6MWD, and improved haemodynamics [21]. However, no significant difference in mortality was observed between patients treated with monotherapy and those receiving combination therapy.

Iloprost and sildenafil act via different pathways (stimulating cyclic adenosine monophosphate [cAMP] production and preventing cyclic guanosine monophosphate [cGMP] breakdown, respectively), but there is evidence of cross-talk between these pathways. Raised cGMP levels inhibit cAMP breakdown, and pre-treatment of erythrocytes *in vitro* with PDE-5 inhibitors potentiated cAMP release in response to treprostinil [22]. In acute haemodynamic testing in patients with PH, the combination of sildenafil and iloprost produced a greater vasodilatory response than either agent alone [5]. There are limited data showing the long-term benefits of combining sildenafil and iloprost, but in a 16-week study the addition of sildenafil to long-term treatment with the prostacyclin epoprostenol improved exercise capacity and haemodynamics among patients with PAH compared with those receiving placebo [23].

In our study, cumulative transplant-free survival was lower for patients who received upfront combination therapy than for those treated with initial monotherapy. At 1 year, the survival rate was 62.9% for those who received combination therapy, compared with 95.1% and 91.8% for those first treated with inhaled iloprost or oral sildenafil monotherapy, respectively. However, before therapy, patients treated with iloprost + sildenafil had higher mean PVR and mean PAP than those who began monotherapy. Therefore, patients with the most severe disease had been assigned to receive upfront combination therapy. This approach, of treating patients with severe PAH with upfront inhaled iloprost and oral sildenafil therapy, was taken in a separate study of eight patients of NYHA functional class IV who were unable to perform a 6MWD test. Following treatment, all patients had an improvement in their functional class and were able to complete a 6MWD test, though one patient later underwent lung transplantation and subsequently died [24]. Similarly,

for the small number of patients for whom measurements were recorded in our study (n = 16), 6MWD significantly increased following combination treatment.

Among patients treated initially with monotherapy, transplant-free survival was higher for those receiving iloprost/sildenafil than for those treated with sildenafil/iloprost. Patients treated with inhaled iloprost also remained on monotherapy longer than patients beginning oral sildenafil monotherapy. When paired recordings were available, the benefit of sequential therapy on exercise capacity was also observed for both drug regimens, with 6MWD significantly higher than pre-treatment values after 3 months of combination therapy. The results from this study suggest that when combining iloprost and sildenafil in a step-wise manner the optimal treatment regimen may be initial monotherapy with iloprost followed by add-on sildenafil if clinical deterioration occurs.
This study has limitations. Owing to the retrospective design, patients were not randomly assigned to each treatment, as highlighted by significant differences in baseline characteristics between the patient groups. Furthermore, complete data were not available for functional class, haemodynamic parameters, and exercise capacity, and there was the potential for selection bias. Unlike treatment with iloprost monotherapy, sildenafil monotherapy resulted in significant improvements in haemodynamics compared with pre-treatment values. Despite this, transplant-free survival was shorter in patients initially treated with sildenafil than among those who received iloprost therapy first. This is difficult to explain, and may reflect differences in the baseline characteristics between these patient groups or be the result of an unrecognized confounding factor. These outcomes also need to be viewed in the context of treatment practices over the course of this study, because patients who received initial iloprost therapy were admitted to the study centre before those first treated with sildenafil. In Europe, iloprost was approved for the treatment of PAH 2 years before sildenafil. Thus, in the early years of the study, inhaled iloprost was the only treatment available and patients may have remained on monotherapy because no other treatment options were available.

Conclusions

The sequence in which patients with PH received combination therapy with iloprost and sildenafil was independently associated with transplant-free survival rate. However, owing to the small size of the study and its retrospective design, further research is required to confirm the external validity of the results.

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5

Discussion

Despite a large amount of research, pulmonary hypertension (PH) is still a disease which causes extensive morbidity and mortality, and which is difficult to diagnose and has a poor prognosis.[1] Targeted therapy for PH is now available in some regions of the world to improve symptoms, but to date, for most of the patients there is no cure. [2]

This thesis describes a comprehensive approach to evaluate various aspects of PH epidemiology including prevalence, risk factors, diagnosis, and prognosis, using data from both a large population-based study and an extensive patient registry. This work reports the prevalence of PH in the general elderly population and the associations of PH with common diseases, including COPD and left ventricular dysfunction. Furthermore, we present data on mortality and prognosis of patients diagnosed with PH. Using information from the large biobank that is linked to the Giessen patient registry, in this thesis we also present data on two biomarkers that were studied within the registry, one with diagnostic potential and one possible prognostic tool.

The findings presented in this thesis may help to improve the understanding of the disease by facilitating the diagnostic process, enabling more detailed information about prognosis, and by improving the possibility of personalizing treatment. Additionally, the findings give further insight into the disease in the pre-clinical stage, at the population level.

This is the first epidemiological study to evaluate the prevalence of PH in an elderly population. [3] By studying both the general population and analyzing data from the largest single center PH registry we were able to integrate various aspects of PH: Significant PH is rare in asymptomatic elderly people. Presence of PH in the general population is associated with concomitant diseases. The PH registry includes sizable groups of WHO groups II+III, which are likely the most prevalent groups in the general population as well.

Discussion of key findings

Studies of Pulmonary Arterial Systolic Pressure in the General Population: The Rotterdam Study

Although previous studies have described the prevalence of the different subtypes of PH using patient registries, much less is known about the overall prevalence of PH in the general population. In daily clinical practice PH is under-diagnosed and undertreated since several years may pass between the first symptoms and the establishment of the diagnosis, which, in itself, is cumbersome and time consuming. [4] Part of the difficulty in diagnosing PH is due to the relatively unspecific early symptoms of PH, including shortness of breath and fatigue.[4] A number of associated factors and underlying or co-occurring morbidities related to PH may be associated with a subclinically increased PASP. Associated factors may precede the occurrence of overt PH, may modify disease severity, and may impact prognosis.[5]

To gain insight into the distribution of echocardiographically measured PASP (ePASP), the prevalence of echocardiographic PH (ePH), and the presence of co-morbidities in the general population, we studied the general elderly population of the Rotterdam Study. As described in **chapter 2.1**, we found a relatively low prevalence of 2.6% for ePH. Previous studies have found higher prevalences of up to 9.1%, mostly in younger populations. [6,7] The highest estimate of 9.1% was calculated in a population referred for echocardiography, so it might be an overestimation of the true prevalence in the general population. [7] A recent study in the population-based Jackson Heart Study used a slightly lower threshold for ePH (35 mmHg vs. 40 mmHg in our study) and excluded patients with absent tricuspid regurgitation from analysis whereas we included those as non-cases, which may, in part, explain the higher prevalence of 6.8% found in the Jackson Heart Study. [6] Our study population arguably represents the „healthier", elderly population because those too sick to come to the research center or to undergo echocardiography were not included in the measurements. Thus, the prevalence in the full population of elderly may be higher. Nevertheless, even in healthy elderly adults, we would not expect the prevalence of ePH to be lower than in healthy younger populations. As mentioned, a reason for the lower estimate may be that we included participants without tricuspid regurgitation or with a tricuspid regurgitation velocity that was too small to measure as non-cases, since we could not estimate the ePASP in these participants and we assumed that is was likely to be low. This may have deflated our estimates. An alternative approach could have been to exclude them from the analysis. Sensitivity analyses without these participants showed a slightly higher, but still relatively low, estimate of 3.4%, thus not completely explaining the differences with other studies.

The European Society of Cardiology recommends that additional right heart characteristics should be used to estimate PASP on echocardiography. [8] We further estimated the prevalence of ePH using both estimated PASP and the dimensions of the right ventricle. This increased the prevalence from 2.6% to 4.5%, indicating that our prevalence estimate may indeed be an underestimation. [9]

In addition, we studied the associations of left heart measures and PASP in 2592 participants of the Rotterdam Study in **chapter 2.2**. In a large multivariable model E/A, E/E´, and left atrial diameter had the strongest associations with PASP (**figure 3b in chapter 2.2).** The strong associations of markers of diastolic function (E/A and E/E´) with PASP reflect the importance of diastolic dysfunction for the development of PH. The pathophysiology of PH due to heart failure with preserved ejection fraction (HFpEF-PH) includes increased LV filling pressures leading to pathologic remodeling of the pulmonary vasculature and involvement of the right ventricle (RV).[10] Among patients with PH due to left heart failure, HFpEF is the most common pathology.[10] One reason may be the increasing incidence and

prevalence of HFpEF in the population. Oktay et al. describe factors that "are likely contributing to both the increasing overall prevalence of HFpEF and the increasing proportion of HF that is due to HFpEF", listing the increased life expectancy and aging of the population, the epidemic of cardiac and non-cardiac comorbidities, and the increased clinical recognition of HFpEF as contributing factors. [11] Survival in patients with HFpEF is strongly associated with RV performance and PASP, underlining the importance of PASP in the prognosis of the underlying disease. [12]

Prevalence of and hospitalizations due to HFpEF are increasing. [13] Physical activity and less sedentary time reduce the risk of developing heart failure, both heart failure with reduced ejection fraction (HFrEF) and HFpEF. [14] In the ARIC Study, the impact of potentially modifiable risk factors on the incidence of heart failure of any kind was analyzed. Among diabetes mellitus, current smoking, systemic arterial hypertension, obesity, and elevated LDL, diabetes mellitus had the highest incidence rate difference. [15] This is also important to consider for the development of PH. Especially as a reduction in the prevalence (or risk on the individual basis) of HF could lead to a reduction in PH due to left heart disease.

Treatment of patients with group 2 PH (PH associated with left heart failure) is always recommended to start with optimized treatment of the left heart disease, irrespective of the specific disease. [8] In addition to optimized treatment of left heart disease, treatment with PH-specific drugs should be considered on an individual basis. However, published trials show disappointing results, albeit in small populations. [16-21] Further studies in larger populations are needed to give further insight into the usefulness and feasibility of PH-specific drugs in patients with left heart failure.

The Giessen Pulmonary Hypertension Registry and biobank: prognosis and diagnosis

By design, population-based studies are best suited to study diseases with the highest prevalence in the population. Conversely, patient registries capture the full spectrum of patients with a particular disease, enabling more detailed assessment of clinical presentation, diagnosis, treatment and prognosis. For PH in particular, these aspects can also be studied for the different etiological subgroups. The Giessen registry is such a patient registry. It includes more than 2500 patients with PH of all etiological groups with deep phenotyping. Additionally, the registry is linked to a biobank with more than 4000 blood and urine samples. Samples are taken from all patients at baseline and during follow-up. Below, we discuss the results of our studies including patients from the Giessen registry, the largest single-center PH registry to date.

In **chapter 3.1**, we present survival data from the Giessen registry. In contrast to most other registries established so far, all etiologic groups are included in the registry and we report differences in survival between the distinct etiologic groups. Prognostic factors

perform differentially between the etiologic groups. The main causes of death among all etiologic groups were right heart failure or respiratory insufficiency, accounting for half of the known causes of death.

Patients in our registry had similar baseline characteristics to those in other national registries in terms of age, six-minute walking test (6MWT), New York Heart Association functional class (NYHA FC), female-to-male-ratio, and hemodynamic parameters. Only minor differences could be detected between the Giessen registry and other registries in terms of survival of PH due to lung disease (LD-PH) and PH due to left heart disease (PVH). [22-25] Within the pulmonary arterial hypertension (PAH) group, patients with PH due to congenital heart disease (CHD) had the best survival. This finding is in agreement with those identified in previous publications, showing good long-term survival in patients with CHD/Eisenmenger syndrome [26]. The survival of patients with idiopathic PAH (IPAH) is very similar to with outcomes from registries in France, UK/Ireland, and the US. [27]

The PH biomarkers identified to date can serve as prognostic tools or are associated with disease severity or progression. Biomarkers can be grouped into markers of endothelial damage, inflammation, right ventricular maladaptation, tissue hypoxia, and end organ failure.[28] There are no established biomarkers to diagnose or exclude PH. To address this lack of established biomarkers, the vascular endothelial growth factor (VEGF) family members placental growth factor (PlGF) and soluble VEGF receptor 1 (sFlt-1) were tested as potential diagnostic biomarkers for PH in **chapter 3.2**. VEGF and its family members are involved in the pathophysiology of PH, as shown in rodent models, and expression studies in human lungs and blood. [29,30] In a relatively large patient cohort of 247 patients with invasively proven or excluded PH of different etiologies, we measured PlGF and sFlt-1 in plasma. Sensitivity and specificity for diagnosis of PH were 62% and 100%, respectively, when both markers were combined. The fact that we did not find a correlation of sFlt-1 and PlGF and invasive pulmonary hemodynamics and other clinical parameters suggests a pathophysiological association not directly linked to increased pulmonary arterial pressure (PAP) or resulting changes of the RV. Immunohistochemical stainings of human lungs from PAH patients compared to healthy donor lungs indicated a pathophysiological link of sFlt-1 and PlGF with the remodeling process. [31] Though these markers seem useful for diagnosis but show no association with hemodynamics, we can speculate that they might reflect an initial disease trigger or even a cause of disease, rather than a signal that drives and worsens the disease.

Screening for a rare disease requires tests with high sensitivity and reasonable specificity as a first step, so, PlGF and sFlt-1 with the cut-offs we chose are not optimal screening tools. After a positive test result and other signs or symptoms suggestive of PH, the patient could undergo RHC sooner, potentially leading to an earlier diagnosis. As such, these measurements may not be used alone as a diagnostic test, but could be part of a diagnostic workup in patients suspected of PH.

On a population level, the test with the cut-off we chose does not seem appropriate to answer epidemiological questions because the moderate sensitivity would miss too many diseased persons. In addition, the test is very costly. In a clinical setting, with their moderate sensitivity and their high specificity for PH (and PAH), sFlt-1 and PlGF are reasonably suitable screening tools, however their main strength lies in their almost unfailing specificity. Therefore, in the decision-making tree of diagnostic workup of patients with suspected PH, in cases of a negative test, confirmation by means of invasive hemodynamic assessment remains obligatory provided other non-invasive tests (e.g. echocardiography) are suggestive of PH. In case of a positive test, sFlt-1 and PlGF are novel tools that confirm PH with high certainty at an early stage of diagnostic evaluation. Nevertheless, a confirmation by right heart catheterization is always needed.

Previous studies have also examined various potential diagnostic markers, including BNP, echocardiography, diffusion capacity of the lung for carbon monoxide (DLCO), computed tomography, oxygen saturation, lung function tests, and ECG and have been reviewed by Bonderman et al. [32]

BNP has been shown to be valuable for diagnosis of acute decompensation of left and right heart failure. [33,34] The potential for BNP alone as a diagnostic marker for stable PH remains poor, but BNP could be utilized in combination with other potential markers as it has a high negative predictive value. [32] In a meta-analysis by Taleb et al. published in 2013, the pooled sensitivity and specificity of using echocardiographically estimated PASP for the diagnosis of PH were 88% and 56%, respectively, using RHC as the gold standard.[35] Bonderman et al. evaluated a combination of ECG, NT-proBNP, and echocardiography as a diagnostic tool for PH. This study investigated the diagnostic accuracy for the differentiation of precapillary PH vs. non-precapillary PH, using a methodologically elaborate approach including a retrospective evaluation cohort, an internal validation with bootstrapping, and a second validation within a prospective cohort. In the prospective cohort, the reported sensitivity was 100%, and specificity was 19%. [36] This reported high sensitivity and the associated low number of false negatives makes it an appropriate tool to detect PH in patients. The low specificity could pose a problem, as low specificity is associated with a high number of false positive results. False positive screening results lead to unnecessary right heart catheterizations with the associated risks and costs.

Translating these findings from a patient registry to the population level is challenging. As the invasive diagnosis or exclusion of PH by RHC in population studies is not feasible and, hence, not available in the Rotterdam Study, a new diagnostic tool or algorithm should be evaluated in a patient registry instead. But for future analysis, ECG data could be combined with echocardiography data and BNP plasma levels to estimate the true prevalence of PH in the general population more accurately. The combination of these diagnostic tools could increase the sensitivity and specificity for detection of PH in the general population. Of course, the accuracy of such a combination of markers for estimating the prevalence of PH in the general population would have to be further evaluated.

Altered glucose metabolism and PAH

To study the association of glucose metabolism and long-term survival, we tested the prognostic relevance of HbA1c at the time of diagnosis for all-cause mortality in patients with PAH. HbA1c was a predictor of all-cause mortality with a hazard ratio of 2.2 per 1-unit increase of HbA1c. The hazard ratio for all-cause mortality was 3.9 for presence of altered glucose metabolism defined as an HbA1c above the median. We found no association of HbA1c with disease severity. [37]

In patients with heart failure, impaired glucose metabolism has been associated with worse clinical parameters and outcomes. [38] For PAH, no long-term prognostic relevance has been shown for HbA1c so far. One study associated glucose metabolism with short-term outcomes and a worse prognosis for female PAH patients with increased insulin resistance was found. [39]

Besides our finding of an association of impaired glucose metabolism with mortality in patients with PAH, in **chapter 2.1**, we also found a borderline-significant association of PASP prevalence with manifest diabetes mellitus. The risk ratio was 1.7 for presence of ePH in patients with diabetes mellitus when compared to non-diabetic persons; however only borderline statistical significance was reached (p=0.07). [3,37] Mechanisms to explain the potential pathophysiological link between PH and altered glucose metabolism speak in favor of PH as the cause rather than the consequence of altered glucose metabolism (AGM).[37] Nevertheless, the association of AGM with increased mortality could lead to the idea of treating non-diabetic PH patients who have an AGM before they develop overt diabetes mellitus. However, before initiation of an interventional trial, more data must be generated to support this idea.

Assessment and Prognostic Relevance of Right Ventricular Contractile Reserve in Patients with Severe Pulmonary Hypertension

Patients with pulmonary hypertension (PH) present with exercise intolerance in daily clinical practice, but the prognostic value of exercise to right heart echocardiography remained unknown. Prospectively, PH patients on stable PH-targeted therapy were evaluated regarding their increase in pulmonary arterial systolic pressure (PASP) in response to exercise in **chapter 4.1**. Our data indicated for the first time that patients with an increase in PASP during exercise showed a significantly better long-term outcome. The ability of the right ventricle (RV) to adapt to exercise (RV contractile reserve) was identified as an independent prognostic factor in PH patients.

Pathophysiologically, patients with PAH exhibit a limited increase in stroke volume due to systolic and diastolic impairment, increased RV afterload and impaired ventriculo-arterial coupling, while the diastolic pressure–volume relationship determines filling and CO. [40,41] Therefore, in terms of clinical decision making, prognostic factors derived from right ventricular dysfunction are valuable.[42] The results of this study indicate for the first time that non-invasive, echocardiographic assessment of RV contractile reserve is a significant predictor of outcome. In our prospective study, exercise-induced PASP-increase was chosen for estimation of RV contractile reserve. A large increase in PASP in response to exercise implies the capacity of the RV to eject a large stroke volume despite high pressures, reflecting a preserved RV contractile reserve. In case of maladaptation to high afterload the RV loses its capability to pump enough blood into the pulmonary circulation. This is clear in the case of a decreasing PAP as a sign of a failing right heart. A limited increase in systemic blood pressure in response to exercise can also be interpreted as a possible sign of severely reduced RV contractile reserve. The pathophysiology behind this may in part be explained by the missing capability of the RV to pump enough blood against the raised pulmonary vascular resistance (PVR) to fill the left atrium. As a consequence, even a healthy LV could not pump sufficient amounts of blood into the systemic circulation to cause an increase in systemic blood pressure. In addition, the dissociation between RV ejection fraction (EF) and PVR was described previously.[43] Thus, a decreased PVR under targeted therapy could be associated with either deterioration or improvement in RV function. [43,44] Notion emerges that PAH is a disease of RV–arterial uncoupling rather than only of pathological pulmonary vascular remodeling. [41] Our study indicates that the increase in PASP during exercise is dependent on maintenance of RV pulmonary arterial coupling and the ability to increase or maintain stroke volume. These two factors are essential for good right ventricular function. So far, there have been no studies on RV contractile reserve in patients with right heart failure due to PAH or chronic thrombo-embolic PH (CTEPH). The concept of estimating RV contractile reserve by PASP-increase during exercise has not previously been used in patients with PH. Therefore we

might conclude that the measurement of RV contractile reserve in patients with PAH may influence clinical decision making, given its independent association with mortality, while prognostic factors are not only important to guide treatment, but also to inform the patient about his or her prognosis. Impairment of RV contractile reserve is likely to be one mechanism underlying the development of right heart failure and frequent assessments might be beneficial in clinical practice. RV contractile reserve has not been used as endpoint in clinical trials yet. Future trials could include RV contractile reserve as a possible endpoint.

Sildenafil versus nitric oxide for acute vasodilator testing in pulmonary arterial hypertension

Right heart catheterization with acute vasodilator testing is mandatory in every newly diagnosed patient with PAH.[8] However, the acute vasoresponse of targeted PAH therapy in comparison with the standard challenge of nitric oxide (NO) and their prognostic value has not been investigated previously. In **chapter 4.2** we compared the acute vasoresponse to inhaled NO (iNO) and oral sildenafil in a large cohort of patients with IPAH and associated PAH (APAH), and assessed the prognostic value for prediction of long-term outcomes in this population. Using current criteria, the detection rate of responders was similar with administration of sildenafil and with inhaled nitric oxide and the prognostic accuracy was similar.

Currently approved targeted therapies for the treatment of PAH have been shown to significantly improve long-term outcome und quality of life. [42] However, treatment response is not uniform. [45] Parameters that help patients and clinicians to anticipate the natural course of a disease are warranted. Acute vasodilator testing has the ability to identify a small subgroup of patients with IPAH with favorable long-term response to treatment with high dose calcium channel blockers. Therefore, performing acute vasodilator testing is mandatory in every newly diagnosed patient with PAH. Performing an acute vasodilator challenge involves drugs that are expensive and difficult to manage, such as iNO or epoprostenol, so it is therefore currently limited to specialized centers in developed countries.[46] With PAH increasingly recognized as a disease that is common in developing countries where resources may be limited, a less complex and costly way of vasoreactivity testing is needed. The aim of our study was to establish an acute vasodilator testing setting with the comparably inexpensive, orally administered, and widely used phosphodiesterase-5 inhibitor sildenafil. Pathophysiologically, sildenafil targets the NO pathway downstream of NO. Sildenafil exhibits systemic vasodilatory effects and animal studies suggest it might have additional long-term effects beyond vasodilation that counteract remodeling processes in the heart and pulmonary vasculature. [47,48] There is evidence that phosphodiesterase-5 inhibitors may directly enhance right

ventricular contractility through cyclic guanosine monophosphate–mediated inhibition of phosphodiesterase-3. [49] As sildenafil also causes systemic vasodilation and has a half-life of four hours, it might induce more adverse effects or more severe adverse effects compared to iNO. We found that administration of sildenafil was safe as no severe adverse effects were observed using 25mg of sildenafil. Our data suggested for the first time that sildenafil may also be used for vasoreactivity testing in patients with IPAH identifying the same number of long-term responders to CCBs as the gold-standard iNO when current criteria are used. Moreover, the intra-individual responses to both drugs were highly correlated and the specificity to identify long-term CCB responders by vasoreactivity testing was similar with both drugs.

Our data indicate that initial vasoreactivity to both iNO and sildenafil, irrespective of the modern specific treatments later applied in the non-responders, might be of prognostic value for the long-term outcome. It has been suggested that patients with good initial vasoreactivity may present in an earlier disease state or exhibit a more benign variant of the disease. [50,51] For further evaluation future prospective studies in larger patient groups are warranted.

Survival with sildenafil and inhaled iloprost in a cohort with pulmonary hypertension

Individualized and goal-oriented therapy has recently emerged as a cornerstone in the treatment of patients with PH. Observational data, especially for combination therapy, regarding long-term outcome are currently lacking. We conducted a retrospective analysis of PH patients who were treated with combination therapy of inhaled iloprost and sildenafil (**chapter 4.3**). The cohort was divided into patient groups receiving up-front combination therapy, monotherapy with inhaled iloprost and add-on oral sildenafil, or monotherapy with oral sildenafil and add-on inhaled iloprost. Our data indicated for the first time that overall survival depended on the chosen treatment regimen, with first line iloprost before addition of sildenafil was associated with the best survival, and in addition that functional class, hemodynamics, and exercise capacity improved after addition of a second drug regardless of the order of drugs.

Combination therapy is often prescribed for long-term use in the modern treatment era despite the absence of broad scientific evidence. While some studies evaluated the short-term efficacy of different combination regiments, long term survival data are lacking. [52] Iloprost and sildenafil address different pathways to evoke their therapeutic potential, but synergistic cross-talk between the cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) cascades has been described. [53] In acute haemodynamic testing in patients with PH, the combination of sildenafil and iloprost produced a greater vasodilatory response than either agent alone. [54] No published data are available for long-

term combination therapy of sildenafil and iloprost, but combination of sildenafil as add-on to parenteral epoprostenol improved exercise capacity and pulmonary hemodynamics. [55,56] Taken together, there is good reason to combine inhaled iloprost with oral sildenafil for the treatment of PH patients. Our study is adding long-term survival data to this field, providing evidence for the first time that combination of inhaled iloprost/sildenafil is associated with a better outcome than with established monotherapy. Our findings are in accordance with two recently published meta-analyses that demonstrated a reduction in the risk of clinical worsening by combination therapies in PAH. [52,57] Also experiences with left heart disease and systemic hypertension speak in favor of combination therapies. [58,59] The question remains which drugs could be good combination partners and what is the best timing for combination. Drug-drug interactions are always an important issue. Individualized therapy is therefore necessary in order to find the optimal treatment regimen for each patient. Our study provided evidence for a combination therapy of inhaled iloprost and sildenafil. However, prospective, randomized long-term data for combination treatment are still lacking.[8] A recently published large, prospective, randomized controlled trial combining Ambrisentan and Tadalafil has shown encouraging results with a significant reduction of clinical events by 50%. [60]

Methodological considerations

Studies in the General Population

The large sample size and the population-based nature are clear strengths of these studies. The standardized echocardiographic assessment, the availability and prospective collection of a large number of covariates are also strong points. However, a number of limitations need to be addressed. The data we used for our analyses were measured cross-sectionally. As a consequence our study may suffer from selection bias, as the persons with severe PH could have died before the echocardiographic evaluation (survivor bias). This could have led to an underestimation of the prevalence of PH in our study.

Echocardiography can only estimate the PASP. The gold standard for measurement of PASP is right heart catheterization. [8] This is a very invasive and costly procedure and, hence, is unsuitable for use in population-based studies. Therefore, we used echocardiography to estimate PASP in our population. Echocardiography is often used as a first step in the PH evaluation or for screening for PH. [8] As discussed earlier, in a meta-analysis the pooled sensitivity and specificity for detecting PH by using echocardiographically estimated PASP for the diagnosis of PH were 88% and 56%, respectively, using RHC as the gold standard.[35] Thus, we believe that echocardiography could be considered an acceptable tool for PH research on the population level. Studies comparing invasively measured PASP with echocardiographic values report both overestimation and underestimation of the true

PASP when using with echocardiography. [61] Also, the largest differences between PASP measured by right heart catheterization and PASP measured by echocardiography have been reported to occur at the high end of the spectrum of PASP, a range which few of our participants were in, but not all reports are in agreement.[62] A limitation of previous studies is that they were not done in the general population, but were usually done in patients with confirmed or suspected PH. It is not clear to which extent they apply to the general population in our studies. Residual confounding is also a possible limitation we have to discuss. As unmeasured potential confounders are not entered into our regression models we could not account for those.
Our study was performed in an elderly population. Hence, the generalizability of our findings to other populations needs to be determined by future studies.

Studies in the Giessen Pulmonary Hypertension Registry

A potential limitation of the studies performed in the Giessen Pulmonary Hypertension Registry is the fact that we studied a single-center cohort. Our reference center is one of the largest in Germany and therefore data may be representative of the overall PH population, though milder cases may not be referred to us. We did not have complete data on right heart catheterization at baseline: some patients came with clinically acceptable right heart catheterization values from secondary centers but these data were not entered into the database. This results in missing data, but is most likely not missing in a particular group more so than in another.
The evaluation of two proteins as novel biomarkers for PH (**chapter 3.2**) has only been done in our study population and needs further validation. We used blood drawn from the pulmonary artery. Future studies will have to determine whether using blood from the cubital vein, which is much easier to access, gives the same results.
The HbA1C study described in **chapter 3.3** has several limitations. Second, we cannot exclude selection bias as HbA1c data were missing at baseline in many patients. We did not analyze differences between patients with missing HbA1c and included patients. We did not perform serial HbA1c measurements during treatment and by that we cannot describe the impact of treatment on HbA1c. We also cannot evaluate if HbA1c is a good marker for follow up. Thus, HbA1c should be evaluated in a prospective manner in future studies and the impact of treatment with PH drugs on HbA1c should be analyzed.
In the study assessing the prognostic relevance of RV contractile reserve (**chapter 4.1**), selection bias may have played a role as we only selected clinically stable patients for the study. This selection may have contributed to the comparably good long-term survival in the described patient group. Only patients with moderate to severe impairment of RV function, mostly WHO functional class III-IV were included. The maximum workload was not defined beforehand. This was done for safety reasons, but may also have an impact

on the maximum PASP increase as higher workload may lead to higher increase in PASP. We included patients who were on stable background therapy, so the prognostic relevance may not be true for newly diagnosed treatment naïve patients. Most other prognostic parameters were assessed at time of diagnosis. The impact of PASP increase at time of diagnosis on survival should be investigated in the future.
Apart from the physiologic rationale for choosing exercise-induced PASP-increase for estimation of RV contractile reserve, there are also technical advantages. Estimation of PASP by echocardiography is easier and more precise than echocardiographic assessments of RV ejection fraction or cardiac output during exercise.[63] However, the best way to measure contractile reserve has not been defined. Catheter or MRI derived pressure volume loops; their intercepts and slopes are within the scope of technical approaches and have shown good performance. [64]

In **chapter 4.2**, in which we studied the potential of sildenafil in acute vasodilator testing, we had a low number of patients in the long-term CCB analyses, which is due to the low rate of positive vasoreactivity. Also, the decision to start long-term CCB treatment was solely based on the hemodynamic criteria achieved with inhaled NO and not with sildenafil. Therefore, we could not provide data on the long-term CCB response of patients that responded to sildenafil, but not to iNO.

The retrospective design leads to the major limitation of missing randomization in our study into survival in patients treated with sildenafil monotherapy and add-on inhaled iloprost, or with inhaled iloprost monotherapy and add-on inhaled sildenafil, or a combination of the two (**chapter 4.3**). As a consequence, there were significant and relevant differences in the baseline characteristics of the treatment groups. Missing data are also a problem. Despite the fact that initial monotherapy (upfront) with sildenafil substantially improved pulmonary hemodynamics, survival was shorter than in the iloprost upfront group. We have no clear explanation for this fact, but the difference in baseline characteristics may be a factor. Selection bias may play a role here too. Sildenafil became available some years after inhalation of iloprost was available for PH patients, so only those patients on inhaled iloprost who survived until the availability of iloprost were included in the study. Confounding by indication may also be an issue. The more severely affected persons may have received one of the treatments over the other or may have received earlier treatment with combination therapy.

Implications and future steps

Overall the findings of this thesis contribute to the understanding of PH. First, in unselected, elderly volunteers living in northwestern Europe the overall prevalence of ePH seems to be low and second, patients with COPD, diabetes or LV systolic or diastolic dysfunction have higher prevalence of ePH than their non-diseased counterparts, although the association was not significant for diabetes. Therefore, our data suggest that patients with these diseases may benefit from careful evaluation for PH and we may have to consider more aggressive treatment and prevention of underlying diseases. In aging populations, increasing the prevalence of LV disease and COPD may cause a rise in the prevalence of ePH, or subclinically increased ePASP, in future years. In the literature, an increased PASP has been associated with an increased mortality in the general population, both in the full population and in people free of cardiopulmonary disease.[65] As a population-based study with detailed follow-up information, the Rotterdam Study is ideal for further evaluation of the association between an increased PASP and different outcomes. Mortality would be a clear primary outcome to analyze. In addition, the unique setting of the Rotterdam Study makes it possible to study associations of PASP or right heart function with function and dysfunction of many further organ systems. Of particular interest might be the association of right heart function or failure with the development of neurological or cognitive disorders, through lowered cerebral perfusion, hypoxemia and resulting tissue hypoxia. In addition, backward congestion to the intestine that may occur in poor right heart function may lead to liver, gastro-intestinal, or endocrine disease. [39]

Besides prevalence, incidence of PH in the general population may also be of interest, especially if we could identify factors associated with a new diagnosis of PH. To study this in the Rotterdam Study, we would have to follow participants with a normal PASP at the first right heart evaluation over time to study if they develop PH. The wide range of information collected in the Rotterdam Study would then allow us to study associated factors. This could be done on a phenotypic level including anthropometric data (e.g. body mass index), systemic circulatory measures (e.g. systemic pressures, oxygen pulse, breathing rate), lung function test results, or metabolic measures such as HbA1c. Also, the genome-wide data on single-nucleotide polymorphisms (SNPs) collected in the Rotterdam Study could be associated with the echocardiographic data, preferably in large collaborations with other similar studies. This approach has already been successfully followed for the left heart. [66] Additionally, French registry data show that in patients with PAH genome-wide association studies (GWAS) can successfully reveal SNPs with potential pathophysiologic relevance. [67]

Reference values for left heart echocardiography are established.[68] For right heart echocardiography the guidelines of the European Society of Cardiology and the American Heart Association report values for which limited published evidence exists according to the authors of those guidelines.[69] Reference values for right atrial dimensions have been published by Gruenig et al. [70] This publication takes sex into account, but not age, ethnicity, or body surface area. Standardization to a measure of body size, such as body surface area, is especially important for echocardiographic measurements. Age and body surface are relevant on the patient level to improve the prognostic relevance of right heart measures, but for the general population these factors may also be important to differentiate patients with normal values from those with pathologies or those at increased risk of disease. Population-based data such as those from the Rotterdam Study can provide important information for such reference values.
Previous work showed that patients with HFpEF had a milder degree of RV systolic dysfunction compared with those with HFrEF. [71] In our analysis of the Rotterdam Study population, to date we have not analyzed the association parameters of LV dysfunction and RV function. Future studies could focus on comparing parameters of LV dysfunction to TAPSE, S´, RV diameter, RV ejection and contraction times, and right atrial size as an indirect measure for right ventricular congestion. This would give further insight into the association with RV function rather than PASP with left heart disease.

A major advantage of the Giessen PH registry is the link to thousands of blood samples drawn from the patients at time of diagnosis and at each follow up right heart catheterization. This enables us to try and answer uncountable research questions using high-throughput techniques including proteomics, metabolomics, and lipidomics, and also study in detail the genetic and epigenetic background of pulmonary hypertension. Such studies may help discover new biomarkers for diagnosis and prognosis and help to guide treatment decisions. A large global database is critical to gain the data needed to determine the intra- and inter-patient genetic and environmental susceptibilities and differences between PH etiologies. A single-center approach has inherent advantages, namely homogeneity of data quality and consistency of standards and procedures. Both types of registries have their advantages and should exist in parallel. Depending on the research question the optimal source of data and trial design should be utilized. As the population ages, so will the population of patients with PH and, with that, the number of comorbidities will rise. This may also be true for our cohort but the finding has not been analyzed, yet. It would be of major interest to see the differences in comorbidities between the etiologic groups. Also the impact of comorbidities on adverse drug reactions could be analyzed and could lead to more effectively personalized treatment.

The association of HbA1c and right heart parameters or outcomes could be evaluated on a population level. One possible protocol could include the measurement of HbA1c and PASP at one scheduled follow-up visit of the Rotterdam Study. This cross-sectional approach has the advantage of earlier completion of data. In a follow-up assessment the change in PASP and HbA1c over time could be analyzed.

Diabetes mellitus and AGM are highly prevalent in the general population and are further increasing. [72] The association of manifest DM with the prevalence of ePH has been shown in **chapter 2.1**. However, that association was not significant, possibly in part due to limited power. Increasing the physical fitness of the general population could lower the prevalence of DM and with that, potentially, the prevalence of PH, if the association is causal. [72]

Personalized medicine is offering hope for patients to receive better treatment with lower risk of unintended drug effects. [73] For pulmonary hypertension, acute vasodilator testing has been found to predict long-term response to oral calcium channel blockers in 1992. [50] Since that landmark result, no further advances for patients with PH with regard to personalized treatment have found their way into daily clinical practice and patient treatment.[8] With our results on acute hemodynamic testing and follow-up of the patients with drug therapy we open the door for further steps to personalized medicine. We could combine the acute hemodynamic response (either in echocardiography or with right heart catheterization) with the follow-up data to predict outcome on the one hand but also gain further insight in the individual response to a specific drug treatment. This individual response could include benefits as well as unintended drug effects. Genetic and epigenetic information could be linked to hemodynamic and biomarker data to predict treatment response.

In conclusion, this thesis casts light on different aspects of pulmonary hypertension: from population cohort to the individual patient, providing insight into pathophysiology and thereby presenting advances in diagnosis and prognosis. The translational approach may aid in taking further steps into better understanding of the disease and optimized and personalized treatment for these patients. General screening in the elderly population is not warranted. However, in patients with underlying cardiac or pulmonary diseases, possibly enriched by the presence of symptoms like dyspnea, further population-based studies may provide valuable information to better identify populations at risk for the development of PH who might benefit from early diagnosis and treatment.

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Summary

Pulmonary hypertension (PH) is a disease of the pulmonary vasculature with high morbidity and mortality in the affected individuals. We carried out research in two different populations: the Rotterdam Study, which is a population-based cohort study, and the Giessen PH registry, a patient population. In the present thesis we aimed to elucidate the prevalence of PH and its associated factors in the general population. We also studied improvement of prognostic and diagnostic evaluation in our patient population by means of biomarkers and exercise testing, and we analyzed the effects of combination therapy and personalized treatment approaches.

The participants of the Rotterdam Study had a mean age of 76.4 years. The prevalence of PH, measured by echocardiography, in this elderly Rotterdam population was 2.6%. We identified associated factors for PH in this population. Among the common diseases, COPD and left heart disease were associated with the highest prevalences of PH (**chapter 2.1**). Among parameters of left heart function and morphology, measures of diastolic function and left atrial diameter showed the strongest associations with pulmonary artery systolic pressure (PASP), measured by echocardiography (**chapter 2.2**).

The Giessen PH registry comprises the largest single center registry and is linked to a biobank. Wepresented baseline and survival data in **chapter 3.1**. Patients in our registry had similar baseline characteristics to those in other national registries in terms of age, six-minute walking test, New York Heart Association functional class, female-to-male-ratio, and hemodynamic parameters. Several prognostic markers were evaluated across all etiologic groups.

A definite diagnosis of PH requires invasive hemodynamic evaluation by means of right heart catheterization. This invasive evaluation carries risks and costs and is in some cases done for exclusion of the disease only. We aimed to find a diagnostic marker for PH to rule out or diagnose PH without invasive tests. In **chapter 3.2**, we found that the biomarkers soluble vascular endothelial growth factor receptor 1 and placental growth factor when analyzed together provide a relatively high diagnostic accuracy. This possibly enables us to obtain diagnostic information earlier in the long process from symptoms via screening to a definite diagnosis (chapter 3.2).

Previous work has shown pathophysiological links between PH and altered glucose metabolism (AGM). AGM and PH were linked to pathophysiological changes such as right heart congestion. Also, changes in signaling pathways, such as the bone morphogenic protein pathway, may play a role. We described the association of AGM with prognosis in patients with PH in **chapter 3.3**. Patients with PH who had an AGM as defined by an

HbA1c above the median had a 3.9 times increased mortality as compared to those with an HbA1c below the median.

The concept of right ventricular contractile (RV) reserve is discussed in **chapter 4.1**. The increase in PASP upon physiological exercise is introduced as a possible measure of RV contractile reserve. An increase in PASP below the median was associated with an increased risk of mortality in a prospective cohort of patients with PH.

In **chapter 4.2** we compared the acute vasoreactivity of patients with PH in response to inhaled nitric oxide and oral sildenafil. Both agents were shown to be of similar prognostic value. Nitric oxide is known to guide therapeutic decisions. We were able to show that sildenafil may also have the potential to personalize therapy.

Combination therapy of oral sildenafil with inhaled iloprost was evaluated in **chapter 4.3**. Sequential addition of a second drug on top of the first can improve functional class, pulmonary hemodynamics, and exercise capacity irrespective of the order in which these are given. Iloprost as a first therapy with later add-on of sildenafil shows the best long term survival in our retrospective uncontrolled study.

The main findings of this thesis are reviewed and discussed in detail in **chapter 5**, as well as relevant methodological issues.

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Samenvatting

Pulmonale hypertensie (PH) is een aandoening van de bloedvaten in de longen die gepaard gaat met een hoge morbiditeit en mortaliteit. Wij hebben deze aandoening onderzocht in twee verschillende groepen: het ERGO onderzoek (Erasmus Rotterdam Gezondheid Onderzoek of de "Rotterdam Study"), een populatie-gebaseerde cohortstudie, en de Giessen PH registry, een patiëntenpopulatie. Het doel van dit proefschrift was om de prevalentie van PH in de algemene bevolking te bestuderen, evenals de factoren die geassocieerd zijn met PH. Daarnaast hebben we in de patiëntenpopulatie onderzocht of het stellen van de diagnose en het inschatten van de prognose verbeterd zouden kunnen worden door het gebruik van biomarkers en inspanningstesten en we hebben de effecten geanalyseerd van combinatietherapie en "personalized" (gepersonaliseerde) behandelingen.

De deelnemers aan het ERGO onderzoek waren gemiddeld 76,4 jaar oud. De prevalentie van PH, gemeten met echocardiografie, in deze oudere Rotterdamse populatie was 2,6%. In het onderzoek hebben wij factoren gevonden die geassocieerd zijn met PH in deze populatie. Chronisch Obstructieve Longziekten (Chronic Obstructive Pulmonary Disease, COPD) en linkszijdige hartziekten gingen gepaard met de hoogste prevalenties van PH (**hoofdstuk 2.1**). Van de metingen van hartfunctie en hartstructuur hadden diastolische functie en diameter van het linker atrium de sterkste associaties met pulmonaal arteriële systolische druk (pulmonary artery systolic pressure, PASP), gemeten met echocardiografie (**hoofdstuk 2.2**).

De Giessen PH registry is het grootste register van PH patiënten uit één centrum en is gekoppeld aan een biobank. In **hoofdstuk 3.1** beschrijven we kenmerken van de populatie en gegevens over de overleving. Patiënten in dit register waren vergelijkbaar met patiënten in andere nationale registers wat leeftijd, zes minuten wandeltest (six-minute walking test), New York Heart Association functionele klasse, de man-vrouw-verhouding en hemodynamische parameters betreft. Wij hebben een aantal prognostische factoren bestudeerd in alle etiologische groepen van patiënten met PH.

Een definitieve diagnose van PH kan alleen gesteld worden met invasieve hemodynamische diagnostiek, via een rechtszijdige hartcatheterisatie. Dit invasieve onderzoek gaat gepaard met risico's en kosten en wordt soms alleen gedaan om de diagnose PH uit te sluiten. Wij wilden een diagnostische marker voor PH vinden die PH zou kunnen uitsluiten of aantonen zonder het gebruik van een invasieve test. In **hoofdstuk 3.2** hebben we laten zien dat de biomarkers soluble vascular endothelial growth factor receptor 1 en placental growth factor samen een relatief hoge diagnostische nauwkeurigheid hebben. Dit zou het mogelijk kunnen maken om al eerder diagnostische informatie te verkrijgen in het lange proces van symptomen via diagnostiek naar een uiteindelijke diagnose.

Eerder onderzoek heeft pathofysiologische verbanden laten zien tussen PH en veranderingen in het glucosemetabolisme (altered glucose metabolism, AGM). AGM en PH gaan allebei gepaard met pathofysiologische veranderingen zoals rechtszijdige stuwing van het hart. Mogelijk spelen ook dezelfde onderliggende mechanismen een rol, zoals veranderingen in het bone morphogenic protein pathway. In **hoofdstuk 3.3** beschrijven we de associatie van AGM met de prognose van patiënten met PH. Patiënten met PH die een AGM hadden, gedefinieerd als een HbA1c boven de mediaan, hadden een 3.9 keer verhoogde sterfte vergeleken met patiënten met een HbA1c onder de mediaan.

Het concept van contractiele reserve van de rechter hartkamer wordt besproken in **hoofdstuk 4.1**. Wij introduceren de toename in PASP in reactie op inspanning als een mogelijke maat van de contractiele reserve van de rechter hartkamer. Een toename in PASP onder de mediaan was geassocieerd met een verhoogd risico op sterfte in een prospectief cohort van patiënten met PH.

In **hoofdstuk 4.2** hebben we de acute vasoreactiviteit van patiënten met PH in reactie op inhalatieve stikstofmonoxide vergeleken met de acute vasoreactiviteit in reactie op orale toediening van sildenafil. Beide medicamenten hadden een vergelijkbare prognostische waarde. Stikstofmonoxide wordt al gebruikt om beslissingen te nemen over de behandeling. Wij hebben nu laten zien dat sildenafil mogelijk ook de behandeling meer op de individuele persoon zou kunnen afstemmen.

Hoofdstuk 4.3 beschrijft combinatietherapie met orale sildenafil en inhalatieve iloprost. Het toevoegen van een tweede medicament bovenop het eerste kan de functionele klasse verbeteren, evenals de pulmonale hemodynamiek en het inspanningsvermogen. Het maakt hierbij niet uit in welke volgorde de medicijnen gegeven worden. Iloprost als eerste behandeling met het daarna toevoegen van sildenafil gaf de beste lange-termijn overleving in onze retrospectieve studie zonder controlegroep.

De belangrijkste bevindingen van dit proefschrift worden beschreven en bediscussieerd in **hoofdstuk 5**, evenals relevante methodologische aspecten.

P I

List of Publications

Chapter 2.1

Moreira EM, **Gall H**, Leening MJ, Lahousse L, Loth DW, Krijthe BP, Kiefte-de Jong JC, Brusselle GG, Hofman A, Stricker BH, Ghofrani HA, Franco OH, Felix JF. Prevalence of Pulmonary Hypertension in the General Population: The Rotterdam Study. PLoS One. 2015 Jun 23;10(6):e0130072.

Chapter 2.2

Billar RJ, Leening MJG, Merkus D, Brusselle GG, Hofman A, Stricker BHCh, Ghofrani HA, Franco OH, **Gall H***, Felix JF*. Left Ventricular and Left Atrial Echocardiographic Measures and Pulmonary Arterial Pressure in the General Population: the Rotterdam Study. *Submitted*
* *equal contributions*

Chapter 3.1

Gall H, Felix JF, Schneck FK, Milger K, Sommer N, Voswinckel R, Franco OH, Hofman A, Schermuly RT, Weissmann N, Grimminger F, Seeger W, Ghofrani HA. The Giessen Pulmonary Hypertension Registry: survival in pulmonary hypertension subgroups. *Submitted*

Chapter 3.2

Tiede SL*, **Gall H***, Dörr O, Troidl C, Liebetrau C, Voss S, Voswinckel R, Schermuly RT, Seeger W, Grimminger F, Zeiher AM, Dimmeler S, Möllmann H, Hamm CW, Ghofrani HA, Nef HM. New Potential Diagnostic Biomarkers for Pulmonary Hypertension. Eur Respir J. 2015 Nov;46(5):1390-6
* *equal contributions*

Chapter 3.3

Belly MJ, **Tiede H**, Morty RE, Schulz R, Voswinckel R, Tanislav C, Olschewski H, Ghofrani HA, Seeger W, Reichenberger F. HbA1c in pulmonary arterial hypertension – a marker of prognostic relevance? J Heart Lung Transplant. 2012 Oct;31(10):1109-14.

Chapter 4.1

Grünig E*, **Tiede H***, Enyimayew EO, Ehlken N, Seyfarth HJ, Bossone E, D'Andrea A, Naeije R, Olschewski H, Ulrich S, Nagel C, Halank M, Fischer C. Assessment and prognostic relevance of right ventricular contractile reserve in patients with severe pulmonary hypertension Circulation. 2013 Oct 29;128(18):2005-15.
* *equal contributions*

Chapter 4.2

Milger K, Felix JF, Voswinckel R, Sommer N, Franco OH, Grimminger F, Reichenberger F, Seeger W, Ghofrani HA, **Gall H**. Sildenafil vs Nitric Oxide for Acute Vasodilator Testing in Pulmonary Arterial Hypertension. Pulm Circ. 2015 Jun;5(2):305-12.

Chapter 4.3

Gall H, Sommer N, Milger K, Richter MJ, Voswinckel R, Seeger W, Grimminger F, Ghofrani HA. Survival with sildenafil and inhaled iloprost in a cohort with pulmonary hypertension. *Accepted BMC Pulmonary Medicine*.

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P |

PhD Portfolio

Name of PhD student	Henning Gall
Erasmus MC department	Epidemiology
PhD period	August 2010 – November 2016
Promotores	Prof. Dr. Oscar H. Franco and Prof. Dr. H. Ardeschir Ghofrani
Copromotor	Dr. Janine F. Felix

Training

Courses and workshops Master Program Health Sciences, Specialization Clinical Epidemiology, NIHES	***ECTS***

2009-2010

Principles of Research in Medicine (ESP01)	0.7
Clinical Decision Analysis (ESP04)	0.7
Methods of Clinical Research (ESP10)	1.4
Clinical Trials (ESP14)	0.7
Genome Wide Association Analysis (ESP29)	1.4
Conceptual Foundation of Epidemiology Study Design (ESP38)	0.7
Case-control studies (ESP40)	0.7
Principles of Genetic Epidemiology (ESP43)	0.7
Introduction to Decision-making in Medicine (ESP49)	0.7
Topics in Health and Diseases un the Elderly (ESP56)	0.7
Markers and Prognostic Research (ESP62)	0.7

Core Curriculum

Study Design (CC01)	4.3
Classical Methods for Data-analysis (CC02)	5.7
Clinical Epidemiology (CE02)	5.7
Methodologic Topics in Epidemiologic Research (EP02)	1.4
Modern Statistical Methods (EP03)	4.3

Advanced Short Courses

Introduction into Clinical Research (EWP01)	1.4
Pharmaco-epidemiology and Drug Safety (EWP03)	1.4
Advanced Topics in Clinical Trials (EWP10)	0.7
Advanced Analysis of Prognosis Studies (EWP13)	0.7
Prognosis Research (EWP16)	0.7
Principles of Epidemiologic Data-analysis (EWP25)	0.7

Skills Courses

English Language (SC01)	1.4
Working with SPSS for Windows (SC04)	0.15
A first glance at SPSS for Windows (SC05w)	0.15

Attended conferences	Year	ECTS
European Respiratory Society Annual Conference, Vienna, Austria Poster: An international survey of current pulmonary arterial hypertension (PAH) management	2012	1.0
Poster: Screening for Biomarkers in Pulmonary Hypertension	2013	1.0
European Respiratory Society Annual Conference, Barcelona, Spain Poster: Evaluation of Angiopoietin-2 and Thrombomodulin as Biomarkers for Pulmonary Hypertension		
3rd Systemic Sclerosis World Congress, Rome, Italy Poster: Autoantibodies targeting angiotensin type 1 and endothelin type A receptors as biomarkers and mediators of systemic sclerosis associated pulmonary arterial hypertension	2014	0.6
European Respiratory Society Annual Conference, Amsterdam, Netherlands Poster: Subtle assessment of quality of life in PH patients on inhaled iloprost treatment	2015	1.0
Annual Conference of the German Society of Cardiology, Mannheim, Germany Oral presentation: Current and future therapies for pulmonary hypertension (Aktuelle und zukünftige Studien bei pulmonaler Hypertonie)	2015	0.8
Oral presentation: Pulmonary hemodynamic response to exercise in chronic thromboembolic hypertension		

Invited Lectures	Year	ECTS
European mechanical circulatory support summit 2014, Bad Oeynhausen, Germany	2014	1.0
German Society of Internal Medicine (Deutscher Internistentag), Wiesbaden, Germany, 2013	2013	1.0

Teaching

Training of echocardiographists (courses, handouts, practical training), PHup2Date, Munich, Germany	2009 – to date	1.0
Seminars for Medical Students about Pulmonary Hypertension	2010 – to date	1.0
Supervision of Medical Doctorate Candidates	2006 - to date	34.0

Parasie, Brita *(Lebensqualität bei Patienten mit Lungenhochdruck*, 2010) Matheis, Christina (*Einfluss der Sildenafil-Langzeittherapie auf die akute Vasoreaktivität*, 2012)
Müller, Henning (*Prädiktion des Sildenafil-Langzeittherapie-Effekts durch akute Vasoreaktivitäts-Testung*, 2012)
Hecker, Franziska (*Überleben der Giessener Lungenhochdruck-Patienten*, 2013)
Menkel, Vera (*Klinischer Verlauf unter inhalativen Ventavis - aktuelle Aspekte*, 2013)
Meier, Julia (*Ein neuer Biomarker zur Diagnose des Lungenhochdrucks* (Biosphere II), 2015)
Janina Heil (*Einfluss der BMPR2-Mutation auf den Krankheitsverlauf der Pulmonalen Hypertonie*, 2015 submitted)
Strauss, Burkhardt (*Echokardiographie in Pulmonaler Hypertonie*, ongoing) Kurz, Fabian (*RV-Funktion Vergleich der Mess-Methoden*, submitted) Thomas Schmidt, Christoph Kempf, Katrin Christ, Insa Randaxhe, Matthias Wassenberg, Dennis Funk, Matthias Maerz, Sebastian Herpel (*Biomarker bei pulmonaler Hypertonie*, ongoing)

Other

Peer review of articles for scientific journals (European Respiratory Journal, Pulmonary Circulation)	2013- to date	0.5

A |

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C |

Curriculum Vitae

Henning Gall was born in Luenen, Germany, on 25th July 1975. He graduated from Giessen Medical School in 2003. From 2003 to 2005 he took part in the international graduate programme “Molecular Biology and Medicine of the Lung” at the Justus Liebig University, Giessen.

Since 2003 he has been working at the Department of Internal Medicine of the University of Giessen, Head Professor W. Seeger, and currently holds a consultant position there. He obtained his doctoral degree in medicine, and the specializations in internal medicine and pulmonary medicine.

Henning participated in the Master of Science programme in Clinical Epidemiology at the Netherlands Institue of Health Sciences. He conducted research at the department of epidemiology under the supervision of Prof.dr. Jacqueline Witteman and dr. Janine Felix. The research focused on the mortality of patients with pulmonary hypertension. He expanded his research project in his current PhD-project to various aspects of the health and disease of the pulmonary vasculature, including epidemiology, prognosis, biomarkers, and therapeutic consequences. It is a joint PhD at the universities of Rotterdam and Giessen under the supervision of Prof.dr. Oscar Franco and Prof.dr. Ardeschir Ghofrani.