Pulmonary hypertension in COPD with focus on pulmonary function and exercise response

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PhD thesis

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Contents

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Summary

Chronic obstructive pulmonary disease (COPD) is one of the most common causes of pulmonary hypertension, and the combined diagnosis is associated with increased morbidity and mortality. Right heart catheterization is necessary to confirm the diagnosis of pulmonary hypertension, but this invasive procedure cannot be performed in the entire COPD population. The present thesis discusses the possibility of identifying COPD patients at risk of developing pulmonary hypertension when they visit pulmonary outpatient clinics where these patients usually attend regular controls. The use of exercise tests to reveal pathology at an early stage of the disease is thoroughly discussed.

Four papers are included in this thesis. All papers are based on studies in a cohort of 100 COPD patients with varying degrees of airway obstruction who were included in a cross sectional study. Patients with pathology affecting left ventricle were excluded to avoid post-capillary contribution to pulmonary artery pressure, as pre-capillary pulmonary hypertension was the main focus.

Paper I addresses the prevalence of pulmonary hypertension in the COPD outpatient population and describes results from right heart catheterization at rest and during exercise. Patients with normal hemodynamics at rest who showed pathological response during exercise were defined as having exercise induced pulmonary hypertension. In paper II we applied tests available at pulmonary outpatient clinics in order to identify the patients diagnosed with pulmonary hypertension. In paper III we applied results from cardiopulmonary exercise test in order to identify patients diagnosed with pulmonary hypertension. In Paper IV we applied results from cardiopulmonary exercise test in order to identify patients with exercise induced pulmonary hypertension. All papers relate to the same context; early diagnosis of pulmonary hypertension in COPD from the perspective of a pulmonary phycisian.

Pulmonary hypertension was present in 27% of the study population. When COPD patients were classified in GOLD stages (Global Initiative for Chronic Obstructive Lung Disease) by the severity of airway obstruction, there was an increasing prevalence of pulmonary hypertension with increasing COPD severity; pulmonary hypertension was observed in 5, 27 and 53% in GOLD stages II, III and IV, respectively. Exercise induced pulmonary hypertension, defined as mPAP<25 mmHg at rest and ΔmPAP/ΔCO slope≥3 mmHg/L/min, was observed in 58% of the patients with normal hemodynamic measurements at rest.

When measurements feasible at the pulmonary outpatient clinic were applied, the importance of PaO_2 was highlighted, as PaO_2 was the only significant predictor of mean pulmonary artery pressure, the variable that defines pulmonary hypertension at rest. PaO_2 below 9.5 kPa at rest

combined with PaO_2 below 8.5 kPa at peak exercise predicted pulmonary hypertension with a detection rate of 76% and a false positive rate of 24%.

When cardiopulmonary exercise test was performed, patients with pulmonary hypertension could be identified by their lower work load and PaO_2. As there were significant differences in work load, we also evaluated the entire course of exercise and observed interesting physiological differences between COPD patients with and without pulmonary hypertension.

When cardiopulmonary exercise test was applied in order to identify COPD patients with exercise induced pulmonary hypertension, we were not able to distinguish between those patients and patients with normal hemodynamic responses at rest and during exercise. However, when we considered the entire course of exercise, there were physiological differences between the groups.

We conclude that it is possible to identify the majority of COPD patients with pulmonary hypertension at the pulmonary outpatient clinics; however a definite diagnosis of pulmonary hypertension can only be obtained by right heart catheterization. We hope that the results provided in this thesis can be helpful for colleagues in evaluation of their COPD patients.

This thesis has provided important information about hemodynamic dysfunction during exercise in COPD patients. The definition of exercise induced pulmonary hypertension was changed during the inclusion period, allowing us to take part in the scientific discussion about the re-definition of exercise induced pathology.

List of abbreviations

AT: anaerobic threshold

CO: cardiac output

COPD: chronic obstructive pulmonary disease

CPET: cardiopulmonary exercise test

*D*LCO: diffusing capacity of the lung for carbon monoxide

EF: ejection fraction

FEV_1: forced expiratory volume in one second

FVC: forced vital capacity

GOLD: Global Initiative for Chronic Obstructive Lung Disease

IC: inspiratory capacity

LMM: linear mixed models

LV: left ventricle

MAP: mean arterial pressure

mPAP: mean pulmonary artery pressure

MVV: maximal voluntary ventilation

*P*aO_2: partial pressure of arterial oxygen

*P*aCO_2: partial pressure of arterial carbon dioxide

PAWP: pulmonary artery wedge pressure

PH: pulmonary hypertension

PP: pulse pressure

PVR: pulmonary vascular resistance

RHC: right heart catheterization

ROC: receiver operating characteristic (curves)

RV: residual volume

RVF: right ventricle failure

SaO_2: arterial oxygen saturation

TLC: total lung capacity

TPR: total pulmonary resistance

W: watt

WU: Wood units

List of papers

This thesis is based upon the following original research papers, which are referred to in the text by their Roman numerals:

Paper

I Hilde JM, Skjørten I, Hansteen V, Melsom MN, Hisdagl J, Humerfelt S, Steine K.
Haemodynamic responses to exercise in patients with COPD.
Eur Respir J 2013; 41: 1031-41

II Skjørten I, Hilde JM, Melsom MN, Hansteen V, Steine K, Humerfelt S.
Pulmonary artery pressure and *P*aO_2 in chronic obstructive pulmonary disease.
Respir Med 2013; 107: 1271-1279

III Skjørten I, Hilde JM, Melsom MN, Hisdal J, Hansteen V, Steine K, Humerfelt S.
Cardiopulmonary exercise test and *P*aO_2 in evaluation of pulmonary hypertension in COPD.
Int J Chron Obstruct Pulmon Dis 2018; 13: 91-100

IV Skjørten I, Hilde JM, Melsom MN, Hisdal J, Hansteen V, Steine K, Humerfelt S.
Exercise capacity in COPD patients with exercise induced pulmonary hypertension.
Manuscript submitted December 2017

1 Background

1.1 COPD

1.1.1 Epidemiology

Chronic Obstructive Pulmonary Disease (COPD) has an estimated global prevalence of 11.7% (1). The prevalence is expected to rise over the next years due to increased tobacco consumption in developing countries (2). Estimated prevalence in Norway is approximately 5% (3). COPD is associated with substantial morbidity and mortality, and it is one of the leading causes of death in most countries (2). In 2011 COPD was the third leading cause of death in the United States, and the same has been observed in Norway (2). COPD progresses slowly and patients need medical treatment for many years. This induces an economic burden (4), as well as the burden of the disease itself experienced by the patients.

1.1.2 Diagnosis and assessment

Global Initiative for Chronic Obstructive Lung Diseases (2) defines COPD as a common, preventable and treatable disease that is characterized by persistent respiratory symptoms and airflow limitation due to airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases (2). Tobacco smoking is the most commonly encountered risk factor for COPD worldwide. Other risk factors include indoor air pollution from biomass fuel, occupational exposure of organic and inorganic dusts, outdoor air pollution, and genetic factors such as alpha-1-antitrypsin (2). Spirometry is essential to diagnose COPD, as airflow limitation is characterized by a low forced expiratory volume in one second (FEV_1) compared to the total forced expiratory volume (FVC) with $FEV_1/FVC<0.7$. Airflow limitation in COPD is not fully reversible and persists after bronchodilator therapy. Spirometric measurements are expressed as percentage of predicted values, and the reference values are based on gender, age, height and ethnicity (2). GOLD classification by spirometric values defines four stages of COPD severity. At the time of study inclusion, partial pressure of arterial oxygen (PaO_2) and partial pressure of arterial carbon dioxide ($PaCO_2$) were also considered in the classification (5) (Table 1). More recently, assessment of symptoms and exacerbations in the ABCD risk score have been emphasized (2).

Table 1

Spirometric classification of chronic obstructive pulmonary disease severity based on post-bronchodilator FEV_1 from 2007-2011

Stage I: mild	$FEV_1/FVC<0.70$
	$FEV_1 \geqslant 80\%$ predicted
Stage II: moderate	$FEV_1/FV <0.70$
	$50\% \leqslant FEV_1 < 80\%$ predicted
Stage III: severe	$FEV_1/FVC<0.70$
	$30\% \leqslant FEV_1 < 50\%$ predicted
Stage IV: very severe	$FEV_1/FVC<0.70$
	$FEV_1<30\%$ predicted *or* $FEV_1<50\%$ predicted plus chronic respiratory failure*

*Respiratory failure: arterial partial pressure of oxygen (PaO_2)<8.0 kPa with or without arterial partial pressure of CO_2 ($PaCO_2$)>6.7 kPa while breathing air at sea level. FEV_1: forced expiratory volume in 1 second. FVC: forced vital capacity. Adapted from (5), Rabe, 2007.

COPD should be considered in patients with relevant exposure who present with cough, sputum production and /or dyspnea (2). Pathological changes occur in the airways, lung parenchyma and pulmonary vasculature. Narrowing of the smaller airways, mucus hypersecretion and ciliary dysfunction all contribute to the symptoms. Destruction of lung parenchyma causes emphysema by an inflammatory process where there is imbalance between proteinases and proteinase inhibitors, as well as negative effects of oxidative stress (6). The loss of alveoli reduces the surface for alveolar-capillary gas exchange, as measured by low diffusing capacity for carbon monoxide (*D*LCO). The loss of alveolar attachment reduces the lumen in the small airways even more. The loss of elastin in alveolar tissue leads to impaired passive recoil of the expanded lung and the airways tend to collapse before expiration is completed. As a consequence of this air trapping, there is an increased residual volume in the lungs at end of expiration (7).

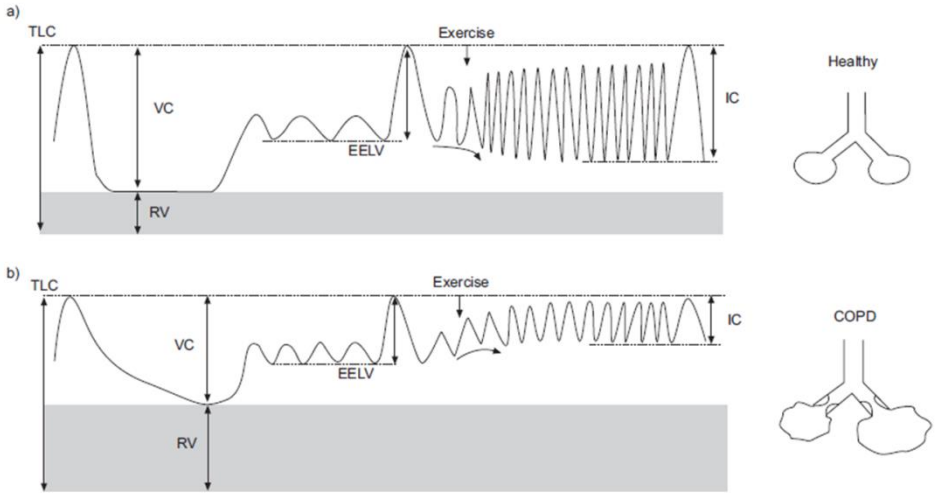
1.1.3 COPD and exercise

COPD patients present with increasing dyspnea and exercise limitation as the disease progresses. Exercise tolerance is reduced due to ventilatory limitation, gas exchange abnormalities, and

deconditioning. Ventilatory exercise limitation in COPD is caused by flow limited inability to increase minute ventilation and/or by dynamic hyperinflation. Dynamic hyperinflation, a temporary increase of operating lung volumes above the resting value, is frequent in COPD patients. The temporary increase of end expiratory lung volume (EELV) evolves when the airways collapse and obstruct before the inspired volume is fully exhaled, thus the inspired volume is a little larger than the expired volume during each respiratory cycle; gradually accumulating more hyperinflation. When EEVL increases, inspiratory capacity (IC) decreases, based on the assumption that total lung capacity (TLC) is constant during exercise. Very low IC is associated with intolerable dyspnea and exercise termination. Dynamic hyperinflation may increase intrathoracic pressure to an extent that affects heart function and pulmonary vasculature.

Figure 1

Dynamic hyperinflation



TLC: total lung capacity; VC: vital capacity; RV: residual volume, EELV: end-expiratory lung volume; IC: inspiratory capacity. Adapted from (8) O'Donnel, 2006

1.1.4 Comorbidity in COPD

COPD is associated with substantial comorbidity (9, 10). Having the same cause, other tobacco related diseases, like coronary artery disease and lung cancer, are frequent. COPD may induce systemic inflammation with resulting cachexia and muscular atrophy. Osteoporosis is associated with COPD due to effects of inflammation, immobility and corticosteroid treatment. Heart failure, diabetes and gastroesophageal reflux are common in COPD, as well as anxiety and depression. Pulmonary hypertension is a comorbidity that has considerable impact on prognosis (11-13) and early diagnosis of this complication is the main objective of this thesis.

1.2 Pulmonary hypertension

1.2.1 Diagnosis and assessment

Pulmonary hypertension (PH) is defined as mean pulmonary arterial pressure (mPAP)≥25 mmHg at rest as assessed by right heart catheterization (RHC) (14). When catheterization of the heart was introduced by Cournand and his colleagues in the 1940s, it was recognized that several diseases are associated with elevated pulmonary artery pressure (15, 16). Normal mPAP at rest is 14±3 mmHg with the upper limit of normal of 20 mmHg (14, 17, 18). The clinical significance of mPAP between 21-24 mmHg is unclear (17). In healthy individuals, there is no difference in mPAP related to gender or ethnicity (18). Age has a significant impact on mPAP at rest, as individuals above 50 years of age has mean mPAP 15±4 mmHg, compared to 13±3 mmHg for younger individuals (<0.001) (18).

Classification of PH categorizes multiple clinical conditions into five groups according to their similar pathological findings and hemodynamic characteristics. The classification was formalized in a consensus meeting in Dana Point in 2008 (19), and revised in a similar meeting in Nice in 2013 (20), see Appendix 1 for details:

1. Pulmonary arterial hypertension
2. Pulmonary hypertension due to left heart disease
3. Pulmonary hypertension due to lung diseases and/or hypoxia
4. Chronic thromboembolic pulmonary hypertension (CTEPH)
5. Pulmonary hypertension with unclear multifactorial mechanisms

Based on combinations of pulmonary artery pressure (PAP), pulmonary artery wedge pressure (PAWP) and cardiac output, we obtain hemodynamic definitions of pre- and post-capillary PH (17). Post-capillary PH is characterized by PAWP>15 mmHg, and represents left heart disease (21, 22). In the study this thesis is based upon, we have made an effort to exclude patients with left heart pathology to avoid any post-capillary contribution to PH. However, exercise may reveal diastolic dysfunction with larger than expected rise in PAWP, even in patients with normal PAWP at rest (23).

Table 2

Hemodynamic definitions of pulmonary hypertension

Definition	Characteristics	Clinical group
PH	mPAP≥25 mmHg	All
Pre-capillary PH	mPAP≥25 mmHg PAWP≤15 mmHg	1 Pulmonary arterial hyperension 3 PH due to lung diseases 4 Chronic thromboembolic PH 5 PH with unclear and/or multifactorial mechanisms
Post-capillary PH	mPAP≥25 mmHg PAWP>15 mmHg	2 PH due to left heart disease 5 PH with unclear and/or multifactorial mechanisms
Isolated post-capillary PH	DPG<7 mmHg and/or PVR≤3 WU	
Combined pre-capillary and post-capillary PH	DPG≥7 mmHg and/or PVR>3 WU	

PH: pulmonary hypertension; mPAP: mean pulmonary artery pressure, PAWP: pulmonary artery wedge pressure; DPG: diastolic pressure gradient (diastolic PAP-mean PAWP); PVR: pulmonary vascular resistance.All measurements at rest. Adapted from (24).

Within group 3 in the classification, pulmonary hypertension due to lung diseases and/or hypoxia, COPD is by far the most frequent diagnosis. PH in COPD is often mild/moderate (13) with slow progression <1 mmHg per year (25). Presence of even moderate PH is a strong predictor of mortality in COPD, and a five year survival rate of 36% has been reported for COPD patients with mPAP>25 mmHg (12). The prevalence of PH in COPD is not well established, and varies between different study populations (26-31). The prevalence increases with the severity of COPD, and in a study comparative to our, PH prevalence was 7% in GOLD 2, 25% in GOLD 3 and 22% in GOLD 4 (32). A small subgroup of COPD patients demonstrate severe PH defined as mPAP≥35 mmHg or mPAP≥25 mmHg combined with cardiac index (CI)<2.0 L/min/m^2 (33, 34). They represent approximately 1% of the COPD population (28, 31, 35). This group was formerly known as “out of proportion pulmonary hypertension in COPD” as their mPAP and pulmonary vascular resistance (PVR) is very high, whereas the airway obstruction is only moderate, indicating that the pathology is mainly related to vascular remodeling in a process similar to pulmonary arterial hypertension (PH classification group 1).

Hypoxic pulmonary vasoconstriction is a physiological mechanism that optimizes gas exchange to reduce ventilation/perfusion (V/Q) mismatch. Alveolar hypoxia induces vasoconstriction in pulmonary vessels, and when chronic in COPD, it causes remodeling of the small pulmonary muscular arteries and arterioles, increasing pulmonary artery pressure. This remodeling includes intimal hyperplasia and muscle hypertrophy. Remodeling of vessels has also been shown in smokers with normal partial pressure of oxygen (36), indicating that inflammatory mechanisms also contribute to PH in COPD patients. Both COPD patients and smokers without airway obstruction show increased number of inflammatory cells infiltrating the adventitia, mostly activated T lymphocytes (37). The remodeling process and inflammation are accompanied by endothelial dysfunction with reduced expression of endothelial nitric oxide synthase (38) and prostacyclin synthase (39), which further favors vasoconstriction and cell proliferation.

A different mechanism of increased mPAP in COPD is dynamic hyperinflation with positive alveolar pressure which compresses the alveolar capillaries (29) and thus increases mPAP.
The loss of capillary bed due to loss of lung parenchyma in emphysema also affects pulmonary hemodynamics (40). Reduction of the vascular bed leads to increased pressure in the remaining pulmonary vasculature, but not until as much as 50% of the vascular bed has been destroyed (41). The pulmonary vessels are distensible and with a small reduction of the vascular bed, the dilatation of the remaining vasculature counteracts the effects of the vanished vessels. When the capacity of further dilatation is exceeded, the pulmonary artery pressure starts to rise (16, 42).

Over the last years there have been advancements in treatment of pulmonary arterial hypertension (group 1) with specific vasodilating agents. Endothelin receptor antagonists, phosphodiesterase 5 inhibitors and prostacyclin analogs in mono- or combination therapy have led to improved survival and exercise tolerance in these patients. When we were planning the study of this thesis prior to 2007, there was ongoing research testing vasodilating agents in COPD patients. We were optimistic that PH in COPD could be candidate for targeted treatment, which gave us a rationale for identifying these patients. However, now there is evidence that vasodilating treatment in COPD patients may actually worsen prognosis and exercise capacity, as PH specific medication dilates the entire pulmonary vascular bed, even in the lung regions with low ventilation. This may increase V/Q mismatch and physiological shunting, resulting in lower PaO_2 and SaO_2 and, eventually, reduced exercise capacity (43, 44). Inhalation of nitric oxide in COPD patients, in order to increase perfusion in well ventilated pulmonary regions, did not improve PaO_2 (45). Although vasodilating agents may increase V/Q mismatch in COPD patients, according to the Cologne consensus Conference 2011 it is recommended to consider such medication in COPD patients with severe PH with mPAP≥35 mmHg or mPAP≥25 mmHg combined with cardiac index <2 L/min/m^2 or PVR>6 WU (34). A recent review article regarding PAH specific medication in COPD shows that there may be

some benefit after all for COPD patients with severe PH (46). Most US PH-centers give PAH specific medication to this group (47). In the future, one can hope for treatment that will reduce progression of the pathological remodeling of pulmonary vessels by affecting the inflammatory process at an early stage of the disease, rather than inducing general vasodilation at a later stage.

1.2.2 Exercise induced pulmonary hypertension

When the present study was designed prior to 2007, pulmonary hypertension was defined as mPAP≥25 mmHg at rest or mPAP≥30 mmHg during exercise as assessed by RHC (48). During the inclusion period, the definition was changed in 2008, and mPAP≥30 mmHg during exercise was omitted from the definition (19). The change in definition was based on a review study of hemodynamic profile in healthy individuals during exercise by Kovacs et al (18), concluding that healthy subjects above 50 years of age often reach mPAP≥30 mmHg. Several small studies were included in the review, as there are no large studies of hemodynamic exercise profiles in healthy individuals, due to the invasive character of RHC. This is much asked for, but due to risk of adverse events, such a study has not been performed for ethical reasons. This implies that the normal ranges for hemodynamic exercise variables are poorly defined, which makes it more difficult to evaluate pathology. There are different opinions on upper limit of normal for PAWP during exercise (18, 49, 50), which makes evaluation of diastolic dysfunction more complex. Regarding mPAP during exercise, there is an agreement that the former definition often failed, however, mPAP≥30 mmHg is still considered pathologic if cardiac output is <10 L/min (16). There has been an ongoing debate whether exercise induced pulmonary hypertension (EIPH) is a condition with clinical relevance (51-53). Among those who consider EIPH as precursor of PH, there are different opinions on how to express and evaluate pathology (54-57), but all agree upon a more complex evaluation of the pulmonary circulation during exercise, where composite variables are calculated. In healthy individuals, the change in mPAP is related to the work performed, as an increase in mPAP is driven by increased cardiac output (CO). Thus, the slope of ΔmPAP/ΔCO has been proposed to define EIPH, as a steep slope indicates pathology (58). A rise in mPAP during exercise beyond what is expected from CO reflects pathology due to reduced vessel distensibility or increased left atrial pressure (16), thus this definition does not discriminate between pre- and post-capillary EIPH.

The change in definition of PH during the study period gave us some challenges, but it also provided a possibility to join the scientific debate about EIPH, as our study included extensive RHC data during exercise. In our study we have chosen the slope of ΔmPAP/ΔCO to express exercise induced pathology and we have defined ΔmPAP/ΔCO slope>3 mmHg/L/min as cut-off for pathology.

As patients with left heart disease were excluded from the study, we consider the post-capillary contribution to ΔmPAP/ΔCO slope minimal.

1.2.3 Cor pulmonale

The term cor pulmonale represents enlargement and failure of the right ventricle caused by pulmonary disease with associated pulmonary hypertension. There are different definitions of cor pulmonale, but the following definition proposed by Weitzenblum has been widely used: “pulmonary arterial hypertension resulting from diseases affecting the structure and/or the function of the lungs; pulmonary arterial hypertension results in right ventricular enlargement (hypertrophy and/or dilatation) and may lead with time to right heart failure” (59).

Pulmonary hypertension increases the work of the right ventricle, which leads to right ventricular hypertrophy with impaired ventricular function. Later, dilatation and right heart failure (RHF) will occur (59, 60). RHF is associated with inability to augment right ventricle ejection fraction during exercise, thus dyspnea is a frequent symptom. Other signs of cor pulmonale include peripheral edema, pulsatile, tender hepatomegaly and elevated jugular venous pressure (60). Although RHF is associated with manifest pulmonary hypertension, our research group has documented that impaired right ventricle systolic function, hypertrophy, and dilation are present even at a slight increase of mPAP, which indicates an early impact on right ventricle function and structure in patients with COPD (61).

2 Study aims and research questions

Early detection of PH in COPD patients is the main objective of this thesis. From the perspective of a pulmonary physician, we have aimed to explore how PH in COPD can be recognized by applying tests readily available in pulmonary outpatient clinics. We have also aimed to explore how exercise influences hemodynamic and ventilatory responses in COPD patients. The present study has been performed in collaboration with cardiologists. The thesis by Janne Mykland Hilde, Pulmonary hypertension and ventricular function in chronic obstructive pulmonary disease (2014, ISBN 978-82-8264-890-5/Nr. 1865), explored different aspects of hemodynamic dysfunction in COPD patients from the perspective of a cardiologist, including echocardiographic assessment. Essential for both theses are the hemodynamic measurements from RHC, as they represent the gold standard for the diagnosis of PH, thus paper I is common for both theses.

2.1 Scientific issues addressed in the four papers

Paper I

•What is the prevalence of precapillary PH in COPD outpatients without left heart disease and comorbidity?

•What is the prevalence of precapillary PH related to GOLD stages?

•Do COPD patients without PH at rest exhibit pathological hemodynamic responses during exercise (EIPH)?

Paper II

•How do pulmonary function tests, arterial blood gases and heamodynamic measurements vary between the different GOLD stages?

•What are the significant predictors of mPAP and PaO_2 in COPD patients when considering measurements feasible at a pulmonary outpatient clinic?

•How can PaO_2 be used as a marker of PH in COPD patients?

Paper III

•How do COPD patients with and without PH perform during cardiopulmonary exercise test?

•Which CPET parameters differ between COPD patients with and without PH when we consider their different exercise levels?

•Can CPET with arterial blood gases discriminate between COPD patients with and without PH?

Paper IV

•Do patients with EIPH, defined as resting mPAP<25 mmHg and ΔmPAP/ΔCO slope>3 mmHg/L/min, perform differently during exercise compared to COPD patients with a normal slope?

•Which CPET parameters differ between COPD patients with EIPH and patients with normal hemodynamics when we consider their different exercise levels?

•Can CPET with arterial blood gases discriminate between COPD patients with and without EIPH?

3 Methods

3.1 Ethics committee approvals

The study protocol was approved by the Regional Committee for Research Ethics Eastern Norway (ref: 274-07127a 1.2007.1085), by the Norwegian Data Inspectorate and the Institutional review board at Oslo University Hospital, Aker. At screening, all potential participants were given an extensive description of the study. The exercise tests and invasive procedures were emphasized and explained in detail. Patients were reassured that participation was voluntary, and that refusing to participate would not influence standard care. Study participants were informed that they could withdraw from the study at any time without providing a reason. All included patients provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki (62).

3.2 Study design, population and sample size

An observational, cross-sectional, single center study was conducted at Oslo University Hospital, Aker. Between 2007 and 2010 we included 112 COPD patients with stable disease, representing the different GOLD stages (2007)(5) of airway obstruction severity.

The pulmonary department, the cardiologic department and the radiologic department collaborated on the study. Cardiologist Janne Mykland Hilde has described the same study population in her thesis “Pulmonary hypertension and ventricular function in chronic obstructive pulmonary disease” (2014), where hemodynamic dysfunction and echocardiographic assessment in COPD patients were explored.

Sample size calculation (Statistical solutions, LLC) was performed with mPAP as the primary outcome. We estimated that we needed to include 120 patients, 30 from each GOLD stage, given a power of 90% and a two-sided significance level of 5%. The calculations were based on mPAP of 35 mmHg in GOLD stage IV versus 20 mmHg in GOLD stage I with standard deviation (SD) of 10 mmHg. A difference in mPAP of 6 mmHg was considered clinically significant. A sample size of 30 in each GOLD stage was calculated based on these assumptions, and we expected that this number would be sufficient to demonstrate differences between patients with and without PH.

From the pulmonary out-patient clinic of Aker University Hospital 86 patients were recruited, this represents approximately 8% of the total population with a COPD diagnosis during the inclusion period. Altogether eight of the included patients were referred from other hospitals, ten patients

were referred from a non-hospital based pulmonary out-patient clinic, four patients were referred from general practitioners and four responded to written or oral information given to the public.

Table 3

Recruitment of patients

Institution	**Patients recruited (n)**
Aker University Hospital	86
Other hospitals*	8
Clinic for Allergy and Lung Diseases, Ullevål Stadion	10
General practitioners	4
Responders to written or oral information to the public**	4
Sum	112

*Includes Rikshospitalet (n= 5), Ullevål (n= 2) and Bærum (n=1) Hospitals.

**Includes Markveien legesenter (n=2), Skullerud sportssenter (n=1) and private acquaintance (n=1)

In addition to the 112 patients in our material, we included seven patients that were later excluded: Two patients withdrew their consents and two were diagnosed with lung cancer, while the others were diagnosed with alcoholism, interstitial pulmonary disease and tuberculosis sequela.

The four papers of this thesis describes the same population with a few exceptions, as number of participants were 98, 95, 93 and 93 in paper I, II, II and IV, respectively. Altogether 100 patients had successful RHC. GOLD stage I patients were not supposed to perform RHC, nevertheless, two patients, diagnosed as stage II at screening, turned out to be stage I at the test day. They were excluded from paper I and II, as those papers categorize participants by GOLD stage. In paper II-IV the two patients who performed coronary angiography were excluded as an extra caution to avoid presence of post capillary PH. One subject was excluded from paper II-IV as she was considered too hypoxemic at rest to perform CPET and peak exercise arterial blood gases. Paper III and IV included the two GOLD stage I patients, but excluded 4 patients (GOLD II n=3, GOLD III n=1) due to mask leak and other technical problems with the CPET registrations, see table 4 for details.

Table 4

Description of subjects excluded from paper I-IV

Subjects excluded	Paper I	Paper II	Paper III	Paper IV
GOLD I (n=2)	-	-	+	+
Coronary angiography (n=2)	+	-	-	-
Too hypoxemic to exercise (n=1)	+	-	-	-
CPET technical problem (n=4)	+	+	-	-
Total n=	98	95	93	93

3.3 Inclusion- and exclusion criteria

Inclusion criteria: COPD verified by spirometry in patients 40-75 years old. Patients had to be free from exacerbation the last two months prior to inclusion. Smokers and ex-smokers with at least 10 pack years of tobacco consumption could be included.

Exclusion criteria: Patients with asthma, pulmonary fibrosis or other acute or chronic pulmonary diseases were excluded from the study. Emphysema was not an exclusion criterion, unless it was combined with fibrosis. Patients with known sleep apnea syndrome were excluded, but we did not screen for this condition. Medical history of pulmonary embolism was reason for exclusion, as those patients may develop chronic thromboembolic pulmonary hypertension, but we did not screen for this. Patients with left ventricular (LV) disease, valvular heart disease, intracardiac shunts, arrhythmias including atrial fibrillation, were excluded, as those patients may have a postcapillary contribution to increases in mPAP. Patients with systemic arterial hypertension with blood pressure>160/90 mmHg despite treatment, were excluded for the same reason. Malignancy, renal failure with glomerular filtration rate <60 ml/min, systemic inflammatory diseases, hyperthyroidism or metabolic conditions except stable diabetes, were all exclusion criteria. Patients on beta-blockers, warfarin or clopidogrel were excluded, as were those unable to exercise on cycle ergometer.

3.4 Pulmonary screening procedures prior to inclusion

COPD patients at the Pulmonary outpatient clinic at Oslo University Hospital, Aker, were screened by one of three pulmonary physicians (Ingunn Skjørten, Morten Nissen Melsom and Sjur Humerfelt) in order to verify the COPD diagnosis and GOLD stage. Spirometry (Vitalograph Spirotrac IV, Ennis, Ireland) was performed according to the guidelines of European Respiratory Society (ERS) / American Thoracic Society (ATS) (63) with the patient sitting upright using a nose clip. Exhalation time was

minimum six seconds. The best of three acceptable forced expiration maneuvers was used for analysis. The laboratory technicians gave standardized instructions to all subjects and performed manual and automatic calibration procedures daily. Reversibility tests were performed with salbutamol aerosol 0.4 mg and spirometry repeated after 15 min. Thereafter ipratropiumbromide aerosol 80 ug was administered and spirometry was repeated 30 minutes after the last inhalation. Norwegian reference values were applied (Gulsvik 2001) (64). Relevant clinical examination was done at the time of screening, including chest radiograph to exclude parenchymal or pleural disease. Six minute walk test (6MWT) was used to assess functional status and mobility. At the time of screening, inhaled medication was optimized, see appendix 2 for details. Eligibility was evaluated according to the inclusion and exclusion criteria.

3.5 Cardiac screening procedures prior to inclusion

Patients who were considered eligible after screening by a pulmonary physician, were then screened by a cardiologist (Janne Mykland Hilde). Blood pressure measurements in triplets and resting electrocardiogram (ECG) were performed. Dynamic exercise test on cycle ergometer (Ergosana ERG911, Germany) was performed to exhaustion with a starting level of 30 W, increasing 10 W per minute. Horizontal or down-sloping ST-segment depression ≥1 mm was not observed, four patients experienced chest discomfort and were examined further for coronary heart disease. Coronary angiography was performed in two of these patients. One had normal angiogram, the other had borderline stenosis. CT coronary angiogram was performed in the other two patients, none of them had significant coronary artery stenosis. Transthoracic echocardiography (Vivid 7, GE Vingmed Ultrasound, Horten, Norway) was performed in patients when there were doubts about efficacy of systemic hypertension treatment or to evaluate a cardiac murmur. Two patients were observed with 24-hour ambulatory blood pressure monitoring, both showed normal values.

3.6 Study program and procedures

A standardized study protocol was established with two different time schedules, allowing us to examine two participants over two consecutive days. For very few patients there was some delay between the tests, due to service on the technical equipment. The short delays were not considered to affect the results in any way. All patients were instructed to take their daily medication on the test days. All tests were performed without supplemental oxygen, even for patients usually receiving oxygen supplement.

Most of the data from clinical tests were initially recorded on paper and records were kept in a locked archive facility. Data were manually entered in a Microsoft Office Excel database. Raw data

from CPET were digitally transformed to Excel database by customized macros. The database was de-identified and contained only a unique study ID number.

3.6.1 Pulmonary function tests

Spirometry (Jaeger, MasterScreen PFT, WUrtsburg, Germany) was performed according to the guidelines of ERS/ATS (63) at the first day of study examinations by all study subjects. Norwegian reference values were applied (64). GOLD classification was performed according to the current guidelines (5), using the best of three spirometric values and PaO_2/$PaCO_2$ on the test day after they had taken their daily medication. Bronchodilator tests were performed at the time of screening, and not repeated at the test day. The guidelines for GOLD classification was changed during the period of publication, as PaO_2<8.0 kPa and $PaCO_2$>6.7 kPa no longer were defining GOLD stage IV. To avoid presenting differences only related to the new GOLD classification (2), we have chosen to use the original GOLD-classification from 2007 (5) in all papers.

Diffusion capacity of the lung for carbon monoxide (*D*LCO) (Jaeger, MasterScreen PFT, WUrtsburg, Germany) was measured in patients with forced vital capacity (FVC)>1.5 liters using single breath method. Measurements were also made when FVC values were between 1.3 and 1.5 liters, but these results are known to be inaccurate. Subjects were sitting, using a nose clip. Standardized instructions were given. Tidal breathing was followed by exhalation to residual volume (RV), then followed by inspiration of test gas to total lung capacity (TLC).The method relies on patient co-operation and the ability of 10 seconds breath hold. The test gas consisted of nitrogen with 0.28% carbon monoxide, 9.3% helium and 20.9% oxygen added. Calibration was performed on a daily basis with automatic and manual procedures using a 3 liter syringe. Norwegian reference values were applied (65). DLCO was obtained for 87 participants, of whom 83 were included in paper III and IV.

Bodypletysmography (Jaeger Master Screen Body, Wurtsburg, Germany) was used to measure total lung capacity (TLC) and residual volume (RV). Subjects were sitting using a nose clip. Standardized instruction was given. The best of 2-3 repetitions was chosen. Calibration was performed on a daily basis with automatic and manual procedures using a 3 liter syringe. European reference values were applied (66). Bodypletysmography was obtained for 95 participants, of whom 90 were included in paper III and IV.

Six minute walk test (6MWT) was performed according to the guidelines of ATS (67) with fingertip pulse oximetry (Nonin, Hudiksvall, Sweden) and Borg 10 dyspnea score. A trained nurse accompanied the subjects. 6MWT was obtained for 96 participants, of whom 92 were included in paper III and IV.

3.6.2 Cardiopulmonary exercise test

Cardiopulmonary exercise test (CPET) (Jaeger Oxycon Delta, Germany and General Electric CardioSoft) was performed with a face mask (Draeger, Germany) on electronically braked cycle ergometer (Ergoline Variobike 550, Jaeger, Germany). Prior to exercise, maximal voluntary ventilation (MVV) was calculated by the measured maximal ventilation in ten seconds x 6. MVV was also estimated as FEV_1 x 37.5, but the measured MVV was usually the highest, most representative value for maximal ventilatory capacity, thus the measured MVV was used to evaluate the ventilatory reserve. All study subjects completed CPET except for one patient who was so severely hypoxemic that exercising without supplementary oxygen was considered inappropriate.

A radial artery cannula was provided for all study subjects for blood gas measurements at rest and during exercise, as well as continuous invasive measurement of systemic blood pressure. One patient had a vasovagal reaction due to arterial cannula insertion, but recovered after lying down and completed examination. We did not experience any other adverse events related to the arterial cannula.

Arterial blood samples were obtained with the patient in supine and sitting positions at rest, during exercise blood samples were obtained every forth minute and immediately prior to termination at maximal exercise. All samples were kept on ice in the syringe until end of exercise and were then analyzed for PaO_2, $PaCO_2$, SaO_2, pH, bicarbonate and lactate (Cobas b 221, Roche, Indianapolis, USA). Invasive, systemic blood pressure was monitored at rest and during exercise (BIOPAC Systems MP 150, Software Aqcknowledge, Transducer RX 104A, California, USA). Blood pressure curves during exercise could be “noisy” due to arm movement, but mean arterial pressure (MAP) sustained robust throughout the test.

The exercise protocol was a stepwise incremental test that could be applied during exercise with right heart catheterization as well, and differed from the more common protocols: Four minutes of unloaded pedaling, four minutes of 25 watts, then increased stepwise with 10 watts every second minute until the patient was exhausted. A cadence of 60 rpm was recommended. Calibration of flow and test gas was performed before every test. Oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were measured breath by breath. Ventilation was assessed by Triple V digital volume sensor, and respiratory frequency registered. Electrocardiogram (ECG) was recorded at rest and during exercise and heart rate denoted. Variables for each exercise level were recorded every 30 seconds, and median value for each level was denoted for statistical use. Peak workload and $\dot{V}O_2$ were defined as the highest level that could be performed for a minimum of 30 seconds. Oxygen pulse was calculated dividing $\dot{V}O_2$ by HR. Ventilatory equivalent for CO_2 ($\dot{V}E/\dot{V}CO_2$) was calculated and $\dot{V}E/\dot{V}CO_2$ nadir was defined as the lowest value on the curve during exercise. Norwegian reference values for CPET were applied (68). Ventilatory reserve was calculated as 1-(ventilation peak/MVV). In COPD

patients, anaerobic threshold (AT) is difficult to evaluate by ventilatory methods (69, 70), as they often terminate exercise at the moment they reach ventilatory compensation point, thus we chose to define AT as the $\dot{V}O_2$ where arterial lactate is 3 mmol/L. From two adjacent measurements, we performed linear interpolation of the point where lactate equaled 3mmol/L with the corresponding $\dot{V}O_2$, well aware that this was a crude estimate, as lactate rises in a curvilinear way, and AT could be slightly underestimated.

3.6.3 Right heart catheterization

According to the protocol, RHC was performed in patients in GOLD stage II-IV, but not in stage I (n=6). In GOLD stage II-IV altogether five patients did not complete the procedure due to difficulty in establishing venous access and one patient denied the procedure. Exactly one hundred patients completed RHC measurements.

No complications were experienced during RHC, but three patients developed superficial arm vein thrombosis at the site of venous access due to compression after the procedure. They all underwent three months of anticoagulation therapy and developed no sequelae.

RHC was performed with the patient in the supine position breathing ambient air. A balloon-tipped 7F Swan-Ganz catheter was inserted in the antecubital (n=95) or femoral (n=5) vein and positioned in the pulmonary artery with guidance of fluoroscopy (Siemens Arco Scope, Erlangen, Germany). ECG and pulse oximetry (SpO_2) were monitored continuously. Automatic calibration was performed prior to pressure measurements. The zero level of the pressure transducer was standardized. Pressure transducers were balanced against atmospheric pressure, and the zero reference level was 5 cm below the sternal angel, corresponding to the mid-thoracic line in the supine patients, representing the level of the left atrium. Measurements were performed at rest and during supine exercise with cycle ergometer (Ergomed 840 L, Siemens, Germany). The stepwise incremental exercise protocol started with four minutes unloaded pedaling at 60 rpm, then 20 W for four minutes, followed by ten W increments every second minute until exhaustion. All pressures were measured at end expiration during a temporary breath-hold, verified by flat respiration curves.

The RHC protocol at rest included measurements of the right atrium pressure (RAP), the systolic and end-diastolic right ventricle pressures, systolic pulmonary artery pressure (PAP) and pulmonary artery wedge pressure (PAWP). Cardiac output (CO) at rest was estimated by thermodilution technique, averaging three or five measurements (17).

During exercise, PAP and PAWP were measured in the last minute of every load level, whereas RAP and CO were only measured at peak exercise. CO was measured once at peak exercise,

as the patients were unable to exercise long enough for three repetitions. Blood samples were collected from pulmonary artery and right atrium. Oxygen saturation was analyzed instantly.

Hemodynamic variables were calculated: Pulmonary vascular resistance (PVR) = (mPAP-PAWP)/CO (WU); total pulmonary vascular resistance (TPR) = mPAP/CO (WU); transpulmonary pressure gradient (TPG) = mPAP – PAWP (mmHg); pulse pressure (PP) = systolic PAP – diastolic PAP (mmHg); stroke volume (SV) = (CO/heart rate) (mL/beat); pulmonary artery compliance (PAC) = SV/PP (mL/mmHg).

Exercise induced increase in mPAP was interpreted relative to increase in blood flow (ΔmPAP/ΔCO) and >3 mmHg/L/min was used as cut off for pathology (58). ΔmPAP/ΔCO slope was calculated from measurements at rest and peak exercise.

Pressure signals, ECG and respiration curves were digitally recorded at a Mac-Lab application (GE Healthcare, Milwaukee, USA). Post processing analyses were made on pressure curves at end-expiration by manually corrected regions of interest if necessary. Computer provided algorithms of mean pressures were used and averaged over three to six cardiac cycles.

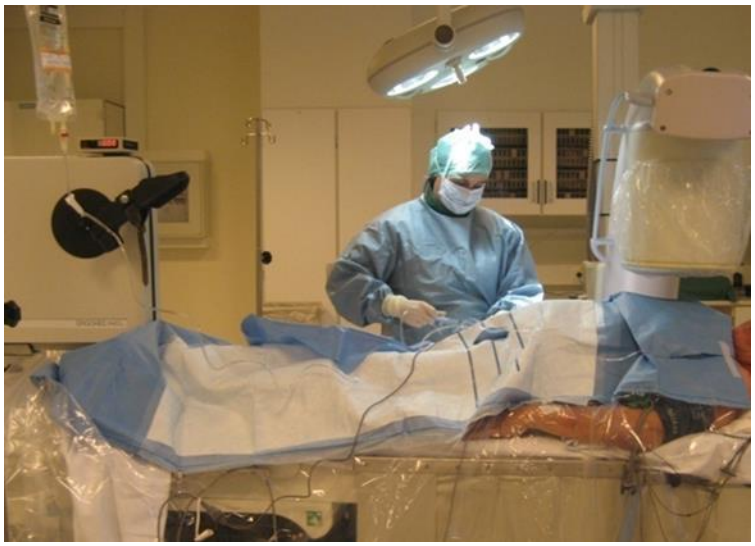
3.6.4 Definition of hemodynamic groups:

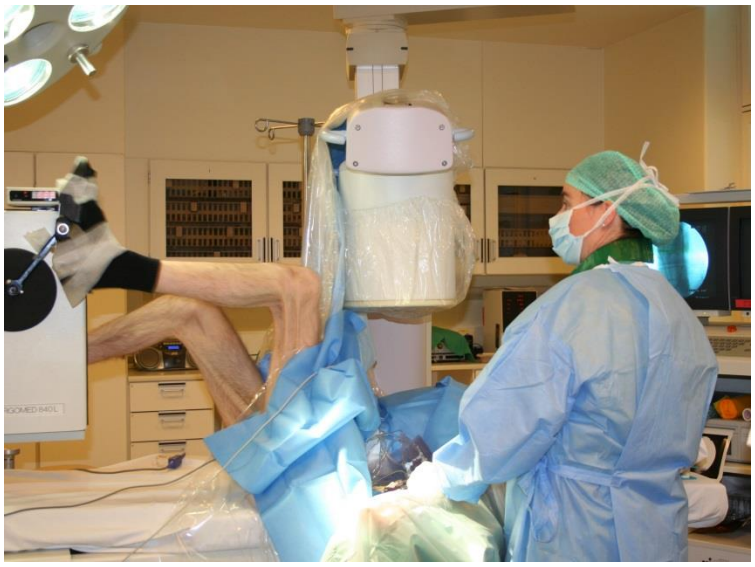
1) COPD-PH: COPD patients with mPAP≥25 mmHg and PAPW<15 mmHg at rest
2) COPD-noPH: COPD patients with mPAP<25 mmHg and PAPW<15 mmHg at rest

   COPD-noPH was divided in two groups by their hemodynamic exercise response, classified as normal or exercise induced pulmonary hypertension (EIPH):

   2.1) COPD-normal: COPD patients with mPAP<25 mmHg and PAPW<15 mmHg at rest and ΔmPAP/ΔCO slope<3 mmHg/L/min during exercise

   2.2) COPD-EIPH: COPD patients with mPAP<25 mmHg and PAPW<15 mmHg at rest and ΔmPAP/ΔCO slope≥3 mmHg/L/min during exercise





Janne Mykland Hilde performs right heart catheterization at rest and during exercise. Private photo with permission

3.6.5 Echocardiography

All patients were examined with Doppler echocardiography (Vivid 7, GE Vingmed Ultrasound, Horten, Norway) by cardiologist Janne Mykland Hilde. Data were stored for offline analyses (EchoPAC, GE, Vingmed). Measurements were performed during breath-hold in end-expiratory phase. Recordings were performed from left parasternal long axis and short axis and apical four-chamber views, as well as from subcostal position. All study subjects had sinus rhythm.

LV ejection fraction and volumes were calculated by the biplane method (modified Simpson's rule) as a measure for LV systolic function using apical four and two chamber views (71). The LV diastolic function was assessed by the size of the left atrium and transmitral pulsed Doppler recordings, which

included peak early filling (E), late diastolic peak filling velocity (A) and the ratio between E and A (E/A) (72). In addition, early diastolic (E´) peak velocity by pulsed tissue Doppler imaging was measured at four-chamber view at the basis of the septal and lateral mitral leaflet and averaged; E/E´ was calculated as surrogate for LV filling pressure (72). Left atrial volume was calculated using the four and two chamber views at end-systole by the method of the area length (71).

3.6.6 Blood samples

Blood samples in fasting condition were obtained from the cubital vein on the start of the first examination day. Standard biochemistry and hematology analyses were performed at the laboratory at Oslo University Hospital, Aker; hemoglobin, leucocytes, platelet count, sodium, potassium, creatinine, blood glucose, CRP, urea, uric acid, ASAT, ALAT, FT4, TSH, Cholesterol (total), LDL, HDL, NT-pro BNP and troponin T. $Alfa_1$-antitrypsine was measured at the laboratory at Oslo University Hospital, Ullevål.

3.7 Statistical analyses

Continuous variables were reported as mean and standard deviation (SD). Normality distribution was evaluated by visual Shapiro-Wilk test and by inspection of histograms and QQ-plots. Crude differences between two groups were assessed with independent sample t-test for normally distributed data, otherwise by Mann-Whitney U test. Paired sample t-test was used to compare the mean difference in a variable under different conditions within the same group. One-way ANOVA with Bonferroni correction for multiple analyses was used to compare three groups when data were normally distributed, otherwise Kruskal Wallis test was performed. A significance level of two-tailed p values <0.05 was used for all analyses.

In paper II multiple linear regression analyses were used to determine independent predictors of mPAP and PaO_2. All significant univariate variables were entered in the full model. Thereafter, backward stepwise eliminations were performed for non-significant ($p>0.05$) variables.

Receiver operating characteristic curve analysis (ROC) was performed to test diagnostic accuracy and optimal cut-off-value for the ability of a variable to discriminate between COPD-noPH and COPD-PH. P-values <0.05 were considered statistically significant.

To analyze between-group differences regarding CPET parameters, linear mixed models (LMM) for repeated measures were fitted. The method was developed to compare groups in longitudinal studies with measurements at several time points, including baseline. The method is suitable for comparing groups during CPET with fixed increments at fixed intervals, as each time point represents a certain exercise level. Measurements at all time points (loads) were considered,

including rest, and LMM therefore adjusts for baseline differences. The model took into account that subjects reached different maximal exercise levels. Models for each outcome included measurement occasion (load level), grouping of subjects (COPD-noPH, COPD-PH, COPD-EIPH) and interaction between load level and group. Further, LMM were adjusted for gender, age and FEV_1 (L) to test for potential confounders. P-values <0.05 were considered statistically significant.

When comparing COPD-PH and COPD-noPH in paper III, a diagonal covariance structure was used to model dependencies among measurements for each subject at multiple time points during exercise. Model fit was better for the exercise levels both groups could perform, so we chose to restrict analyses up to 85 W.

When comparing COPD-PH, COPD-EIPH and COPD-normal in paper IV, an unstructured covariance matrix was used to model dependencies among measurements for each subject at multiple time points during exercise, and all exercise levels were included in the analyses. COPD-EIPH was our defined reference group. Estimates of intercept and slope, as well as the difference between COPD-EIPH and the two other groups, were calculated. The intercept represents the calculated level of a variable at baseline, and is not the same as the measured baseline value, as all measurements during exercise were considered in the calculation. The slope represents the change in a CPET variable with increasing load.

3.8 Ethical considerations

One of the major ethical issues in this study was that the invasive procedures and exercise tests could potentially cause adverse events. All patients were thoroughly informed about the procedures and possible adverse events and were reassured that they had the opportunity to withdraw at any time if they changed their mind regarding any procedures. Only one patient used the opportunity to withdraw from RHC. To reduce the risk of adverse events as much as possible, the teams performing the invasive procedures and exercise tests were experienced and dedicated. The study complied with the hospitals handbook and procedures and emergency equipment was available during interventions.

The participants were informed about the test results, including a diagnosis of PH. As PH is associated with increased morbidity and mortality in COPD patients, it may be difficult for patients to have a diagnosis of PH confirmed. Specific treatment for PH in COPD is not recommended (17), except for patients with severe PH (34), and none of our patients were offered such treatment, thus the diagnosis of PH had no immediate medical implications. However, the patients diagnosed with PH were carefully followed by a pulmonary physician after study participation, in order to assure optimal medical care.

Scientific ethical issues have been related to the fact that two PhD candidates have shared the same data with discussions regarding use of data collected by the other part. All authors participated in the research process. Raw data and databases were available for the authors. All authors contributed to writing of the manuscripts and approved the final versions.

4 Results

4.1 Paper I

In this cohort of 98 COPD outpatients, where LV dysfunction, co-morbidities and exacerbations were thoroughly excluded, we observed pre-capillary PH at rest in 27% of the patients. The prevalence of pre-capillary PH increased with advancing GOLD stage from % in moderate disease (GOLD II), 27% in severe disease (GOLD III) to 53% in advanced COPD (GOLD IV). PAWP at rest was significantly higher in the PH group compared to the no-PH group, 11±3 versus 9±3 mmHg, respectively ($p<0.05$), but all subjects were within normal limits. PVR was elevated (>1.5 WU) in all patients with PH, and mean PVR in this group was 3.3±4 WU. In patients without PH mean PVR was 2.0±0.9 WU, as 69% in this group demonstrated elevated PVR. PAC was significantly reduced in the PH group ($p<0.01$) compared with the no-PH group.

Functional capacity, assessed by six minute walk test, was reduced with 9.5 m for every mmHg increase in mPAP (95% CI -14.3, -4.5 m; $p<0.01$) when adjusted for age, sex, height, weight, FEV_1 and PAWP. 6MWD was reduced by 29.5 m for every WU increase in PVR (95% CI -48.9, -10.1 m; $p<0.01$) when adjusted for SpO_2 and the above mentioned variables.

Assessment of hemodynamic profile during exercise revealed that there was no difference in the absolute mPAP increase between patients with and without pulmonary hypertension, although there was significant difference in the workload achieved; 50±20 W in PH group versus 74±25 W in no-PH group. We observed a linear relationship between maximal workload and % increase in CO from rest to maximal exercise. Slopes for ΔmPAP/ΔCO were calculated and they were 7.2mmHg/L/min in the PH-group versus 4.6 mmHg/L/min in the no-PH group. In the COPD-noPH group EIPH was defined by a slope of ΔmPAP/ΔCO>3 mmHg/L/min , and as much as 58% of the COPD patients without PH demonstrated pathological exercise response. Significant differences were observed between patients with normal exercise response and EIPH; in EIPH workload, PaO_2 and PAC were lower and 6MWT shorter, whereas age and PVR at rest were higher ($p<0.05$ for all).

4.2 Paper II

The focus of this paper was detection of PH in COPD patients based on measurements feasible at pulmonary outpatient clinics. Altogether 95 patients with moderate to very severe COPD were included. PH was observed in 25 subjects (26%). A gradual increase in mPAP was observed with increasing COPD severity, and the average mPAP was 18, 20 and 25 mmHg in GOLD stage II, III and IV, respectively. All subjects had PAWP below 15 mmHg. Cardiac index (CI) was normal and similar in all GOLD stages, whereas heart rate was significantly higher (84 beats/min) in GOLD stage IV compared

to stage II and III (p=0.002). PVR was significantly higher in GOLD stage IV (3.3 WU) compared to stage II and III (p<0.001).

Respiratory failure, defined as PaO_2<8 kPa, was observed in 14 patients and seven of these qualified for long term oxygen treatment (LTOT). A decline in PaO_2 during exercise was observed in 73% of the subjects, whereas 27% experienced no change or an increase in PaO_2. The mean change in PaO_2 from rest to peak exercise (ΔPaO_2) was most pronounced in GOLD stages III and IV, and patients with most severe hypoxemia at rest experienced the largest fall in PaO_2 during exercise.

Univariate linear regression analyses for mPAP revealed FEV_1, RV/TLC, heart rate, and PaO_2 as predictors, but in multiple regression analyses including the mentioned predictors as well as gender, age and height, only PaO_2 was a significant predictor of mPAP (p<0.001) accounting for 35% of the variation. For PaO_2 at rest and PaO_2 at peak exercise, both FEV_1 and mPAP were significant predictors in multiple regression analyses (p<0.001).

ROC curve analyses for PaO_2 at rest and peak exercise were applied to determine cut-off values for prediction of PH. Area under the curve was 0.77 (95% CI 0.66, 0.88) and 0.81 (95% CI 0.70, 0.93) for PaO_2 at rest and at peak exercise, respectively. By combining cut-off values of PaO_2 at rest ≤9.5 kPa and at peak exercise ≤8.5 kPa, a detection rate of 76% and a false-positive rate of 24% were obtained.

4.3 Paper III

Altogether 93 COPD outpatients performed cardiopulmonary exercise test with arterial blood gases to evaluate their exercise capacity. PH was observed in 24%. The majority had mild to moderate elevation of mPAP (range 25-35 mmHg) and PCWP<15 mmHg. Three patients, characterized by severe emphysema, had mPAP in the range 35-40 mmHg, and were classified as severe PH.

COPD-PH patients had more advanced pulmonary disease, with more airway obstruction, hyperinflation, impaired gas diffusion capacity and hypoxemia. Ventilatory limitation was the main reason for exercise termination, as 69% of patients experienced a ventilatory reserve <15%. Hypoxemia was strongly contributing to the low exercise capacity in COPD-PH, as 14 subjects (64%) developed PaO_2<7.0 kPa. Exercise capacity was severely reduced in COPD-PH compared to COPD-noPH, with maximal workload of 40±21 W versus 72±31 W, respectively (p<0.001). This was accompanied by lower $\dot{V}O_2$ in COPD-PH compared to COPD-noPH, 13.9±3.0 mL/min/kg versus 17.6±4.3 mL/min/kg (p<0.001). PaO_2 was lower in COPD-PH compared to COPD-noPH; at rest 8.1±1.5 kPa versus 9.8±1.2 kPa (p<0.001) and at peak exercise 6.7±1.1 kPa versus 9.4±1.7 kPa (p<0.001). When applying the linear mixed model analyses, we found that the observed difference in $\dot{V}O_2$/kg between the COPD-PH and COPD-noPH group was not related to PH, but to load level and gender, as

well as FEV_1. There was no difference in HR at peak exercise between the two groups, but by LMM analyses we found that PH affected HR significantly (p= 0.01). We observed that although multiple variables differed at rest and peak exercise, only PaO_2, $PaCO_2$, SaO_2, pH, heart rate, ventilation, and respiratory frequency differed in a statistical model that considered the entire course of exercise and adjusted for gender, age and FEV_1 (all $p<0.05$).

A striking difference between patients with and without PH was the difference in the maximal workload, as none with PH were able to bicycle more than 85 W, whereas 30 % of COPD-noPH reached a higher load. To make a simple algorithm in order to predict PH, we focused on the patients who performed 85 W or less. We applied ROC curves for data obtained at unloaded pedaling. The ability to discriminate between COPD-noPH and COPD-PH was best for PaO_2 as ROC curve demonstrated an area under the curve (AUC) of 0.86 (95% CI 0.78, 0.95, $p<0.001$). The cut-off value of 8.1 kPa predicted PH with a sensitivity of 86% and a specificity of 78%.

4.4 Paper IV

In this study 93 COPD patients were categorized as COPD-PH, COPD- EIPH and COPD-normal based on mPAP at rest and ΔmPAP/ΔCO slope during exercise as previously described in chapter 3.6.4. Exploring differences between COPD-EIPH and COPD-normal was the main objective. PH was observed in 24%, EIPH was observed in 45%, whereas only 31% of the study population experienced normal hemodynamic responses to exercise. At rest mPAP was 18±4, 18±3 and 28±5 mmHg in COPD-normal, COPD-EIPH and COPD-PH, respectively. During exercise mPAP was 34±7, 39±7 and 49±7 mmHg in COPD-normal, COPD-EIPH and COPD-PH, respectively. During exercise, there were no differences in mean peak PAWP for the three groups, but there were significant differences in PVR between all groups, 1.4±0.2 WU, 2.7±1.0 WU and 4.0±1.6 WU in COPD-normal, COPD-EIPH and COPD-PH, respectively. The normal physiological decrease in PVR during exercise was observed in COPD-normal only.

There were no differences between COPD-EIPH and COPD-normal regarding spirometric parameters. Compared to COPD-normal, COPD-EIPH had higher residual volume and lower *D*LCO, indicating more emphysema in the COPD-EIPH group. The EIPH group included 62% women, which was significantly different from COPD-normal with 31% women. The EIPH group was also significantly older compared to the other groups.

Maximal work load was lower in COPD-EIPH compared to COPD-normal, 60±31 W versus 89±23 W, respectively ($p<0.001$). However, there were no difference in % predicted values for $\dot{V}O_2$, ventilation, oxygen pulse, and lactate at peak exercise, but one or both groups differed from COPD-PH. Linear mixed model analyses were applied and adjusted for the differences in work load, gender

and age, as well as FEV_1. COPD-EIPH was our defined reference group. In LMM analyses COPD-EIPH demonstrated higher increase in $\dot{V}O_2$, ventilation, respiratory frequency, HR and lactate compared to COPD-normal ($p<0.05$). For the same variables, there were no differences between COPD-EIPH and COPD-PH. COPD-EIPH demonstrated a modest, but significant, reduction in pH compared to COPD normal ($p=0.005$). For PaO_2, we observed that the intercept was lower ($p<0.001$) and the decline steeper ($p<0.001$) in COPD-PH compared to COPD-EIPH, whereas there were no differences between COPD-EIPH and COPD-normal. For $PaCO_2$, we observed a more pronounced increase during exercise in COPD-PH compared to COPD-EIPH, but no differences between COPD-EIPH and COPD-normal.

5 Discussion

The discussion of this thesis will be structured in three main parts. Methodological considerations will be addressed in 5.1. Discussion of the results in papers I-IV will be addressed in 5.2. Clinical consequences of the results will be discussed in 5.3.

5.1 Methodological considerations

5.1.1 Design

An observational study with cross-sectional design was performed and multiple variables were collected at one point in time. This design is useful in order to investigate associations between chosen variables, however it cannot reveal causality.

5.1.2 Internal and external validity

In observational research the validity of a study must be evaluated. Internal validity is defined as the ability of a study to measure what it is set out to measure (73). The relevant question is whether the study is free from systematic errors, such as selection bias, confounding and information bias.

In a clinical study like the present, selection bias may occur if the investigator systematically avoids offering inclusion to patient groups who fulfills the inclusion criteria or if certain patient groups are more likely to deny participation in the study. In the present study, we were very committed to the inclusion and exclusion criteria, encouraging all eligible patients to participate. Surprisingly few patients turned down the offer to participate in this physically demanding study with invasive procedures. The few patients who did not want to participate, gave two main reasons; either the study was too demanding, or they could not skip work. This implies that both patients with more severe and with less severe disease declined participation, which is an advantage regarding selection bias compared to a situation where only those with a severe disease denied participation

Confounding is a blurring or mixing of effects; a researcher attempts to relate an exposure or condition to an outcome, but actually measures the effect of a third factor, the confounding variable (73). When assessing exercise performance by the presence of PH, pulmonary function may represent a confounder; the differences observed is related to emphysema and airway obstruction rather than PH. Confounding can be controlled by matching, stratification and multivariate analyses. In the present study we stratified by GOLD stages and we performed sophisticated multivariate

analyses to avoid the effect of known confounders, however, there is always a risk of unknown confounders.

Information bias can best be summarized by the question: Are we measuring what we intend to measure? With every diagnostic test there is a chance of error that can be expressed by sensitivity and specificity. Information bias results from incorrect determination of exposure/condition, outcome or both (73). In the present study we have performed many and complex procedures with different analyzing errors. Most of the test results vary in a random manner, which has less effect on the final conclusion compared to a systematic error. We have been confronted with the possibility of a systematic overestimation of mPAP due to increased intrathoracic pressure at the point of hemodynamic measurements, resulting in biased PH classification. This is a serious concern that will be discussed in the next sections. On the other hand, we have followed the investigation protocol strictly, in order to minimize errors due to careless performance. Information bias may also result from errors occurring when plotting data in the database. The database has been checked thoroughly several times by at least two investigators and plotting errors have been discovered. A plotting error of a CPET variable was detected when the figures in paper III and IV were made, as the value at a certain time point for one individual was very different from the adjacent measurements.

External validity is the ability to generalize from the study population to the reference population. This implies that the study population is a representative sub-sample of the reference population. As we have included COPD patients without relevant comorbidity, our results cannot be extrapolated to the general COPD population. However, our study population should be representative of COPD outpatients without comorbidity. The restricted external validity of our study regarding the general COPD population was intended, as causes of PH other than COPD had to be excluded.

5.1.3 Patient selection

We aimed to include 30 patients form each GOLD stage, representing stable COPD patients without significant comorbidity visiting the pulmonary out-patient clinic at a university hospital. We were not able to include 30 patients in GOLD stage I, as these patients have few symptoms and seldom are referred to a hospital clinic. GOLD stage I is less frequent than stage II, as normal FEV_1 and lower than normal FEV_1/FVC requires higher than predicted FVC, otherwise patients go directly to stage II.
Although we only included eight patients with mild COPD, the selection is representative of hospital and private specialist based out-patient clinics. As patients in GOLD stage I were not supposed to perform RHC, the main results of the study have not been affected by few participants in this group.

Altogether five patients under evaluation for lung transplant were admitted to the study from Oslo University Hospital, Rikshospitalet. Except for younger age (55±3 versus 64±6, p=0.008), these patients did not differ from the other COPD patients in GOLD stage IV.

The strict exclusion of left heart disease, arrhythmia, obstructive sleep apnea and other comorbidities prevents our study from being representative of the entire COPD cohort, but this selection was intended from the start, in order to study hemodynamic dysfunction caused by COPD and emphysema only. As almost every patient who was asked to participate in the study agreed to be included, there was no selection bias regarding this aspect. Overall, the study population represents the source population, consisting of stable COPD patients without comorbidity admitted to a local hospital outpatient clinic.

5.1.4 Right heart catheterization and hemodynamic evaluation

The gold standard in diagnosing pulmonary vascular disease is RHC. For measurements at rest there are recommended standard procedures (17, 74). As exercise may reveal pulmonary vascular dysfunction at an earlier stage, RHC was also performed during dynamic exercise. At the time the catheterizations were performed, there were no standard procedures for exercise measurements. When we were planning the study, the following were discussed; i) exercise protocol, ii) concomitant RHC and CPET, iii) thermodilution or Fick method for CO measurements, iv) measurement of CO at all exercise steps versus at peak exercise only, v) use of esophagus balloon catheter.

The exercise protocol including unloaded pedaling as described in the Methods chapter, ensured that all patients were able to perform some exercise and allowed for measurements at every load level. Concomitant RHC and CPET were not performed as the equipment was in different laboratories and moving the CPET apparatus back and forth could have disturbed the measurements. We also believed that too many monitoring systems attached to the patient at the same time, would have been unpleasant. The Fick method for estimating CO relies on measurement of oxygen uptake, but as CPET was not performed concomitantly, estimating CO by thermodilution was considered satisfactory. This technique has proven reliable even at low CO or with severe tricuspid regurgitations (75). To obtain reliable measurements of CO with thermodilution technique is time consuming, and we decided to obtain measurements only at peak exercise, to ensure enough time for measurements of mPAP and PAWP at every exercise level. If CO had been measured at every exercise level, it would have increased the accuracy of the mPAP/CO slope.

Retrospectively, we see that the use of an esophagus balloon to measure intra-thoracic pressure would have been preferable. The change in intraplural and intrathoracic pressure during the respiratory cycle is more pronounced in patients with COPD and hyperinflation, and the pressure

fluctuations are even more augmented during exercise in patients experiencing dynamic hyperinflation. The intrathoracic pressure is most positive at end-expiration, where we have performed our hemodynamic measurements, and this may add to the intravascular pressure, with a potential risk of overestimating mPAP and PAWP. At the time the catheterizations were performed, it was less focus on the respiratory pressure swings in COPD patients, and the papers by Boerrighter and by Kovacs stating methodological concerns regarding this issue (76, 77), were published after we had performed RHC in all participants. We performed pulmonary artery pressure measurements at end-expiration according to the standard procedure at the time (78, 79). Other studies of PH in COPD patients from the same period have, similar to us, measured pressures at end-expiration (80). As measurements were performed at end-expiration during temporary breath-hold, it is not possible to reanalyze catheterization data averaging pressure over the respiratory cycles. The use of an esophagus balloon would have made us able to correct the intravascular pressure measurements for the extravascularly transmitted pressure caused by the respiration, but we believed that the esophagus balloon would have been too uncomfortable for the volunteering patients.

5.1.5 Cardiopulmonary exercise test

The exercise protocol chosen, as described in the Methods section, was similar for all patients, regardless of their physical ability. This implied that patients with moderate disease exercised for a much longer time than patients with more severe disease, often for longer than the recommended test duration of 8-17 minutes (81). The advantage of using the same protocol for all was the ability to compare differences between patients at identical exercise levels. We also planned to compare measurements at the same load during RHC and CPET. However, after making the CPET protocols with 25, 35 and 45 W, we realized that the bicycle used during RHC could only perform 20, 30 and 40 W. By the time we discovered this, we had also observed that the different body positions, supine for RHC versus upright for CPET, affected exercise performance much more than the 5 W differences between the protocol steps, thus we chose to keep the CPET protocols as originally composed.

The use of a face mask instead of a mouthpiece increased the dead space ventilation. This increase is insignificant in healthy individuals, but for the COPD patients with severe ventilatory exercise limitation, it may have had a small impact on the results. On the other hand, the use of a face mask allows ventilation with pursed lip breathing, applying positive end-expiratory pressure in the airways and diminishing dynamic hyperinflation. Patients who experienced a mask leak were excluded from the CPET papers.

The equipment we used was unable to perform correct gas exchange measurements if supplementary oxygen was given, and this was the main reason for testing all patients when they

were breathing ambient air. This also gave similar test conditions for all patients. If some patients had received oxygen while others did not, this could have influenced our results. One can discuss whether it is unethical to let COPD patients with severe respiratory failure on LTOT to exercise to their maximal limit without supplementary oxygen, but as all vital signs were thoroughly monitored during exercise, we regarded the CPET procedure to be safe. However, one patient was excluded from the CPET studies, as her PaO_2 at rest (5.0 kPa in supine position) was considered too low to exercise.

Serial arterial blood samples were collected during CPET, and they were kept on ice until the test was finished. They were then analyzed after careful mixing of the content. Ideally, the samples should have been analyzed at once, but that was not possible as the analyzing unit was stationed in a different floor and we did not have personnel to bring the samples for analyses every forth minute. The samples were cooled quickly in a cup filled with ice cubes, thus the results are considered adequate. No blood samples were analyzed later than 35 minutes after it had been drawn, for the majority of the patients the time was much shorter (15-20 minutes). Although PaO_2 may increase and $PaCO_2$ decrease when the sample is stored, the delay is too short to influence the results substantially (82).

Measurement of inspiratory capacity (IC) during exercise was not performed, as this was not part of the standard CPET procedure at the time the study was performed. The equipment used in the study did not have suitable software for measuring IC during exercise. IC could have provided important information about dynamic hyperinflation. In healthy individuals, IC increases during exercise (83, 84). In patients with airway obstruction, especially in those with emphysema, IC may be gradually reduced during exercise (8). The airways collapse in the expiratory phase before all the inspired air is exhaled, gradually increasing the volume and intrathoracic pressure. As dynamic hyperinflation increases intrathoracic pressure, with the risk of overestimating mPAP and PAWP, identifying these patients would have been preferable (85, 86). End tidal CO_2 ($PETCO_2$) measurements could have been helpful in evaluation of the source of V/Q mismatch (69, 87, 88), but the equipment used did not provide an opportunity to measure this.

5.2 Discussion of main results

The four papers all explore different aspects of ventilatory and hemodynamic dysfunction at rest and during exercise. In Paper I we identified COPD patients with PH and and EIPH. These findings represent the fundament for the study, as the other papers rely on the hemodynamic measurements and categorization of PH and EIPH. The prevalence of PH and EIPH will be discussed in relation to paper I, as will the concept of EIPH and the different ways of expressing exercise pathology. The

relevance of hypoxia in the development of PH and the importance of PaO_2 in the diagnosis of PH in COPD is discussed in detail in relation to paper II and confirmed in paper III. Advantages and challenges by the use of CPET in detecting PH and EIPH is discussed in relation to paper III and IV.

5.2.1 Paper I

Prevalence of pulmonary hypertension in COPD.

Prior studies of COPD patients have reported a wide range of prevalence of PH, varying from 30 to 90%. The definition of PH has varied, as earlier studies have used mPAP>20 mmHg, whereas more recent studies have used mPAP>25 mmHg. The study populations have differed, and several studies have included COPD patients with end-stage disease who are candidates for volume reduction surgery or lung transplant (11, 31, 89-92). Lung transplant candidates undergo routine RHC as part of their pre-transplant evaluation, which has provided interesting data in this group. There are few studies of COPD outpatients with a wide range of airway obstruction regarding the prevalence of PH. With the current definition of PH, we found an overall prevalence of 27%. This is in line with studies by Evers et al and Keller et al who found prevalence of 33 and 35%, respectively, in cross-sectional studies on stable COPD patients, (93, 94) where the slightly higher prevalence may be explained by the definition of mPAP>20 mmHg used in those studies. Portillo et al have used the same PH definition as the present paper in a retrospective study of stable COPD patients in GOLD stages II-IV and found an overall prevalence of 18% (32). The PH prevalence observed in our study was lower compared to a prevalence of 36% in Danish lung transplant candidates (13), as would be expected as that study was restricted to GOLD stage III and IV. A comparable prevalence of 39% could be reported if GOLD stage II patients were excluded from our prevalence calculations.

We observed increasing prevalence of PH with increasing GOLD-stage. In GOLD stages II, III and IV a prevalence of 5%, 27% and 53% was found, respectively. For GOLD stage II and III this is comparable to the prevalence of 7% and 25% reported by Portillo et al (32), however, they reported a lower prevalence of 22% in GOLD stage IV.

Hemodynamic responses to exercise.

Exercise stresses the pulmonary circulation and may reveal pathology that is not manifest in the resting condition. Exercise testing provides an opportunity for earlier detection of abnormality in the pulmonary vascular bed. In healthy individuals during exercise, CO increases linearly with $\dot{V}O_2$ and workload, resulting in a physiologic increase in mPAP and decrease in PVR. The increase in mPAP is flow dependent, and a rise in mPAP during exercise beyond what is expected from CO may reflect pathology due to reduced vessel distensibility, increased left atrial pressure or increased intrathoracic pressure (16).

After the change in the definition of PH in 2008, where mPAP>30 mmHg during exercise no longer were regarded as pathology, redefining EIPH was still new and controversial when this article was published, with several proposals for expressing pathological exercise response. Similar for all proposals were a more complex evaluation of the pulmonary circulation during exercise, where composite variables were calculated. The slope of ΔmPAP/ΔCO had been proposed to define EIPH, as a steep slope indicates pathology with a cut-off value>3 mmHg/L/min (58). Experts in the field stated that "Exercise stress hemodynamic investigations should report on measurements of the components of the PVR equation, that is; mPAP, pulmonary wedge pressure, and CO to allow for a differential diagnosis of pulmonary vascular disease versus left heart failure" (16). We chose to emphasis the change in mPAP relative to CO, and applied ΔmPAP/ΔCO slope>3 mmHg/L/min as our definition of EIPH, as this fitted our data well. As patients with left heart disease were excluded in our study, we considered this definition suitable for evaluation of EIPH in our cohort, even though it did not implement changes in left atrial filling pressure, represented by PAWP.

Exercise capacity was low both in no-PH and PH-groups, 74 and 50 W respectively. In both groups PVR increased during exercise, in contrast to the reduction seen in healthy individuals. This reflects that vascular distensibility is affected not only in COPD patients with manifest PH, but even in patients with normal or borderline mPAP at rest. PAC was reduced during exercise in both groups, whereas an increase is seen in healthy individuals. The drop in PAC was relatively larger than the increase in PVR in the no-PH group, indicating that reduction in PAC could be an early marker of pathology in the pulmonary circulation.

When ΔmPAP/ΔCO was calculated for the no-PH and PH group, the mean slopes were 4.6 and 7.2 mmHg/L/min, respectively. A steep slope was expected for the PH group, but we also found that as much as 58% of the patients with normal mPAP at rest experienced ΔmPAP/ΔCO slope>3 mmHg/L/min and were categorized as having EIPH. Similar prevalence of EIPH, defined by mPAP>30 mmHg during exercise, was observed by Christensen et al (95), who found EIPH in 65% of COPD patients. The different definitions for EIPH are congruent for CO<10 L/min, thus the two studies are comparable. The prevalence of EIPH, combined with the low exercise performance, implies that the majority of COPD patients experience pathological circulatory stress many times a day during activities of daily living, with potential negative effects on the right ventricle.

Our definition of precapillary EIPH is susceptible to misclassification, as PAWP is not part of the definition. During exercise in healthy individuals, PAWP and CO increases in a linear manner with a slope of approximately one, indicating a nearly one to one upstream transmission of PAWP on mPAP (16). All patients had normal PAWP at rest, and by echocardiography there were no signs of overt systolic or diastolic dysfunction of the left ventricle. However, during exercise 14 patients experienced a rise in PAWP larger than expected with PAWP peak in the range 21-26 mmHg. As there

is no common definition of upper limit of normal for PAWP during exercise at the moment, we chose not to exclude patients with peak PAWP above 20 or 25 mmHg from our analyses. There are two possible mechanisms that may contribute to the larger than expected rise in PAWP; 1) latent diastolic dysfunction unmasked by exercise or 2) increased respiratory pressure swings caused by dynamic hyperinflation, where positive intrathoracic pressure adds to intravascular pressure.

We cannot completely rule out diastolic dysfunction in a few of our study subjects, and one could argue that some of the patients with EIPH have been misclassified with pre-capillary pathology. However, echocardiographic data did not indicate overt diastolic dysfunction in any of the patients and PVR indicated precapillary pathology in the majority of patients. In the 42 patients with EIPH, seven patients had high PAWP peak, of whom four demonstrated PVR>1.5 WU indicating predominantly pre-capillary pathology, however only one demonstrated PVR>3 WU with definite precapillary PH. The change in intrapleural and intrathoracic pressures during the respiratory cycle are more pronounced in patients with COPD and hyperinflation, and the pressure swings are even more augmented during exercise in patients experiencing dynamic hyperinflation (96). The intrathoracic pressure is most positive at end-expiration, where we have performed our hemodynamic measurements, and this may add to the intravascular pressure, with a potential risk of overestimating mPAP and PAWP (76, 77). This could explain why some patients without any signs of diastolic dysfunction demonstrate higher than expected peak PAWP.

The positive intrathoracic pressure at end-expiration may also affect the slope of ΔmPAP/ΔCO and this complicates hemodynamic evaluation of COPD patients (16, 76). In our study the slope is based on measurements at two single occasions, at rest and peak exercise. If both measurements were equally overestimated, the slope may not be affected; however, it is reason to believe that the exercise measurements are more overestimated than the resting measurements due to dynamic hyperinflation, thus this is a source of error to our categorization of EIPH. Nevertheless, our categorization is meant to describe the impact of COPD on the pulmonary circulation and the right heart, and the intrathoracic pressure fluctuations are important for the total load.

The results of the study have contributed to more insight in the pulmonary circulation in COPD patients, and it has provided us an opportunity to join the scientific debate about EIPH.

5.2.2 Paper II

The focus of this paper was detection of PH in COPD patients based on measurements feasible at pulmonary outpatient clinics. We observed that only PaO_2 was a significant predictor of mPAP when adjusted for demographic data and pulmonary obstructive indices. When assessing the presence of PH in COPD, the combined evaluation of PaO_2 at rest and at peak exercise proved useful.

Consistent with numerous previous studies in COPD patients, we observed a clear inverse relationship between mPAP and PaO_2 (13, 28). This is due to hypoxic vasoconstriction and vascular remodeling of small pulmonary arteries. In our study, only PaO_2 was a significant predictor of mPAP, whereas in a study of COPD patients with end-stage disease evaluated for lung transplantation, both PaO_2 and $PaCO_2$ were significant predictors (13). This discrepancy may be explained by the lower $PaCO_2$ with less variance both in the group with and without PH in our cohort. Indices of airway obstruction failed to predict mPAP. This is in contrast to a study of COPD patients with severe emphysema where FEV_1, and not PaO_2, was a predictor of mPAP (29). However, severely hypoxic patients were excluded from their study and supplementary oxygen was provided during RHC.

We were surprised that PH was observed in several patients without respiratory failure, and that the cut-off value for PaO_2 at rest was as high as 9.5 kPa. The higher PaO_2 could be related to our strict exclusion criteria, as no patients with LV disease, interstitial parenchymal disease or other diagnoses known to affect gas exchange were included. It also points to the fact that other mechanisms than hypoxia is involved in development of PH in COPD, where vascular remodeling related to inflammation and endothelial dysfunction contribute (97). Our observed mean PaO_2 in subjects with PH was comparable to previous COPD studies (13, 25, 26, 30, 95). However, only two of these studies (13, 95) have reported mPAP and PaO_2 on an individual level. Similar to us, they observed surprisingly high PaO_2 in some subjects with PH, suggesting that there is a large variation in PaO_2 in COPD-PH patients, implying multifactorial causes of vascular remodeling.

Arterial blood gas analyses at rest are standard procedure at pulmonary outpatient clinics, whereas arterial blood gas analyses during exercise requires more effort to obtain. We did not measure SpO_2 with pulse oximetry concomitantly, as arterial samples provided more reliable results. To be able to apply our suggested cut-off value for PaO2 at peak exercise, an arterial cannula is necessary. Without a cannula it is difficult to draw arterial samples due to arm movement. Arterial blood samples drawn immediately after exercise will usually not reflect the maximal desaturation, as blood gases quickly returns to normal in COPD patients after termination of exercise.

We have shown that PaO_2 is superior to pulmonary obstructive indices and GOLD-classification in detection of PH in COPD. When combining cut-off values of PaO_2 at rest ≤9.5 kPa and PaO_2 at peak exercise ≤8.5 kPa, a detection rate of 76% and a false-positive and false-negative rate of 24% were obtained. These cut-off values may guide the pulmonary physician when considering referral to echocardiography for further PH evaluation, as COPD-PH patients should be identified due to their worse prognosis (11, 98).

5.2.3 Paper III

When the cardiopulmonary system is challenged during exercise, pathology may be relieved at an earlier stage compared to measurements at rest. During CPET, it often becomes obvious whether it is the ventilation or the circulation that limits exercise (87, 99). Patients with COPD exhibit a typical pattern during exercise where the ventilatory reserve is low, dynamic hyperinflation present, hypoxemia and hypercapnia occurs, and exercise terminates at the anaerobic threshold (69, 100). In pulmonary arterial hypertension (group 1) a different CPET response is observed: The ventilatory reserve is normal and there is no dynamic hyperinflation. Hypoxemia is accompanied by low $PaCO_2$ and anaerobic threshold occurs early in the course of exercise (69, 101, 102). When both COPD and PH are present, the pattern is much more difficult to interpret, as the development of a specific variable may point in different directions caused by the two pathological processes. However, the study shows that CPET can be a valuable tool in detecting PH in an outpatient COPD population. Although we were not able to discriminate fully between patients with and without PH, as results were overlapping, certain CPET findings were highly indicative of PH.

When we compare our results to other studies, the picture is diverging. Unlike our study, most studies have not been able to demonstrate significant differences in peak work load and $\dot{V}O_2$ between COPD patients with and without PH (89, 103, 104). However, Thirapatarapong et al found significant differences in work load and $\dot{V}O_2$ in a study of comparable size including 98 COPD/emphysema patients with FEV_1<50% predicted evaluated for lung transplant, volume reduction surgery or rehabilitation (92). Like our study, Thirapatarapong also demonstrated that O_2-pulse was significantly lower in COPD-PH, although they found lower values in both groups than we did, 5.0 versus 7.5 mL/beat in COPD-PH and 5.2 versus 9.4 mL/beat in COPD-noPH. Other studies have not found differences in O_2-pulse (89, 103). $\dot{V}E/\dot{V}CO_2$ is an indicator of ventilation/perfusion mismatch, and similar to our observations, Holverda et al found significantly higher $\dot{V}E/\dot{V}CO_2$ nadir in COPD-PH in a study of 25 patients with moderate to severe COPD (103). The lower PaO_2 and oxygen saturation at rest and peak exercise in patients with PH in our study is also demonstrated in other studies (103-105), emphasizing the important role of blood gases in evaluation of PH in COPD.

Including patients with a wide range of airway obstruction gave us information about exercise capacity in patients usually seen in a hospital outpatient clinic. However, due to the study design, where we also included patients with moderate COPD less prone to have developed COPD, we observed significant differences in airway obstruction indices between the patients with and without PH. One can argue that the observed differences in CPET parameters between the two groups, such as work load, $\dot{V}O_2$, O_2-pulse, ventilation, PaO_2, SaO_2 and $PaCO_2$, are more related to differences in airway obstruction than to pulmonary hemodynamics. The difference in maximal work load affects some of the CPET variables, thus it was relevant to control for both airway obstruction

and work load. Linear mixed model analyses proved useful in this respect, and provided interesting data about physiological differences between patients with and without PH. However, as these differences appear after complex calculations, they cannot be implemented in routine CPET evaluation and have more theoretical than clinical consequences.

The observation that none of the patients with PH were able to exercise above 85 W is more clinically relevant, as PH is unlikely in patients who achieve a higher maximal load. In altogether sixteen patients unloaded pedaling was the maximal load achieved and PH was observed in 50% of those. As unloaded pedaling was the only work load conducted by all participants, variables obtained at this level were investigated and ROC curves applied. Once again PaO_2 was the best parameter to identify the patients with PH, as *P*aO2<8.1 kPa identified PH with a sensitivity of 86% and a specificity of 78%. With these results, we regard CPET as a valuable tool in detecting PH in COPD patients.

The importance of PaO_2 justify the use of arterial cannula during CPET, even if it is time consuming and somewhat unpleasant for the patients. Repeated arterial blood samples with lactate measurements also allowed us to determine the anaerobic threshold more accurately, as the ventilatory CPET methods are of little value in patients who reach their ventilatory limitation close to the anaerbobic threshold. It is typical for COPD patients to abort exercise when lactic acid starts to accumulate as H^+ stimulates ventilation. Due to flow limitation, these patients are not able to increase ventilation as required, instead dyspenea occurs and they terminate exercise (100). This is in contrast to patients with circulatory exercise limitation, such as pulmonary arterial hypertension group 1, who often reach anaerobic threshold early and have high levels of lactate at the end of exercise (69, 87, 101). The pattern observed during CPET indicated that the majority of patients experienced exercise limitation due to ventilatory limitation caused by COPD, some were limited by deconditioning, but none experienced classic circulatory exercise limitation with pathological O_2-pulse curve or reduction in systemic blood pressure. Even the patients with mPAP>35 mmHg were considered to experience ventilatory rather than circulatory exercise limitation. End tidal CO_2 ($PETCO_2$) measurements could have been helpful in evaluation of the source of V/Q mismatch; emphysema or pulmonary hypertension with remodeled vessels. Patients with PH usually have lower than normal $PETCO_2$ and lower $PETCO_2$ compared to $PaCO_2$ in arterial blood (69, 87, 101). Unfortunately the equipment we used could not provide $PETCO_2$, however, there is reason to believe that our conclusion would have been the same; the majority experienced primarily ventilatory exercise limitation.

5.2.4 Paper IV

In this study of COPD outpatients where left ventricular disease was excluded in advance, we have identified a large group of patients characterized by normal mPAP at rest accompanied by an increased rise in mPAP relative to CO during exercise, representing EIPH. During CPET, COPD patients with ΔmPAP/ΔCO slope>3 mmHg/L/min differed from COPD patients with normal hemodynamic responses in $\dot{V}O_2$, ventilation, respiratory frequency, heart rate, lactate and pH in a statistical model that included measurements at every load level and adjusted for gender, age and FEV_1.

Definition and prevalence of EIPH in our study, as well as methodological and hemodynamic considerations regarding intrathoracic pressure fluctuations and diastolic dysfunction are discussed in paper I, chapter 5.2.1 and will not be repeated here.

When patients without PH were categorized as COPD-EIPH or COPD-normal, we observed significant differences in age and gender that needed to be addressed, as both gender and age affect exercise performance during CPET. COPD-EIPH was significantly older (p=0.02, t-test) and with female dominance (p=0.01) compared to COPD-normal. All patients were ≥50 years old and 70% of patients ≥70 years were defined as COPD-EIPH. A steeper ΔmPAP/ΔCO slope with advancing age is in line with the findings in healthy individuals (49, 106, 107). We therefore have to include age as an important factor for the present observation of EIPH.

The difference in gender distribution, with significantly more women in COPD-EIPH compared to COPD-normal, was more unexpected. In a study based on exercise stress echocardiography, Argiento et al found similar mPAP-flow relationship for both genders (106). During exercise, healthy males achieved higher workload and CO, accompanied by higher mPAP, compared to healthy females, but the ΔmPAP/ΔCO slope was similar. Calculation of distensibility coefficient α demonstrated that women below the age of 50 have more distensible vessels, an effect that tapers off after menopause (106). All our patients were older than 50 years and studies comparing gender differences in ΔmPAP/ΔCO slope for healthy subjects above this age are scarce. It could be that the mPAP/CO slope is steeper in older women compared to men, but unfortunately there is not enough normality data to answer this for the time being. We have discussed whether the lower CO in women makes them more likely to have a steeper slope. We have speculated whether CI would be better than CO in the definition of EIPH, as CI accounts for the relatively lower CO due to smaller size in women. However, a recent study by Weatherald et al could not confirm this (108). It is possible that the gender difference observed in our study actually reflects that the women included had more dysfunctional pulmonary circulation compared to the men, as women were dominant even in the PH group.

Due to gender and age differences, CPET variables were compared as percent of predicted. There were no differences between COPD-EIPH and COPD-normal in $\dot{V}O_2$, ventilation, heart rate,

oxygen pulse, $\dot{V}E/\dot{V}CO_2$ nadir, or lactate, indicating that CPET is unable to distinguish COPD-EIPH from COPD-normal. However, for several of the CPET variables, there was a stepwise increase/decrease from one group to the next, but these differences were difficult to prove significant, possibly due to smaller sample size with three groups. This could be the reason why PaO_2 and $PaCO_2$ both at rest and at peak exercise are not significantly different between COPD-EIPH and COPD-normal.

As there were some important significant and non-significant differences between the hemodynamic groups, we applied LMM analyses to our data to explore if there were significant differences between the groups after all, when adjusting for gender, age and FEV_1, as well as considering all exercise levels. For this paper, LMM results were split in two different effect measures; level/intercept and slope. In the adjusted model, the increase in $\dot{V}O_2$, ventilation, respiratory frequency, heart rate and lactate were higher in COPD-EIPH compared to COPD-normal. The higher $\dot{V}O_2$ for the same external bicycling work implies increased "internal work" in COPD-EIPH compared to COPD-normal. This could represent respiratory work. The EIPH group had higher residual volume and lower *D*LCO than the hemodynamic normal group, which indicates more emphysema in COPD-EIPH. Dynamic hyperinflation could then explain the higher ventilation and respiratory frequency in COPD-EIPH. This indication of dynamic hyperinflation brings us back to the methodological concerns regarding positive intrathoracic pressure, especially in the expiratory phase, as a source of error in mPAP measurements. The higher ventilation in EIPH-COPD could also be related to V/Q mismatch, caused by either emphysema, vascular dysfunction or both.

In paper II and III we have emphasized the importance of PaO_2 in predicting PH in COPD. We were surprised to observe that PaO_2, as well as $PaCO_2$, were similar in COPD- EIPH and COPD-normal. Similar PaO_2 in EIPH and hemodynamic normal patients was also observed by Degani-Costa et al in a study of patients with interstitial lung disease (109).

Due to differences in gender, age and pulmonary function between the three groups, a direct comparison of data was difficult, and we were not able to discern CPET differences between COPD-normal and COPD-EIPH. After adjusted analyses, there were differences between the groups, but as they only appeared after correcting calculations, they have more theoretical than clinical value; thus we must conclude that CPET is not a suitable method to identify COPD-EIPH.

5.3 Clinical consequences and future perspectives

This thesis has highlighted that PH is common in COPD patients, and PH is observed even in patients with moderate airway obstruction. We have provided simple tools readily available for the pulmonary physician when considering PH in a COPD patient, emphasizing the importance of PaO_2. We recommend CPET with arterial blood gas measurements to determine the reason for exercise

limitation in COPD patients with suspected PH. Very low exercise performance and PaO_2<8.1 kPa at unloaded pedaling indicates PH. However, we must conclude that echocardiography and RHC are necessary to confirm the diagnosis of PH, and we recommend that cardiologists are more involved in the care of COPD patients. Diagnosing PH in COPD may seem irrelevant today, as these patients are offered nothing but standard COPD care (17). However, if effective treatment would be available, this would completely change the picture. Medication that inhibits the vascular remodeling without increasing V/Q mismatch could be beneficial for the large COPD population, and should be the target of pharmaceutical research. Until then, the majority of COPD patients with PH should be given optimal COPD treatment, including smoking cessation and rehabilitation (110). An important exception is patients with severe PH combined with only moderate reduction in pulmonary function, as they may benefit from specific vasodilating treatment (34, 111). None of the patients in our study were offered such treatment, as all the patients with severe PH also had very severe reduction in pulmonary function.

The thesis has also provided important information about hemodynamic dysfunction during exercise in COPD patients with normal hemodynamic measurements at rest, allowing us to take part in the scientific discussion about the redefinition of EIPH. Within this field, there are still many questions to be answered by future research. Hemodynamic exercise data for healthy persons above 50 years of age are difficult to obtain, but very much asked for, as the limits of normal must be defined before evaluation of pathology can take place. There is still an ongoing debate on how to express EIPH (108, 112); more studies comparing the different methods in different populations would be useful. A study by Godinas et al showed full agreement for three methods in 78% of cases when comparing multipoint mPAP/CO slope, two point ΔmPAP/ΔCO slope and mPAP peak>30 mmHg+TPR peak>3 (113), but studies in different populations should confirm this and one specific method should be agreed upon. Standard, unified RHC procedure and protocol for obtaining correct hemodynamic measurements during exercise should be available, including references on how to avoid inaccurate measurements due to respiratory pressure fluctuations in COPD patients. By an ironic coincidence, the European Respiratory Society has published an official statement on exercise hemodynamics in November 2017 (114), simultaneously as this thesis is completed.

As further prospects for our own study population, we are planning to analyze high sensitive troponin I to compare COPD patients with and without PH. It would also be very interesting to investigate all-cause mortality and to establish prognostic factors in the cohort, thus an application for such a study has been submitted.

6 Conclusions

The studies of this thesis have provided more insight in the pulmonary circulation at rest and during exercise in COPD patients. We have shown that hemodynamic dysfunction is common in COPD and that it is possible to identify the majority of patients at risk by investigation at the pulmonary outpatient clinic. The physiological changes during exercise, both ventilatory and hemodynamic, have been thoroughly characterized for COPD patients.

To answer our main research questions of the four papers we conclude:

Paper I

•The prevalence of precapillary PH in COPD outpatients without left heart disease and comorbidity was 27%.

•PH was present in 5, 27 and 53% in GOLD stage, II, III and IV, respectively.

•EIPH, defined as mPAP<25 mmHg at rest and ΔmPAP/ΔCO slope≥3 mmHg/L/min, was observed in 58% of the patients with normal hemodynamic measurements at rest.

Paper II

•Hemodynamic measurements of PVR and mPAP were higher in GOLD stage IV compared to II and III. PAWP were similar in all GOLD-stages. Pulmonary function tests and PaO_2 became more pathologic with increasing GOLD stage.

•PaO_2 was the only significant predictor of mPAP when considering measurements feasible at a pulmonary outpatient clinic.

•FEV_1 and mPAP were significant predictors of PaO_2.

•PaO_2 below 9.5 kPa at rest combined with PaO_2 below 8.5 kPa at peak exercise predicted PH in COPD patients with a detection rate of 76% and a false-positive rate of 24%.

Paper III

•During exercise, COPD patients with PH have significantly lower work load, $\dot{V}O_2$, oxygen pulse, ventilation and PaO_2, as well as higher $VE/\dot{V}CO_2$ and $PaCO_2$ compared to patients without PH.

•When considering the entire course of exercise during CPET and adjusting for airway obstruction, age and gender, patients with PH experience significantly higher ventilation, respiratory frequency, heart rate, $PaCO_2$ and lower PaO_2, SaO_2 and lactate compared to patients without PH.

•CPET with arterial blood gases cannot fully identify COPD patients PH, but very low exercise performance and PaO_2< 8.1 kPa during unloaded pedaling indicates PH.

Paper IV

- During exercise, there were no differences in CPET parameters at peak exercise when comparing patients with ΔmPAP/ΔCO slope>3 mmHg/L/min to COPD patients with a normal slope.
- When considering the entire course of exercise during CPET and adjusting for airway obstruction, age and gender, the increase in $\dot{V}O_2$, ventilation, respiratory frequency, heart rate and lactate were higher in COPD patients with EIPH compared to COPD patients with normal exercise response.
- CPET with arterial blood gases is not a suitable method to identify COPD patients with EIPH.

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Disclosure

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Appendices

Appendix 1

Updated Classification of Pulmonary Hypertension (5th WSPH Nice 2013)

1. Pulmonary arterial hypertension

1.1 Idiopathic PAH
1.2 Heritable PAH
1.2.1 BMPR2
1.2.2 ALK-1, ENG, SMAD9, CAV1, KCNK3
1.2.3 Unknown
1.3 Drug and toxin induced
1.4 Associated with:
1.4.1 Connective tissue disease
1.4.2 HIV infection
1.4.3 Portal hypertension
1.4.4 Congenital heart diseases
1.4.5 Schistosomiasis
1' Pulmonary veno-occlusive disease and/or pulmonary capillary hemangiomatosis
1''. Persistent pulmonary hypertension of the newborn (PPHN)

2. Pulmonary hypertension due to left heart disease

2.1 Left ventricular systolic dysfunction
2.2 Left ventricular 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 pulmonary diseases with mixed restrictive and obstructive pattern
3.4 Sleep-disordered breathing
3.5 Alveolar hypoventilation disorders
3.6 Chronic exposure to high altitude
3.7 Developmental lung diseases

4. Chronic thromboembolic pulmonary hypertension (CTEPH)

5. Pulmonary hypertension with unclear 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

BMPR = bone morphogenic protein receptor type II; CAV1 = caveolin-1; ENG = endoglin; ALK= activin-like kinase; KCNK: potassium channel subfamily K; HIV = human immunodeficiency virus; PAH = pulmonary arterial hypertension

Ref: Simonneau et al. Updated clinical classification of pulmonary hypertension. Journal of the American College of Cardiology. 2013;62(25 Suppl):D34-41.

Appendix 2

COPD treatment at the time of inclusion (n=112)

Therapy for COPD	**n (% of total)**
Long term oxygen treatment	12 (11)
Ambulatory oxygen treatment	4 (4)
Inhaled ipratropium	53 (49)
Inhaled tiotropium	45 (40)
Inhaled short acting $beta_2$ agonists when needed	62 (55)
Inhaled long acting $beta_2$ agonists without ICS	13 (12)
Inhaled long acting beta agonists with ICS	70 (63)
Oral theophyllin	13 (12)
Oral corticosteroids	18 (16)
No COPD medication	10 (9)
Antihypertensive treatment of systemic arterial hypertension	
Calcium antagonist	8(7)
Angiotensin receptor antagonist	1(1)
Angiotensin receptor antagonist with hydrochlorhiazid	15(13)
Loop diuretics	7(6)

ICS: inhaled corticosteroids. A total of 31 patients used medical treatment for systemic hypertension, while 33 were diagnosed with arterial hypertension, of whom two did not use their medication as prescribed at inclusion.

Papers I-IV