Chronic obstructive pulmonary disease. Aspects of smoking, biomarkers and cardiovascular disease.

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Chronic obstructive pulmonary disease

Aspects of smoking, biomarkers and cardiovascular disease

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LUND
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Abstract

Chronic obstructive pulmonary disease (COPD) is a common and preventable disease characterized by airflow obstruction and emphysema development. COPD often coexist with cardiovascular disease (CVD) and both diseases have common risk factors i.e. age and smoking and shared features i.e. chronic inflammation. Smoking is of particular interest as it substantially increases the risk of developing COPD and CVD and is known to elicit systemic inflammatory responses. COPD (and CVD as well) often go unnoticed for years in the preclinical or early stage of disease so arguably, early disease detection is of great importantance and vital to improve prognosis. Plasma biomarkers of inflammation could be instrumental in this task and in recent years many new biomarkers have been described, but very few make it into clinical use.

This thesis deals with COPD and its comorbidity (CVD) and their common risk factor smoking, with focus on the role of new and established biomarkers of COPD and atherosclerosis.

All papers in this thesis are cross sectional studies based on the ROLLS study population. ROLLS was an invitational study including 450 subjects on the basis of self-reported smoking habits and pulmonary symptoms. All participants underwent a one day study protocol consisting of transthoracic echocardiography, lung function tests, carotid ultrasound examination, resting blood pressure measurement, blood sample analysis and a questionnaire.

In paper I the aim was to study the impact of smoking status on echocardiographic variables of cardiac function in a population without clinically evident cardiopulmonary disease. Here, no convincing effect of current or prior smoking on echocardiographic variables of systolic and diastolic function could be detected, even if smokers had significantly higher cardiac index due to a slightly increased heart rate.

In paper II the aim was to examine whether brain natriuretic peptide (BNP), an established marker of cardiac dysfunction, could be used as a biomarker of COPD in a population with normal cardiac function. The results showed no significant differences in BNP levels between subjects with COPD and controls. Thus, the clinical value of BNP in this setting is limited.

Paper III evaluated matrix metalloproteinases (MMP´s) as biomarkers of COPD and atherosclerosis and the impact of smoking. The results showed that MMP´s are influenced by smoking status and that MMP-1 and MMP-12 are independent predictors of concomitant COPD and carotid plaque even when adjusting for traditional risk factors.

In paper IV the aim was to explore whether a novel inflammatory biomarker, fibroblast growth factor 23 (FGF23), was influenced by COPD status and impaired pulmonary function. The findings showed that FGF23 was associated with the presence of COPD even after adjusting for traditional risk factors, and that FGF23 was associated with impaired pulmonary function and gas exchange.

In conclusion, this thesis evaluated the impact of smoking on cardiac function and MMP´s and explored the current (BNP) and future (MMP and FGF23) value of biomarkers in COPD and CVD.

Key words

Smoking, COPD, atherosclerosis, biomarkers

Classification system and/or index terms (if any)

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This thesis is dedicated to my grand parents

Ein Schwert verhieß mir der Vater,
ich fänd' es in höchster Not

Sigmund, från Valkyrians 1:a akt, Richard Wagner

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List of papers

This thesis is based upon the following papers which will be referred to by their Roman numerals in the text.

I. **Kraen M,** Frantz S, Nihlén U, Engström G, Löfdahl, C-G, Wollmer P, Dencker M :

Echocardiographic consequences of smoking status in middle-aged subjects

Echocardiography 2017; 34: 14 14-19

II. **Kraen M,** Frantz S, Nihlén U, Engström G, Löfdahl, C-G, Wollmer P, Dencker M :

Brain natriuretic peptide levels in middle aged subjects with normal left ventricular function in relation to mild-moderate COPD

Clinical Respiratory Journal 2017; 1-7

III. **Kraen M,** Frantz S, Nihlén U, Engström G, Löfdahl, C-G, Wollmer P, Dencker M :

Matrix Metalloproteinases in COPD and atherosclerosis with emphasis on the effects of smoking

PLoS ONE 2019, 14(2): e0211987

IV. **Kraen M,** Frantz S, Nihlén U, Engström G, Löfdahl, C-G, Wollmer P, Dencker M :

Fibroblast growth factor 23 is an independent marker of COPD and is associated with impairment of pulmonary function and diffusing capacity

Submitted

Abbreviations

A	Late diastolic mitral inflow
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
AU	Arbitrary Units
BMI	Body Mass Index
BNP	Brain Natriuretic Peptide
BP	Blood Pressure
BSA	Body Surface Area
CI	Cardiac Index
COPD	Chronic Obstructive Pulmonary Disease
CO	Cardiac Output
CP	Cor Pulmonale
CVD	Cardiovascular Disease
$D_{L,CO}$	Diffusing Capacity of the Lungs for Carbon Monixide
DT	Deceleration Time
E	Early diastolic mitral inflow
e`	Peak early diastolic mitral annular velocity
eGFR	Estimated Glomerular Filtration Rate
ERS	European Respiratory Society
FEV_1	Forced Expiratory Volume in one second
FGF23	Fibroblast Growth Factor 23
FVC	Forced Vital Capacity
GOLD	Global Initiativ for Chronic Obstructive Lung Disease

HbA1c	Glycated Haemoglobin
HDL	High-Density Lipoprotein
hsCRP	High sensitive C-Reactive Protein
ICA	Internal Carotid Artery
IL	Interleukin
IVS	Interventricular Septum
LA	Left Atrium
LDL	Low-Density Lipoprotein
LLN	Lower Limit of Normal
Ln	Natural Logarithm
LVEDD	Left Ventricular End-diastolic Dimension
LVEDV	Left Ventricular End-diastolic Volume
LVM	Left Ventricular Mass
LVSDV	Left Ventricular End-systolic Volume
LVOT	Left Ventricular Outflow Tract
LVOT-VTI	Left Ventricular Outflow Tract Velocity Time Integral
MDRD	Modification of Diet in Renal Disease
MMP	Matrix Metalloproteinases
PAH	Pulmonary Arterial Hypertension
PN	Predicted Normal
PWT	Posterior Wall Thickness
ROLLS	Role Of Low Lung function Study
RV	Residual Volume
SD	Standard Deviation
SV	Stroke Volume
TLC	Total Lung Capacity
TNF-α	Tumor Necrosis Factor-α
VC	Vital Capacity

Introduction

Chronic obstructive pulmonary disease (COPD) is according to WHO the third most common cause of mortality claiming 3 million lives world-wide in 2016 (www.who.int). COPD often coexist with cardiovascular disease (CVD) which in the same statistics is ranked as the number one cause of mortality. Age and smoking are the most important common risk factors for COPD and CVD and the latter is responsible for an estimated 480,000 preventable deaths each year in the USA alone [3]. COPD (and CVD as well) often go unnoticed for years in the preclinical or early stage of disease so arguably early disease detection is vital to improve prognosis [1, 2]. Plasma biomarkers (indicators of disease measured in blood) could be instrumental in this task and in recent years many new biomarkers have been described, but very few make it into clinical use [4]. This thesis deals with COPD and its most important comorbidity and risk factor and the role of new and established biomarkers of COPD and CVD.

General background

Chronic obstructive pulmonary disease

COPD is defined as "a preventable disease with some significant extra-pulmonary effects that may contribute to the severity in individual patient. Its pulmonary component is characterised by airflow limitation that is not fully reversible....usually progressive and associated with abnormal inflammatory response of the lung to noxious particles or gases" [5]. COPD is a common disease affecting 10-25 % of adults >40 years of age but is unfortunately severely underdiagnosed especially in the early stages of disease [6, 7]. COPD is diagnosed and staged according to the GOLD criteria which advocates a fixed post bronchodilator ratio of $FEV_1/FVC<0.7$. This is a simple and widespread measure, but due to a progressive age-related decline in FEV_1 it is known to over-diagnose COPD in elderly subjects [8]. As an alternative strategy the 5^{th} percentile of the FEV_1/FVC ratio (the lower limit of normal, LLN), which takes age and gender into account, has also been proposed [9].

The principal features of COPD are airflow obstruction and emphysema development. The airflow obstruction is primarily a result of wall thickening and mucus hyper secretion in the small conducting airways and is often accompanied by emphysema development [5, 10]. Emphysema is characterized by a progressive non-fibrotic destruction of the terminal airways leading to alveolar enlargement and hyperinflation due to the loss of elastic recoil of the lung [11]. What causes the disease is not completely understood but it is probably a multifactorial process triggered by inhaled particles that leads to a progressive and exaggerated inflammatory response [5].

COPD and smoking

Smoking is the most important preventable risk factor for COPD and CVD. Cigarette smoke contains an abundance of noxious particles that can cause direct injury to airway epithelial cells or elicit highly complex inflammatory responses involving a multitude of cytokines and proteases [5]. Lung function naturally declines with age [10], but cigarette smoke accelerates aging of the lungs [12] and furthermore promotes matrix destruction and cellular apoptosis [13] eventually leading to emphysema.

COPD and comorbidity

COPD is a pulmonary disease but it is associated with numerous other conditions, most notably lung cancer and CVD [14, 15]. Importantly, when concomitant CVD is present this increases morbidity and mortality [14, 16]. Chronic systemic inflammation is a common feature of COPD and CVD [17-19] and in this context smoking plays an important role [20]. Several inflammatory biomarkers have been associated with COPD but whether this is because of systemic inflammation or primarily reflects inflammation of the lung is unclear [5].

COPD and biomarkers

A biomarker can be defined as a measurement of any biological molecule that is associated with, and pathophysiologically related to, a relevant clinical outcome or disease [4]. Biomarkers can be related to a certain disease in several ways and pertain diagnostic or prognostic information or be valuable in assessing risk of disease, severity of disease or response to therapy [21]. In the routine clinical assessment of a CVD patient several biomarkers such as LDL, HbA1c, brain natriuretic peptide (BNP), troponin or hsCRP can be measured. In the case of COPD none are in clinical use all though COPD have been associated with many biomarkers of systemic inflammation (e.g. IL-6, IL-8, fibrinogen, hsCRP or TNF-

α) [22]. This illustrates an important problem with many biomarkers i.e. their lack of specificity [21, 23].

Specific background

Paper I

In paper I the aim was to explore the long-term effects of current and prior smoking on cardiac systolic and diastolic performance using transthoracic echocardiography. Smoking is known to have significant effects on heart rate, blood pressure and peripheral resistance and it is the most important common risk factor for COPD and CVD, but the effects on cardiac hemodynamics in cardiopulmonary "healthy " smokers is not well studied. Previous studies have mainly focused on the acute effects [24-27] and only a few studies have evaluated the chronic effect of smoking status on echocardiographic variables [28, 29].

Paper II

Brain natriuretic peptide (BNP) is produced in the left and right ventricles as a response to pressure and volume overload [30, 31]. It is well known that BNP is elevated in different states of cardiac disease [32-35], but BNP is also raised in patients with advanced COPD when pulmonary arterial hypertension (PAH) or cor pulmonale (CP) is present [36-38]. In these patients BNP has even been shown to be a prognostic marker [39-41]. While BNP has an established role in the diagnosis and management of left sided heart failure the clinical value of BNP measurements in patients with stable, less advanced COPD is questionable. Previous studies have shown BNP to be elevated in the acute setting [33, 37, 42] or in the setting of advanced COPD [36, 38, 40], but concomitant cardiac disease has rarely been accounted for. COPD. Thus the aim of this paper was to investigate whether BNP levels are influenced by COPD in subjects without cardiac disease.

Paper III

Matrix metalloproteinases (MMP´s) are a group of enzymes collectively involved in the degradation of extracellular matrix proteins [43]. In recent years, MMP´s have been associated with different pathophysiological aspects of COPD. Especially MMP-9, and to a lesser extent MMP-1,-10 and -12, have been implicated in the underlying disease mechanisms of small airways obstruction, emphysema development, mucus hypersecretion and inflammation [43-48]. So MMP´s could be

potential biomarkers of COPD. Some MMP´s are also known biomarkers of atherosclerotic disease [49, 50] and plaque development and rupture [51, 52] and furthermore, smoking is known to affect the concentration and activity of MMP´s [53-57]. Therefore, when evaluating MMP´s in the context of COPD, smoking and CVD need to be taken into consideration. Thus the aim of the paper III was to study the impact of smoking on selected MMP´s (MMP-1,-3,-7,-10 and -12) and to investigate their value as serum biomarkers of COPD and atherosclerosis.

Paper IV

Fibroblast growth factor 23 (FGF23) is a bone derived hormone governing phosphate metabolism that acts via renal receptors to decrease phosphate reabsorption [58-60]. In recent years though, FGF23 has been shown to influence numerous other tissues (e.g. heart, liver and blood cells), especially in the context of chronic kidney disease where circulating levels are high [61-64]. FGF23 receptors are also present in the lung and recently it has been shown that FGF23 and cigarette smoke can elicit bronchial inflammatory responses involving interleukin-1,-6 and -8 [65-67]. Intriguingly, FGF23 has a co-receptor (α-klotho) which possesses strong anti-ageing properties and α-klotho deficient mice develop premature ageing [68] and emphysema [69]. Furthermore, α-klotho has bronchial anti-inflammatory properties [66, 70] and it is down regulated in COPD subjects [71]. A comprehensive and unifying hypothesis describing the role of FGF23 and α-klotho in inflammatory lung disease still remains to be formulated, but FGF23 could potentially be a novel biomarker of COPD. Epidemiological data, however, on FGF23 and COPD are very scarce, so the purpose of paper IV was to explore whether FGF23 is associated with the presence of COPD or impaired pulmonary function.

Objectives

The main objectives of this thesis were:

1) To study the impact of smoking status on echocardiographic measurements of systolic and diastolic function in a population without clinically evident cardiopulmonary disease.
2) To examine whether BNP can be used as a biomarker of COPD in a population without systolic or diastolic dysfunction.
3) To evaluate MMP´s as biomarkers of COPD and atherosclerosis and the impact of smoking on MMP levels.
4) To explore whether FGF23 is associated with COPD and impaired pulmonary function.

Materials and methods

The papers in this thesis are based on the study population recruited for the Role Of Low Lung function Study (ROLLS) which took place at the Department of Clinical Physiology, Skåne University Hospital, Malmö between June 2004 and May 2007. The ROLLS study was approved by the local ethics committee of Lund University and a additional approval was obtained for subsequent biomarker analysis. All participants gave their written consent prior to enrolment.

Study population

In 2000, a questionnaire concerning smoking habits and respiratory symptoms was mailed to 11933 citizens aged 17-77 years in Malmö and surrounding communities. The response rate was 78.1 % and the 9316 responders served as a basis for recruiting subjects to the ROLLS study [72]. One of the objectives of this study was to investigate the impact of smoking and cardiovascular comorbidity in asymptomatic smokers and subjects with respiratory symptoms/disease. For that purpose 870 subjects were selected with the aim of including 120 never smokers and 560 smokers without respiratory symptoms and 190 subjects with a self-reported diagnosis of chronic bronchitis, emphysema or COPD. Of the 870 invited subjects 450 accepted the invitation and completed the one day study protocol consisting of transthoracic echocardiography, lung function tests, carotid ultrasound examination, electrocardiogram, resting blood pressure measurement, blood sample analysis and questionnaire. The examinations performed are described in details below

Methods

Lung function tests

Both spirometry and body plethysmography were performed according to the recommendations of the European Respiratory Society and European reference values were used [73]. A spirometer (Master Screen, Viasys GmbH - Erich Jaeger,

Hoechberg, Germany) was used to measure FEV_1 and vital capacity (VC), while TLC and RV were measured with a body plethysmograph (Master Screen, Viasys GmbH - Erich Jaeger, Hoechberg, Germany). All measurements were performed 15-45 minutes after inhalation of 1.0 mg of terbutaline (Bricanyl® Turbuhaler®). Diffusing capacity for carbon monoxide ($D_{L,CO}$) was measured using the single-breath technique (Master Screen, Viasys GmbH - Erich Jaeger, Hoechberg, Germany) [74] and the reference values for $D_{L,CO}$ were corrected for haemoglobin values according to established procedures [75]. For papers II-IV the diagnosis of COPD and severity staging was performed according to the 2010 recommendations by GOLD (Global initiative for chronic Obstructive Lung Disease). For diagnosis a post-bronchodilator $FEV_1/VC<0.7$ is required and severity is graded using FEV_1. Stage I (mild) is defined as FEV_1 >80% of predicted normal (PN), stage II (moderate) as $80\% \geq FEV_1 > 50\%$ PN, stage III severe as $50\% \geq FEV_1 \geq 30\%$ PN and stage IV (very severe) as $FEV_1 < 30\%$ PN. For paper I a stageing procedure was not performed but instead an age-adjusted FEV_1 /FVC ratio below the 5^{th} percentile was also required for a diagnosis of COPD [8].

Smoking habits

All participants filled out a detailed questionnaire regarding smoking habits and tobacco consumption. Current smokers and subjects reporting smoking with in the last 12 months were classified as current smokers. Subjects who reported previous smoking on a daily basis for more than one month, but not within the last 12 months were classified as ex-smokers. Finally subjects who had never smoked or who reported smoking for less than one month were classified as never-smokers. The term ever-smokers incorporates both current smokers and ex-smokers. Tobacco consumption was calculated in pack years which is defined as 20 cigarettes a day for a year. Smoking habits and consumption were entirely based on self-reporting.

Transthoracic echocardiography

Transthoracic echocardiography was performed by experienced sonographers on a Sonos 5500 (Philips, Andover, MA, USA). The participants had been resting for 15 minutes prior to the examination. A single observer performed all echocardiography measurements three times on separate cardiac cycles, and the mean value was used in all analyses. The following measurements were made in 2-dimensional echocardiography from a parasternal long-axis view: interventricular septum thickness (IVS), left ventricular end-diastolic diameter (LVEDD) and posterior wall thickness (PWT). Left ventricular mass (LV mass) was calculated as LV mass = 0.8 x 1.04 x $[(LVEDD+IVS+PWT)^3 - (LVEDD)^3]$ + 0.6g [76]. Moreover, left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume

(LVESV), and left atrial maximal volume (LA) were calculated using the biplane summation of disks method from apical 4 and 2 chamber views [76]. Cardiac dimensions were indexed for body surface area (BSA) to adjust for differences in body size. Pulsed Doppler measurements were acquired from the apical four-chamber view at the tip of the mitral leaflets. Peak early diastolic (E) and late diastolic (A) mitral flow velocities and deceleration time of E-wave (DT) were measured, and E/A ratio was calculated. Pulsed tissue Doppler of the septal and lateral mitral annulus was obtained from the apical four-chamber view. Peak early diastolic mitral annular velocities (e´) were measured both at the septal and lateral annulus. The ratio for E/e´ was calculated, both for the septal and the lateral wall, and with e´ averaged from the septal and lateral wall [77]. The E/e´ ratio together with left atrial size was used to estimate left atrial filling pressures [78]. Left ventricular outflow tract velocity time integral (LVOT-VTI) and left ventricular stroke volume (SV) were measured, left ventricular cardiac output (CO), and cardiac index (CI) were calculated according to contemporary guidelines [79]. In order to control for the impact of respiration, all pulsed Doppler and tissue Doppler measurements were performed at respiratory arrest after an end-expiration [80].

Ultrasonography of the carotid artery

Carotid ultrasound examinations were performed on an Acuson Sequoia system by experienced sonographers following a standardized protocol used for routine clinical evaluation with two-dimensional and Doppler imaging. This allows for visualization of the distal segments of the common carotid artery, the bifurcation and the proximal and medial segments of the internal carotid artery (ICA). For study purposes, a plaques was defined as a regional thickening of 2 mm or more relative to adjacent segments and protruding in to the arterial lumen, and was treated as a dichotomous variable.

Blood samples and biomarker analysis

Blood samples were drawn at resting and non-fasting condition. Total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and glycated haemoglobin (HbAlc) were measured using routine methods. Creatinine was determined using Jaffe`s method and estimated glomerular filtration rate (eGFR) was calculated using the MDRD formula for SI units: $175x\ (creatinine/88.4)^{-1.154}\ x\ (age)^{-0.203}\ x\ (0.742\ if\ female)$ [81]. In order to correct for actual body surface area this was multiplied by 1.73/BSA.

Plasma BNP was measured with the Triage BNP Assay from Biosite Inc, San Diego, CA using the UniCel™ immunoassay System from Beckman Coulter Inc, Brea, Ca. Lower limit of detection was 1 pg/mL (0.29 pmol/L).

Plasma EDTA samples were stored in -80 °C. MMP-1,-3,-7,-10,-12 and FGF23, IL-1, IL-6 and IL-8 were analysed by the Proximity Extension Assay technique using the Proseek Multiplex CVD 96x96 reagents kit (Olink Bioscience, Uppsala, Sweden). The coefficients of variance (CoV) of the various biomarkers are as follows (intra- and inter-assay variation): MMP-1 (5%, 19%), MMP-3 (9%, 14%), MMP-7 (7%, 11%), MMP-10 (5%, 28%), MMP-12 (8%, 10%), FGF23 (9%, 26%), IL-1 (7%, 18%), IL-6 (6%, 8%) and IL-8 (6%, 15%) . Data are presented as arbitrary units (AU). Values can be transformed to actual concentrations using transformation algorithms on the Olink Bioscience website (www.olink.com). The conversion, however, is not exact [82, 83].

Blood pressure

Blood pressure was measured manually in supine position under resting condition and the average of two measurements was used. Hypertension was defined as systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg.

Statistical analyses

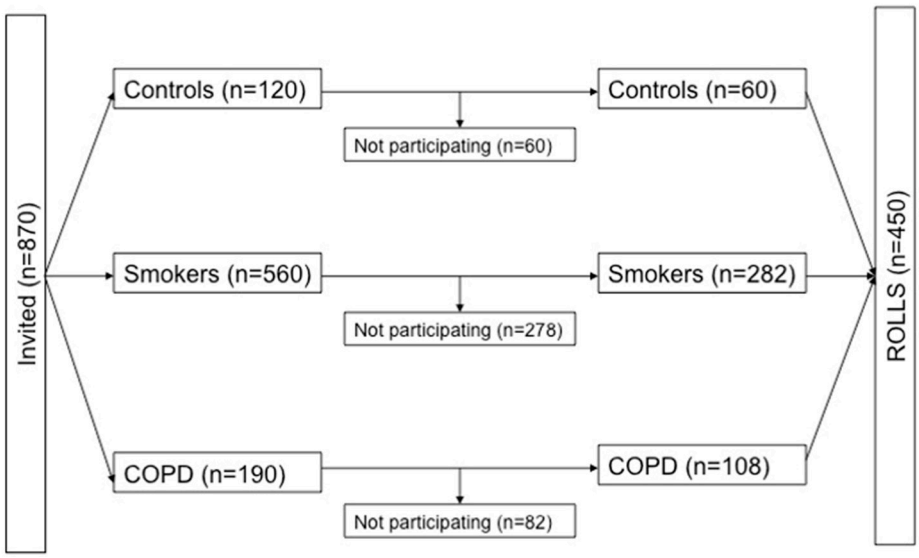
All statistical analyses were performed by the principal author aided by the co-authors using Statistica version 12 and 13 (papers I and II) and SPSS version 24 (paper III and IV). In all papers, continuous variables and their distributions are presented as means with standard deviations (SD) and categorical variables are characterized by percentages or numbers. Positively skewed distributions (BNP) were log-normalized to obtain normal distribution (paper II). Student´s *t*-test (paper II and IV) and ANOVA/ANCOVA analyses (paper I-III) were used for group comparison of parametric data and chi^2 for non-parametric data. In paper II and IV multivariable linear regression analyses were used for assessing associations. Bivariate (paper IV) and multivariate (paper III) logistical regression models were used for predicting group affiliations. Bonferroni or Scheffe was used as post hoc test to adjust for multiple comparisons and generally, whenever possible, age-adjusted p-values were reported.

Results and discussion

Study group

As previously mentioned all papers in this thesis are based on the ROLLS study population and therefore some general considerations about the inclusion procedure, which has been briefly described in the Material and Methods section, are warranted. The participation was invitational and based on the response received from a prior questionnaire. As illustrated by Figure 1 the inclusion was based on a mixture of perceived respiratory symptoms, self-reported respiratory diagnosis and self-reported smoking status.

Figure 1
Diagram illustrating the inclusion procedure of ROLLS. Reprinted with kind permission from Sophia Frantz.



The objective was to create a reasonably sized control group and a large number of asymptomatic smokers in order to study COPD and CVD in the early stages of disease. The dropout rates were similar across the three invited groups (50%, 50% and 57 % respectively). A drop out analyses showed that the vast majority either did not respond (n=283) or declined the invitation (n=102).

At the study visit some expected reclassification took place. In the group of symptomatic subjects (n=108) only 34 subjects fulfilled the GOLD criteria for COPD but on the other hand in the group of "healthy" smokers (n=282) 98 subjects had spirometric evidence of airflow obstruction. Since four years had passed between the questionnaire and the inclusion some participants had stopped smoking and were now ex-smokers.

Table 1 displays selected background data that characterizes the study population in total and stratified by smoking status. The age ranges from 46 to78 years with a mean of 61.5 years and a slight female preponderance (59%). Current smokers constitute 49 % with a sizeable group of ex-smokers with relatively heavy prior cigarette consumption (mean of 24 pack years). In both current and ex-smokers $D_{L,CO}$ is markedly reduced as a sign of impaired gas exchange The majority of COPD subjects (n=132) are in the early stages of disease (stage I 67%, stage II 28 %, and stage III and IV 5%). As expected in this age group the prevalence of carotid plaque and hypertension were quite high (51 and 39% respectively). Ejection fraction (EF) was normal.

Table 1.
General characteristics of the study population stratified by smoking status. Continuous variables are presented as means ±standard deviation (SD). Categorical variables are presented as numbers and percentages.

	Never-smokers (n=89)	Ex-smokers (n=141)	Current smokers (n=220)	Total (n=450)
Sex (men/women)	31/58	58/83	96/124	185/265
Age (yrs)	62.3±8.2	62.2±7.3	60.7±7.5	61.5±7.6
Pack years	0	24±17***	30±18***	22±19
BMI (kg/m^2)	26.3±4.9	28.2±5.1**	25.5±4.8	26.5±5.0
HbA1c (%)	4.6±0.4	4.8±0.8**	4.7±0.7*	4.7±0.7
LDL (mmol/L)	3.8±0.9	3.7±1.0	3.8±1.0	3.8±1.0
Hypertension	47 (53%)	55 (39%)*	72 (33%)**	174 (39%)
COPD	7 (8%)	51 (37%)***	74 (34%)***	132 (30%)
FEV_1 (%PN)	109±17	97±19***	97±16***	99±18
$D_{L,CO}$ (% PN)	90±13	83±19**	78±16***	82±17
Carotid plaque	29 (33%)	68 (48%)*	134 (61%)***	231 (51%)
LVEF (%)	66.5±5.2	66.3±4.7	65.2±5.0	65.8±5.0

General linear model (ANOVA). * Indicates p<0.05, ** indicates p<0.01 and *** indicates p<0.001 compared to never-smokers.

Echocardiographic consequences of smoking status (paper I)

The objective of paper I was to explore the echocardiographic consequences of smoking status. For this purpose, subjects with prevalent cardiac disease or moderate-severe COPD were excluded. The diagnosis of COPD was based on the spirometry findings (n=132) but an FEV_1 /VC ratio below the 5th percentile was also required for exclusion (n=70) Cardiac exclusion criteria were history of cardiovascular diseases, valvular dysfunction and regional left ventricular hypokinesia or reduced left ventricular ejection fraction on the echocardiographic examination (n=19). Another 6 subjects were excluded due to digital storage failure (n=5) and inadequate acoustic window (n=1). Subjects on stable medication for hypertension (n=70, 20%) were not excluded.

The characteristics of the remaining 355 subjects stratified by smoking status are displayed in Table 2. The current smokers were significantly younger than never-smokers with a higher tobacco consumption and a lower blood pressure.

Table 2
Clinical variables of the study population. Continuous variables are presented as means ±SD. Categorical variables are presented as numbers

Variable	Never-smokers (n=81)	Ex-smokers (n=102)	Current smokers (n=172)
Sex (male/female)	28/53	43/59	73/99
Age (yrs)	62.2±8.2	60.9±7.5	59.9±7.2*
Height (cm)	168±9	170±9	170±9
Body mass (kg)	74.8±16.5	81.0±15.9*	74.5±16.4
BMI (kg/m^2)	26.3±4.9	28.1±5.2*	25.8±5.0
BSA (m^2)	1.8±0.2	1.9±0.2*	1.8±0.2
Systolic BP (mmHg)	142±18	139±16	133±16***
Diastolic BP (mmHg)	79±11	78±10	76±11*
Pack years	0±0	22.1±15.5*	28.4±15.7*

General linear model (ANCOVA) with age-adjusted p-values. * Indicates p<0.025 compared to never smokers.

As previously described complete lung function tests and thorough transthoracic echocardiograms were performed in all subjects and results are summarized in Table 3.

Table 3
Data on echocardiography and lung function tests. Values are presented as means ±SD.

Variable	Never smokers (n=81)	Ex-smokers (n=102)	Current smokers (n=172)
Cardiac index(L/min/m^2)	2.42±0.49	2.54±0.54	2.61±0.52**
CO (L/min)	4.4±1.00	4.86±1.18*	4.81±1.01*
SV (mL)	70±16	73±15	71±14
Heart rate (bpm)	64±10	67±9	68±10**
LVOT diameter (cm)	19.4±1.8	19.9±1.8	19.8±1.7
LVOT vti (cm)	23.4±3.7	23.4±3.5	22.9±3.8
LVM/BSA (g/m^2)	78±20	79±18	76±15
LVEDV/BSA (mL/m^2)	55±11	53±10	55±9
LVESV/BSA (mL/m^2)	18±5	18±4	19±5
LA/BSA (mL/BSA)	30±8	29±6	28±6
E/e´avg	10.6±2.6	9.9±2.7	9.5±2.6**
E (cm/s)	76±16	74±14	74±15
e´avg (cm/s)	7.7±2.0	8.0±1.6	8.4±2.0*
E/A	1.0±0.2	1.0±0.2	1.0±0.3
DT (ms)	240±58	230±41	232±42
$D_{L,CO}$ (%PN)	90±13	88±16	80±15***
VC (%PN)	115±16	110±16	109±14**
FEV_1 (%PN)	110±16	103±14**	101±14***
FEV_1/VC (%PN)	102±6.5	100±7.6*	98±7.1***
RV (%PN)	106±18	100±16*	106±22
TLC (%PN)	105±12	102±12	102±11

General linear model (ANCOVA) with age-adjusted p-values. * Indicates p<0.025, **p<0.01 and *** p<0.001 compared to never smokers.

Current smokers exhibited a significantly higher cardiac index (2.61±0.52 L/min/m²) as compared to never-smokers (2.42±0.49 L/min/m²) with ex-smokers

falling in between (2.54±0.54 L/min/m²). This was due to a higher heart rate in current smokers as the stroke volumes did not differ between groups.

Never-smokers had a slightly lower e´, which in part could be an age-related finding since e´ declines with advancing age. Apart from that, no other significant difference in any other echocardiographic variable could be detected.

Smokers had significantly lower diffusing capacity, FEV_1, VC and FEV_1/VC as compared to never-smokers whereas ex-smokers had significantly lower FEV_1 and FEV_1/VC as compared to never-smokers. These results were expected as they are signs of subclinical deterioration of lung function in ever-smokers. There were no significant correlations between CI and lung function variables to confound the differences in CI described above (data not shown).

Interestingly, smokers showed significantly lower systolic and diastolic blood pressure which is an unexplained but not uncommon epidemiological finding [84].

Impact of smoking on cardiac function

Cigarette smoking has many short and long-term effects on the cardiovascular system and on cardiac hemodynamics [85-89]. The latter can be broken down into effects on diastolic and systolic cardiac function, heart rate, peripheral vascular resistance and blood pressure. Cardiac output indexed for body surface area (cardiac index, CI) is a relevant but often neglected parameter of global cardiac performance as it encompasses several of the above mentioned components. In this study, a small but significant increase in heart rate resulted in a higher CI in current smokers, which is a previously unreported finding. Acute nicotine exposure stimulates sympathetic nerve activity and increases heart rate [90, 91] so even though the subjects were instructed to refrain from smoking on the day of examinations the increase in CI could possibly be an effect of acute nicotine exposure.

Prior studies on the acute effects of smoking on diastolic function have quite concordantly found a prolonged deceleration time (DT) and an increase in late mitral inflow (A) leading to a decrease in E/A ratio [24-27, 92, 93]. The results from studies of the chronic effects of smoking on diastolic function are more ambiguous, but also generally points to impaired relaxation of the left ventricle [27, 94, 95].The data presented here could not support these findings. On the contrary, no smoking related differences in left atrial volume, left ventricular mass, size and function or E, E/A, DT could be detected. Evaluating diastolic function with echocardiography is notoriously troublesome, because of the difficulties in obtaining, measuring and interpreting data, and as a consequence new variables and staging algorithms are often proposed. In the last decade the development of speckle tracking echocardiography and myocardial deformation analysis have led to improved sensitivity for detection of subclinical alteration of systolic and diastolic myocardial

function [96, 97]. These analyses were not performed in the present study and could arguably limit the diastolic assessment.

In conclusion, no convincing effect of current or prior smoking on echocardiographic variables of systolic and diastolic function could be detected, even if smokers had significantly higher CI due to a slightly increased heart rate.

Brain natriuretic peptide levels in middle aged subjects with normal left ventricular function in relation to mild-moderate COPD (paper II)

The aim of paper II was to evaluate the levels of BNP in COPD subjects with normal cardiac function. For this purpose subjects with a history of cardiac disease or EF<55% (n=26), echocardiographic signs of elevated left sided filling pressure (n=75) or advanced COPD with GOLD stage III and IV (n=5) were excluded. The E/e´ ratio together with left atrial size was used to estimate left atrial filling pressures [78] which was the proposed algorithm at that time. Thirteen subjects were excluded due to missing data, so the final population (n=331) consisted of 245 controls and 86 subjects with COPD (Gold stage I/II, 65/21). Table 4 summarizes the data.

Table 4
Characteristics of the population. Continuous variables are presented as means ±SD. Categorical variables are presented as numbers. P-values are derived from unpaired Student`s *t*-test or chi^2.

Variable	Control (n=245)	COPD (n=86)	P-value
Sex (male/female)	95/150	45/41	0.03
Age (yrs)	59.3±7.5	63.4±6.6	<0.001
BSA (m^2)	1.9±0.2	1.9±0.2	0.20
Heart rate (bpm)	67±10	69±11	0.11
Pack years	18.9±16.9	31.5±22.0	<0.001
Medication (yes/no)	61/184	29/57	0.34
Hypertension (yes/no)	88/157	25/61	0.22
BNP (pmol/L)	8.0±7.1	8.8±8.1	0.43
LnBNP (pmol/L)	1.8±0.7	1.9±0.7	0.47
Hemoglobin (g/L)	138±11	141±11	0.88
Creatinine (µmol/L)	72±15	76±24	0.08
FEV_1 (%PN)	106±14	90±14	<0.001
FEV_1/VC (%PN)	102±6	84±7	<0.001
RV (%PN)	102±19	119±22	<0.001
TLC (%PN)	102±11	109±12	<0.001
RV/TLC (%PN)	95±13	102±13	<0.001
$D_{L,co}$ (%PN)	85±14	77±18	<0.001

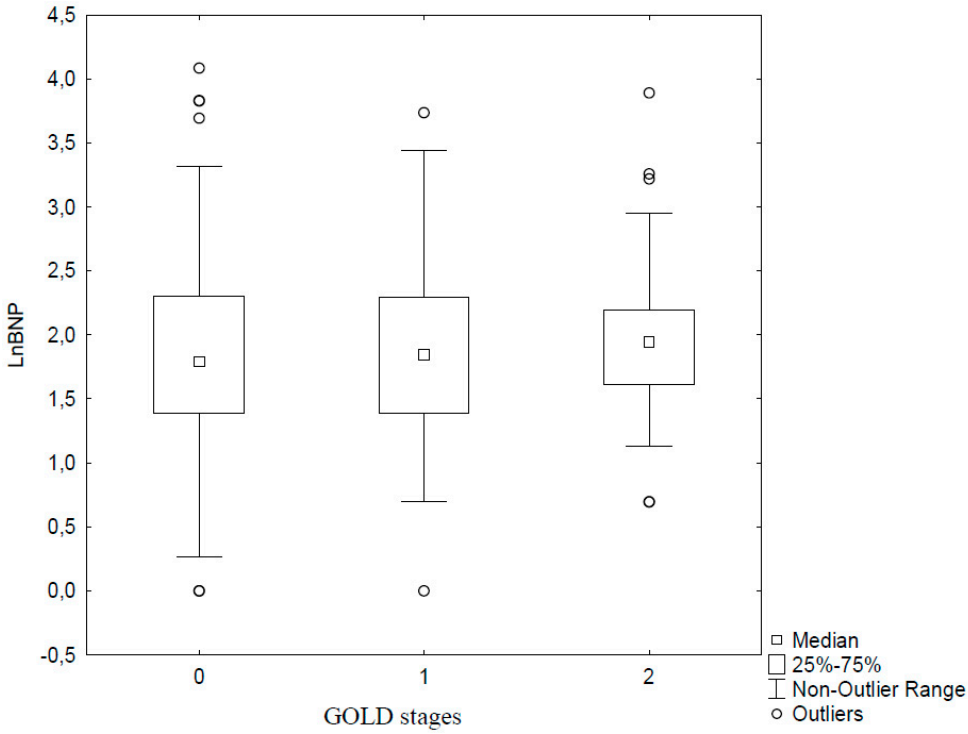
The COPD subjects were significantly older, with higher tobacco consumption and a relative male preponderance. As expected the COPD group had impaired pulmonary function with significantly increased RV and TLC and reduced $D_{L,CO}$. Importantly, lnBNP did not differ between the two populations (p=0.47). BNP levels are known to be influenced by numerous factors such as age, sex and heart rate [98], and BMI [99]. Therefore all variables that correlated with lnBNP (p<0.10) in linear regression analyses were tested in a multivariable setting together with pack years and all pulmonary variables. Table 5 displays the results.

Table 5
Multivariable linear regression analysis with lnBNP as dependent variable (n=326).

Variable	β*	p-value
Left atrial volume (mL)	0.34	<0.001
Age (yrs)	0.29	<0.001
Hemoglobin (g/L)	-0.17	0.005
BSA (m^2)	0.23	0.003
Sex	0.10	0.20
Left ventricular mass (g)	0.07	0.35
Heart rate (beats per minute)	-0.10	0.05
RV (%PN)	0.08	0.61
TLC (%PN)	-0.18	0.42
FEV_1 (%PN)	-0.02	0.85
VC (%PN)	0.13	0.44
$D_{L,co}$ (%PN)	-0.09	0.17
Pack years	0.03	0.64

Age, left atrial volume, BSA (positively), and haemoglobin (negatively) remained significantly associated with lnBNP. The severity of COPD did not affect BNP levels as illustrated in Figure 2.

Figure 2
Box-and whisker plot of lnBNP stratified by presence and severity of COPD.



BNP in COPD and heart failure

BNP is elevated in patients with impaired systolic or diastolic cardiac function as a result of increased preload and afterload [100, 101]. A normal BNP value is known to have a high negative predictive value and can be used to reliably rule out heart failure in patients presenting with dyspnoea [101, 102]. Regarding COPD several studies have shown that BNP is elevated and a marker of disease severity and prognosis [40, 103], especially, in the case of more advanced disease with elevated pulmonary artery pressure and impaired right ventricular function [36, 38, 40, 104]. In this scenario, the BNP elevation is probably due to increased preload and afterload in the right atrium and ventricle [38, 105]. Exertional dyspnoea is a common presenting symptom in patients with heart failure or COPD, and it can be clinically challenging to interpret BNP results in these patients bearing in mind that COPD and CVD often coexist [14, 15]. In the acute setting BNP can be used to discriminate between cardiac and pulmonary causes [37, 42] if higher cut off values

are chosen. In the outpatient setting smaller studies have reported elevated BNP levels but in more advanced disease and not always accounting for cardiac comorbidity [36, 103, 104]. In the present study care was taken to minimize the influence of cardiovascular disease by excluding subjects with depressed ejection fraction and/or signs of elevated filling pressure. Furthermore, spirometry was used to ensure a correct COPD diagnosis and staging. These features make this study unique and in this population, which could mimic an outpatient setting of symptomatic or asymptomatic smokers, BNP measurements could not rule out pulmonary disease.

In conclusion, no significant differences in BNP levels between subjects with mild-moderate COPD and controls could be detected in a population with normal cardiac function. Thus, the clinical value and discriminative power of BNP in this setting is limited.

Matrix Metalloproteinases in COPD and atherosclerosis with emphasis on the effects of smoking (paper III)

In paper III the aim was to explore whether MMP-1,-3,-7,-10 and -12 could be associated with COPD and atherosclerosis, when taking smoking habits into account. The study group consisted of 417 participants as 33 subjects were excluded due to missing data on biomarkers (n=22), $D_{L,CO}$ (n=6) or lipids (n=5). The participants were subsequently divided into four different groups according to the presence or absence of COPD or plaque in the carotid artery. Group I (n=157, no plaque and no COPD), group II (n=136, plaque but no COPD), group III (n=43, COPD but no plaque) and group IV (n=81, plaque and COPD). Of the 124 COPD subjects, the majority were in the mild stages of disease (GOLD I, n=84, II, n=35, III, n= 4, IV, n= 1). For study purposes plaque in the carotid artery was used as a surrogate measure of atherosclerosis. The clinical characteristics of the total population and the different groups are displayed in Table 6.

Table 6
Data on anthropometrics, pulmonary, clinical and biochemical variables. Group I (no plaque or COPD), group II (plaque without COPD), group III (COPD without plaque), group IV (plaque and COPD). Values are mean ±SD or numbers.

Variable Group	I (n=157)	II (n=136)	III (n=43)	IV (n=81)	Total (n=417)
Sex (male/female)	47/110	63/73*	19/24	45/36**	174/243
Age (yrs)	58±7.3	63±7.1***	63±7.1***	66±6.2***	62±7.6
BMI (kg/m^2)	27±5.3	27±5.2	26±4.5	26±4.6	27±5.1
Smoking (n/ex/cu)	48/49/60	24/37/75**	4/19/20	3/28/50***	79/133/205
Pack years	14±14	24±18***	23±16*	35±23***	23±19
Systolic BP (mmHg)	134±17	141±18*	137±20	143±20**	138±18
HbA1c (%)	4.6±0.4	4.8±0.8	4.7±0.5	4.9±0.9*	4.7±0.7
LDL (mmol/L)	3.8±0.9	3.8±1.0	4.0±0.8	3.6±1.1	3.8±1.0
FEV_1/VC (%PN)	102±6	101±6	83±9***	81±11***	96±12
RV (%PN)	100±17	105±20	123±21***	118±26***	108±22
TLC (%PN)	102±11	102±11	112±12***	105±13	104±12
RV/ TLC (%PN)	94±11	97±14	103±17**	105±17***	98±14
$D_{L,CO}$ (%PN)	87±14	83± 16	81± 21	70±15***	82±17
MMP-1 (AU)	1.25±0.86	1.46±0.95	1.57±0.94	1.71±0.85 **	1.44±0.91
MMP-3 (AU)	1.85±0.79	1.96±0.73	1.98±0.94	2.23±0.84 **	1.97±0.81
MMP-7 (AU)	5.85±0.61	6.09±0.71*	5.85±0.51	6.17±0.74 **	5.99±0.67
MMP-10 (AU)	7.54±0.70	7.59±0.69	7.49±0.56	7.53±0.77	7.55±0.70
MMP-12 (AU)	6.46±0.70	6.90±0.75 ***	6.55±0.77	7.20±0.89 ***	6.76±0.81

ANOVA were used for calculating p-values. Scheffe was used as post hoc test for multiple comparisons. * Indicates p<0.05, ** indicates p<0.01 and *** indicates p<0.001 compared to group I

Smoking status was significantly skewed with a majority of smokers in groups II-IV and very few never-smokers in groups III and IV (9% and 4% respectively). Consequently, tobacco consumption was also significantly higher in groups II-IV. Furthermore, subjects in groups II-IV were older and with a relative male predominance. Importantly, all MMP levels (except MMP-10) were significantly elevated in group IV and MMP-7 and-12 were also higher in subjects with plaque.

All MMP´s correlated positively with tobacco consumption and age (MMP-3,-7 and -12). Interestingly, MMP-1 and -12 correlated negatively with FEV_1 and $D_{L,CO.}$ (data published in paper III, Table 2).

Smoking status, both current and prior smoking, significantly influenced all MMP´s (except MMP-3) as displayed in Table 7.

Table 7
ANCOVA analysis of MMP values stratified by smoking status with age-adjusted p-values. Bonferroni was used as post hoc test due to multiple comparisons. Values are mean ±SD

	Never-smokers (n=79)	**Ex-smokers (n=133)**	**Current smokers (n=205)**
MMP-1 (AU)	1.16±0.96	1.31±0.88¤¤	1.64±0.86***
MMP-3 (AU)	1.89±0.82	2.11±0.90*	1.92±0.72
MMP-7 (AU)	5.83±0.56	5.98±0.69	6.06±0.67**
MMP-10 (AU)	7.30±0.60	7.46±0.73¤¤	7.69±0.68***
MMP-12 (AU)	6.40±0.68	6.62±0.81*, ¤¤¤	6.98±0.79***

* Indicates p<0.05, ** indicates p<0.01 and *** indicates p<0.001 compared to never-smokers. ¤ Indicates p<0.05, ¤¤ indicates p<0.01 and ¤¤¤ indicates p<0.001 compared to current smokers

Finally, a multivariate logistic regression model was build using known risk factors for CVD and COPD (age, gender, smoking status, pack years, blood pressure, HbA1c and LDL) with subsequent addition of the MMP´s individually. The results are shown in Table 8. Age, smoking, pack years and systolic blood pressure were strong predictors of combined CVD and COPD, but even MMP-1 and MMP-12 contributed significantly to the standard model with odds ratios of 1.64 and 1.60 respectively.

Table 8
Multivariate regression analysis with group comparison. Group I (n=157, no plaque and no COPD), group II (n=136, plaque but no COPD), group III (n=43, COPD but no plaque) and group IV (n=81, plaque and COPD). MMPs were added individually to the standard model that included sex, age, smoking status, pack years, systolic BP, HbA1c and LDL.

Group	II vs I OR (95% CI)	III vs I OR (95% CI)	IV vs I OR (95% CI)
Male sex	1.34 (0.78-2.33)	1.40 (0.73-3.42)	1.40 (0.63-2.53)
Age (per year)	1.10 (1.06-1.14)***	1.12 (1.06-1.18)***	1.17 (1.11-1.23)***
Ex-smokers vs never-smokers	0.87 (0.36-2.12)	3.33 (0.91-16.66)	3.44 (0.79-15.03)
Current vs never-smokers	1.73 (0.68-4.41)	3.81 (0.96-14.85)	6.78 (1.39-28.38)*
Pack years (per year)	1.04 (1.02-1.07)***	1.03 (1.00-1.06)	1.06 (1.03-1.09)***
Systolic BP (per mmHg)	1.03 (1.01-1.04)**	1.01 (1.00-1.03)	1.03 (1.01-1.05)**
HbA1c (per %)	1.00 (0.67-1.65)	0.85 (0.45-1.71)	1.22 (0.72-1.91)
LDL (per mmol/L)	0.96 (0.73-1.26)	1.34 (0.88-1.87)	0.79 (0.57-1.13)
MMP-1 (per unit)	1.26 (0.94-1.70)	1.48 (0.99-2.22)	1.64 (1.13-2.36)**
MMP-3 (per unit)	0.70 (0.46-1.02)	0.61 (0.42-1.22)	0.95 (0.55-1.44)
MMP-7 (per unit)	1.34 (0.86-2.01)	0.69 (0.41-1.37)	1.27 (0.76-2.06)
MMP-10 (per unit)	0.90 (0.61-1.30)	0.69 (0.44-1.28)	0.70 (0.40-1.08)
MMP-12 (per unit)	1.37 (0.93-2.02)	0.79 (0.46-1.32)	1.53 (1.01-2.56)*

* Indicates $p<0.05$, ** indicates $p<0.01$ and *** indicates $p<0.001$ compared to group I.

MMP´s as biomarkers of COPD, atherosclerosis and smoking

As mentioned in the Introduction MMP´s have been associated with both CVD [49-52, 106-108] and COPD [43-48] and are known to be influenced by smoking status [53-57]. In the present study MMP-1, -3, -7, -10 and-12 were scrutinized regarding these associations. MMP-1, (which degrades collagen) and MMP-12, (which degrades elastin) are of special interest as they have been strongly implicated in the development of smoke-induced emphysema in animal models [54, 109, 110], and have been found to be elevated in COPD subjects [53, 111, 112]. Generally, the data confirmed that MMP-1, -7 and -12 are associated with atherosclerosis and concomitant COPD, and that all MMP´s (except MMP-3) are influenced by

smoking status. The latter finding is not surprising since MMP´s are biomarkers of inflammation, but should be considered when studying MMP´s in the context of atherosclerosis and COPD. What this study adds to the current knowledge is that MMP-1 and -12 independently contribute to disease prediction and correlate with signs of impaired gas exchange in the lungs.

In conclusion, MMP´s are influenced by smoking status and MMP-1 and MMP-12 are independent predictors of concomitant COPD and carotid plaque in multivariate regression models. Thus, MMP-1 and MMP-12 carry information about pulmonary and vascular disease even when accounting for traditional risk factors, especially smoking habits.

Fibroblast growth factor 23 is an independent marker of COPD and is associated with impairment of pulmonary function and diffusing capacity

In paper IV the objective was to explore whether FGF23 was associated with COPD and impairment of pulmonary function. From the ROLLS population 44 participants were excluded due to missing data on blood biomarkers (n=22), lung function measurements (n=7) or renal impairment with estimated glomerular filtration rate (eGFR) <45ml/min/m^2 (n=15). Based on the spirometry results the remaining 406 participants were divided into a COPD group (n=117) and a non-COPD group (n= 289). The majority of COPD subjects were in the mild to moderate stages of disease (GOLD I, n=81, II, n=31, III, n= 4, IV, n=1). The clinical characteristics of the two groups and the total population are displayed in Table 9.

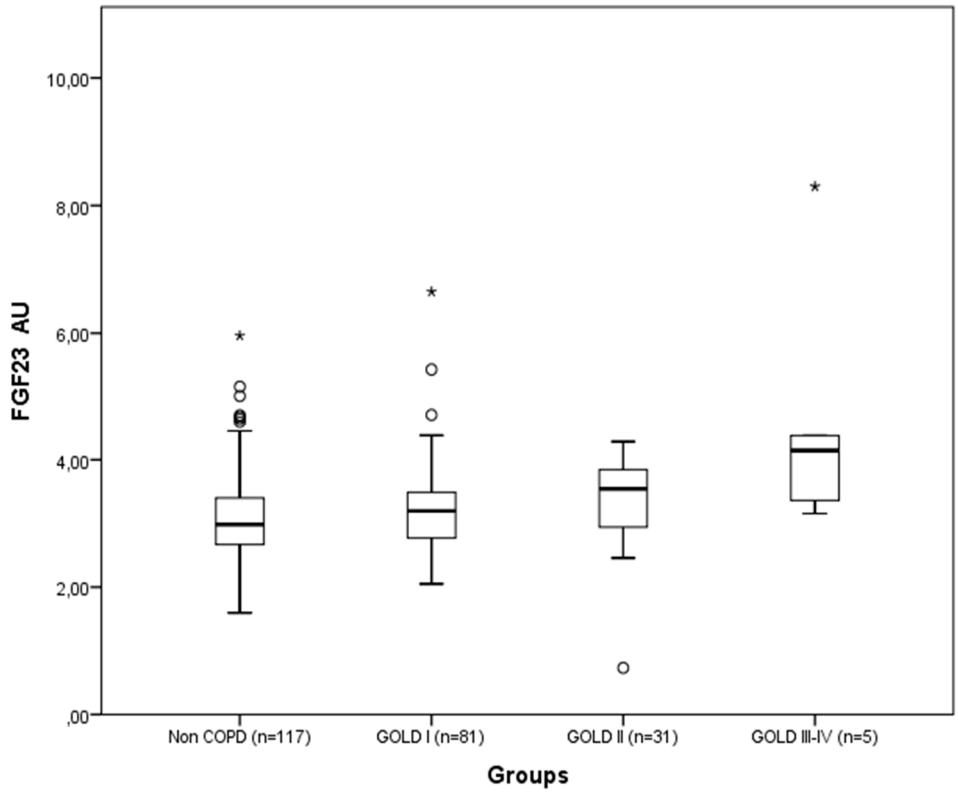
Table 9
Data on anthropometrics, pulmonary and clinical variables according to COPD status. Values are mean ±SD or numbers. Student`s *t*-test or chi ^{2} was used for calculating p-values.

Variable	Non-COPD (n=289)	COPD (n=117)	Total (n=406)
Sex (male/female)	109/180	59/58*	168/238
Age (yrs)	60.1±7.5	64.5±6.6***	61.4±7.5
BMI (kg/m^2)	26.7±5.1	26.1±4.6	26.5±4.9
Systolic BP (mmHg)	137±17	141±20	138±18
Pack years	18.7±16.9	31.3±21.8***	22.0±18.8
Ever-smokers (yes/no)	218/71	110/7***	328/78
FEV_1 (%PN)	105±14	86±18***	100±17
VC (%PN)	110±15	111±17	111±15
FEV_1/VC (%PN)	102±6	82±11***	96±12
RV (%PN)	102±19	121±24***	108±22
TLC (%PN)	102±11	108±13***	104±12
RV/TLC (%PN)	96±13	105±16***	98±14
eGFR (mL/min/m^2)	79.7±17.8	76.7±16.8	78.8±17.5
FGF23 (AU)	3.1±0.6	3.3±0.8***	3.1±0.7
IL-1 (AU)	4.1±0.6	4.1±0.6	4.1±0.6
IL-6 (AU)	4.2±0.9	4.3±1.0	4.2±0.9
IL-8 (AU)	4.9±0.7	5.1±0.8	5.0±0.8

* Indicates p<0.05, ** indicates p<0.01 and *** indicates p<0.001 compared to non-COPD.

The COPD subjects were significantly older, predominantly male and with a higher tobacco consumption and decreased pulmonary function. Estimated GFR did not differ between the groups. Importantly, FGF23 levels were higher in COPD subjects while IL-1, IL-6 and IL-8 levels were unaffected by group status. There was a trend for FGF23 to increase with increasing disease severity as displayed in Figure 3. This, however, was not statistically significant after adjusting for age and multiple comparisons (Bonferroni).

Figure 3
Box-and-whisker plot of FGF23 stratified by presence and severity of COPD.



Inter-quartile ranges with ° and * representing outliers. AU denotes arbitrary units.

FGF23 correlated weakly with age, tobacco consumption, systolic blood pressure and creatinine and weak to moderate correlations existed with IL-1,-6,-8 and with variables of pulmonary function, the strongest being for $D_{L,CO}$. (β= -0,30, see paper IV, Table 2). In multivariable linear regression analyses with pulmonary variables added individually to a standard model consisting of age, eGFR, blood pressure and pack years, FGF23 remained significantly associated with FEV_1, VC, RV/TLC and $D_{L,CO}$ (Table 10). Again the strongest relationship was with $D_{L,CO}$ (β= -0.33, $p<0.001$).

Table 10
Relationships between FGF23 and pulmonary variables with adjustments for age, eGFR, systolic blood pressure and pack years. Linear regression models with FGF23 as the dependent variable.

Variable	Standardized β	p-value
TLC (%PN)	-0.056	0.25
RV (%PN)	0.025	0.60
RV/TLC (%PN)	0.120	0.02
VC (%PN)	-0.110	0.03
FEV_1 (%PN)	-0.194	<0.001
FEV_1/VC (%PN)	-0.125	0.01
$D_{L,CO}$ (%PN)	-0.325	<0.001

Table 11 displays the results of multivariable logistic regression analyses for predicting COPD status using three different models. Model 1 consisted of FGF23, age, gender and eGFR. Model 2 incorporated inflammatory biomarkers (IL-1,-6 and -8) and in model 3 smoking status and tobacco consumption were added. As expected, age (OR=1.09), tobacco habits (OR=3.86) and consumption (OR=1.02) were strong predictors of COPD. Notably, FGF23 was a significant predictor in all three models with OR= 1.60 in model 3.

Table 11
Multivariable logistic regression analysis with COPD as outcome variable. Model 1: sex, age and eGFR. Model 2: sex, age, eGFR, IL-1, IL-6 and IL-8. Model 3: sex, age, eGFR, IL-1, IL-6, IL-8, ever-smoking and pack years.

Variable	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
FGF23 (per unit)	1.58 (1.11-2.25)*	1.68 (1.14-2.50)**	1.60 (1.07-2.39)*
Age (per year)	1.08 (1.05-1.12)***	1.08 (1.05-1.12)***	1.09 (1.05-1.13)***
Male sex	1.65 (1.03-2.64)*	1.57 (0.97-2.53)	1.16 (0.68-1.99)
eGFR (per mL/min/m^2)	1.00 (0.99-1.02)	1.00 (0.99-1.01)	1.00 (0.98-1.01)
IL-1 (per unit)		0.62 (0.41-0.96)*	0.62 (0.40-0.95)*
IL-6 (per unit)		1.03 (0.78-1.36)	0.89 (0.66-1.21)
IL-8 (per unit)		1.13 (0.81-1.59)	1.13 (0.79-1.61)
Ever-smoking			3.86 (1.47-10.15)**
Pack years (per years)			1.02 (1.00-1.04)*

FGF23 as a novel biomarker of lung disease

Data on FGF23 and COPD are scarce and conflicting. The findings in paper IV are supported by two previous reports of elevated FGF23 levels in subjects with COPD [66, 113], but contradicted by another study reporting no significant difference [114].The present study is to date by far the largest, but the numerical differences between groups are small and the findings still need validation in another large population. As mentioned earlier in the Introduction the reason for serum FGF23 to be elevated in COPD also remains to be elucidated, but circumstantial evidence is connecting FGF23 to the pathophysiologic inflammatory processes that occur in the airways after exposure to cigarette smoke [65-67, 70, 71].

Regarding correlations between FGF23 and variables of pulmonary function data are very scarce with only two rudimentary reports of a negative correlation with FEV_1 [113, 114]. Paper IV confirms this in a multivariable setting and furthermore for the first time shows FGF23 to be associated with decline in RV/TLC, VC, FEV_1/VC and $D_{L,CO}$ as well. $D_{L,CO}$ is a marker of pulmonary gas exchange and it is known to correlate with the degree of emphysema as assessed by histology [115]. Since mice with FGF23 co-receptor deficiency develop structural changes resembling emphysema [68], FGF23 could possibly be a novel biomarker for the

emphysematous phenotype of COPD. Again further studies are needed to validate this interesting observation.

In conclusion, paper IV shows that FGF23 is associated with the presence of COPD even after adjusting for traditional risk factors, and that FGF23 is associated with impaired pulmonary function and gas exchange. Further studies are needed to validate these findings and to investigate whether FGF23 could serve as a useful biomarker of COPD or emphysema development.

Conclusions

Paper I

No convincing effect of current or prior smoking on echocardiographic variables of systolic and diastolic function could be detected, even if current smokers had a higher cardiac index due to a slightly increased heart rate.

Paper II

In subjects with normal cardiac function and only mild-moderate COPD, no significant BNP elevation could be detected. Thus, the clinical value of BNP in this setting is limited.

Paper III

MMP-1 and MMP-12 are independent predictors of concomitant COPD and carotid plaque and carry information about pulmonary and vascular disease even when accounting for traditional risk factors. Most MMP´s are influenced by smoking status, which should be taken into consideration when interpreting MMP values.

Paper IV

FGF23 is an independent predictor of COPD, and FGF23 is associated with impaired pulmonary function and gas exchange. Further studies are needed to validate these findings and to investigate whether FGF23 could serve as a useful biomarker of COPD or emphysema development.

Future perspectives

As stated in the Introduction COPD and CVD and their most important preventable risk factor, smoking, are among the leading causes of morbidity and mortality worldwide. To discourage people from smoking would be a major step forward in a global health perspective and that is the ultimate goal to pursue. Meanwhile, it is important to detect detrimental health effects of smoking and diagnose smoking related disease at an early stage when treatments are most effective. This is a central aspect of this thesis, but it is in no respect an easy endeavor.

In paper I the echocardiographic effects of smoking on cardiac function was very modest, but arguably newer techniques like three-dimensional echocardiography, strain analysis and cardiac magnetic resonance all have higher sensitivities for detecting small changes in systolic and diastolic function and could possibly have altered the results.

In papers II-IV circulating biomarkers were evaluated regarding their associations with COPD and CVD. This is an important area of research because a good biomarker potentially offers a cheap and reliable non-invasive test of disease with diagnostic, therapeutic or prognostic implications. This said, the road from discovery to integration into guidelines is very long and obstacle-ridden and as a consequence only a few biomarkers make it into clinical use. BNP is one such exemption and the findings in paper II actually increases its clinical usefulness by showing its specificity for cardiac disease.

The MMP´s evaluated in paper III have been associated with CVD and smoking and this has been replicated in other cohorts, but as they are non-specific markers of inflammation it is unlikely that they will make it into clinical use as diagnostic biomarkers. Regarding therapeutic implications, the first trial with specific MMP-12 inhibition showed a reduced inflammatory response in mice exposed to cigarette smoke [116] but in human inhibition of MMP-9 and MMP-12 had no clinical effects after 6 weeks of treatment [117].

Finally, FGF23 has only just passed the first step confirming its association with COPD. Now replication in another large cohort is required and a quantitative test (i.e. Elisa) should be used in order to establish relevant cut off values for disease detection and to see if this adds to predictive models of already known risk factors.

So for this biomarker there is literally speaking still a long way to go, but this is what science is often about, breaking new ground and finding new paths.

Kämpfen ist meine Sache nicht

Papageno, från Trollflöjten, Emmanuell Schikaneder

Populärvetenskaplig sammanfattning

Bakgrund

Kronisk obstruktiv lungsjukdom (KOL) är en sjukdom som ger försämrad lungfunktion och som orsaker symtom i form av hosta, andfåddhet och ökat slemproduktion. KOL är en allvarlig sjukdom som drabbar många människor och den rankas av världshälsoorganisationen (WHO) som den tredje vanligaste dödsorsaken i världen. För att ställa diagnosen och bedöma svårighetsgraden utförs en lungfunktionsundersökning, en så kallad spirometri. Tidigare eller nuvarande rökning är tillsammans med ålder de viktigaste risk-faktorerna för utveckling av KOL.

Hjärtkärlsjukdom, som primärt beror på åderförkalkning, är också en vanlig sjukdom och den rankas av WHO som den vanligaste dödsorsaken i världen. Förekomsten av hjärtkärlsjukdom kan bland annat undersökas med hjärtultraljud, kärlultraljud och blodtrycks-mätning. KOL och hjärtkärlsjukdom finns ofta hos samma patient och detta kan bero på att de har gemensamma risk-faktorer, nämligen ålder och rökning.

Biomarkörer är biologiska ämnen, till exempel proteiner, som man bland annat kan mäta i blodet och som kan förknippas med en sjukdom. Biomarkörer kan användas för att upptäcka eller bedöma svårighetgraden av en sjukdom och kan ibland säga något om prognosen. En bra biomarkör kan ge läkaren möjlighet att upptäcka sjukdom i ett tidigt skede och kanske undvika andra mer komplicerade undersökningar. Olika biomarkörer har funnits en tid och används flitigt hos patienter med hjärtkärlsjukdom men spelar ännu inte någon större roll för patienter med KOL. Generellt upptäcks KOL och även hjärtkärlsjukdom ofta när man har haft sjukdomen en tid och därför är det viktigt att försöka hitta nya och bättre biomarkörer.

Syftet med denna avhandling är att utvärdera eventuella nya biomarkörer för KOL och studera sambanden mellan KOL och hjärtkärlsjukdom med särskild fokus på den gemensamma risk-faktorn rökning.

För att kunna studera detta närmare samlades en grupp på 450 frivilliga försökspersoner som antingen kända sig friska eller hade symtom på lungsjukdom. Merparten var rökare eller före detta rökare. Alla försökspersoner undersöktes med

hjärt- och kärl-ultraljud, blodtrycksmätning, blodprov och biomarköranalys och komplett lungfunktionsbedömning. Resultaten från dessa undersökningar har sedan analyserats och ligger till grund för avhandlingens delarbeten och slutsatser.

Fynd

I delarbete I undersökte vi med hjälp av hjärtultraljud om hjärtfunktionen påverkades av rökning. Vi fann at den inte gjorde det i nämnvärd grad, men att rökare hade en lite högre puls och att detta medförda att hjärtats samlade arbete (hjärtminutvolym) ökades lite. Detta är intressant eftersom andra studier har visat att hjärtfunktion påverkas av rökning

I delarbete II ville vi undersöka i fall BNP, som är en biomarkör för sänkt hjärtfunktion, också kunde användas för att hitta personer med KOL. Detta är intressant eftersom patienter med KOL eller sänkt hjärtfunktion ofta har samma symtom, nämligen andfåddhet. Vi fann att BNP inte kunde användas för det ändamålet när man tog hänsyn till personens hjärtfunktion.

I delarbete III undersökte vi om fem olika biomarkörer (så kallade MMP) var förknippade med förekomst av KOL och åderförkalkning. Två av dem visade sig vara det även när man tog hänsyn till saker som ålder, kön och rökning som man vet är förknippade med dessa sjukdomar. Detta är ett nytt fynd, men det är ännu för tidigt att säga om detta går att använda till något i det kliniska arbetet med patienter.

Slutligen har vi i delarbete IV visat att en ny biomarkör, FGF23, är förknippat med KOL och försämrad lungfunktion. Detta är delvist nya rön och det är tänkbart att FGF23 kan upptäcka lungsjukdom och tecken till försämrad lungfunktion i ett tidigt skede. Men flera studier krävs för att fastställa detta.

Sammanfattningsvist kan man säga att vi i en grupp av personer som överlag kände sig friska kunde påvisa en skadlig effekt av rökning på lungorna och kärlen, men inte på hjärtfunktion. Vi visade dessutom att flera biomarkörer kunde förknippas med KOL och hjärtkärlsjukdom och att rökning påverkade nivåerna av dessa. Förhoppningsvist kan man i framtiden möjligen med hjälp av biomarkörer upptäcka KOL och åderförkalkning i ett tidigare skede och därmed förbättra prognosen för patienterna.

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Erratum

In paper I, in the section: Study population, it is incorrectly described that subjects with GOLD stage II or more (n=70) were excluded. The correct description is that subjects with COPD and FEV_1/VC below the 5th percentile (n=70) were excluded.

In paper II, Table 4, the β* for BSA is incorrectly stated to be -0.23. The correct value should be positive 0.23.

Throughout paper III, whenever the term ***serum*** MMP´s is used the correct term should be ***plasma*** MMP´s.

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