AKERSHUS UNIVERSITY HOSPITAL

UiO : Faculty of Medicine
University of Oslo

Cardiovascular comorbidity in chronic obstructive pulmonary disease:

Biomarkers, vascular function and effects of statin treatment

Anke Meta Christina Neukamm, M.D.

Department of Pulmonology

Division of Medicine

Akershus University Hospital

and

Institute of Clinical Medicine

University of Oslo

ExtraStiftelsen
Helse og Rehabilitering

Center for
Heart Failure Research

Series of dissertations submitted to the
Faculty of Medicine, University of Oslo
No. 2106

ISBN 978-82-8333-137-0

Cover: Hanne Baadsgaard Utigard.
Print production: John Grieg AS, Bergen.

Produced in co-operation with Akademika Publishing.
The thesis is produced by Akademika Publishing merely in connection with the thesis defence. Kindly direct all inquiries regarding the thesis to the copyright holder or the unit which grants the doctorate.

“Not everything that can be counted counts,

and not everything that counts can be counted.”

Albert Einstein

TABLE OF CONTENTS

PREFACE AND ACKNOWLEDGEMENTS

I started working in Norway in 2005 at "Glittre", a pulmonary rehabilitation hospital. Working at a rehabilitation clinic, I was mainly working with patients with chronic obstructive pulmonary disease (COPD). It became increasingly obvious to me that COPD is a common and complex disease, both with regard to its pathophysiology and treatment. Therefore, when I became aware of a new PhD project at the Akershus University Hospital that would include working with COPD patients, it immediately attracted my attention. I was glad to be given the opportunity to assess pulmonary and cardiovascular features in this patient group, both with an epidemiological and an interventional approach.

The initial acronym of the trial was "ROMEO", but it had to be changed to "RODEO" when we realized that ROMEO had been used previously. Seemingly I went from a "romantic" approach to a somewhat rougher, but maybe more appropriate description of the entire PhD project. According to Wikipedia, rodeo is known as "a sport that involves a committed individual getting on a large bull with the attempt to stay seated while the animal tries to buck off the rider. The bull then bucks, rears, kicks, spins, and twists in an effort to throw the rider off. This continues for some time until the rider falls off the bull or successfully dismounts after completing his ride. "

I am very thankful to my two supervisors, **Torbjørn Omland** and **Vidar Søyseth** for choosing me and their continuous support to complete this work. Vidar with his broad expertise in epidemiology and statistics has shown incredible patience with the progression of my statistical understanding and knowledge over the years. Torbjørn is an international capacity in the field of cardiovascular biomarkers and I am very grateful to learn and benefit from his ideas, knowledge and experience.

Patient recruitment turned out to be more challenging than expected, but thanks to the good help and valuable support from **Gunn Seim Ekeland** and **Arne Didrik Høiseth**, even during my maternity year 2011/2012, we managed to complete the trial. Arne

Didrik was also the first author and main contributor to paper I in this thesis when I made my first steps and experiences in manuscript review and preparation during patient recruitment. I am very grateful for the continuous and profound help and support by **Gunnar Einvik** who was a fundamental contributor with steady advice and profound knowledge since the first days of the project. I am also thankful to **Ragnhild Røysland, Anna Randby** and the other **PhD students** for being wonderful colleagues and friends.

I want to thank **Vigdis Bakkelund**, who, together with **Marit Jørgensen** and **Annika Lorentzen**, collected blood samples and assisted with the EndoPat and electrocardiographic measurements and who always made things possible with a consistent cheerful and positive attitude.

A great thank you also goes to all the helpful nurses of the pulmonary outpatient clinic at the Akershus University Hospital for their help with the spirometric measurements and performance of the six-minute walk tests.

Thanks also to **Sverre Lehmann**, **Eli Nordeide** and the staff at the pulmonary outpatient clinic at the Haukeland University Hospital who contributed with patients to the interventional study.

For the interventional part of the project, I received valuable help from the Department of Good Clinical Practice (GCP), Oslo University Hospital, by **Birgitte Lid Adamsen** and colleagues, monitoring the trial and giving continuous advice during the study period.

A great thank you also goes of course to all the committed COPD patients and volunteers who were willing to contribute to the studies. Thanks to a grant from the Norwegian Extra Foundation for Health and Rehabilitation I was able to initiate and perform the studies. Thanks also to Astra Zeneca who provided study medication for the trial.

Through my employment as a research fellow at the University of Oslo I was able to finish the trial and I was so lucky to work with and get support from **Berit Lund Opheim**, **Vibeke Solberg Bjørklund**, **Ellen Elisabeth Westgaard** and **André Øien.**

Being a research fellow at the university I was given the opportunity to teach undergraduate medical students in internal medicine and clinical communication and I want to thank all the students, patients and my mentor in communication, **Pål Gulbrandsen,** for this giving experience beside my PhD work.

Finally, I want to thank my beloved family for their support, my parents **Antje and Ernst Neukamm** for giving me their love and profound back-up throughout my life, my brothers **Christian** and **Thorsten** for always giving me something to lean on and reach out for and of course my better part **Frode** who takes me for who I am and shares all ups and downs with me and our wonderful daughters **Annika** and **Eline**.

ABBREVIATIONS

ACS	Acute coronary syndrome
AECOPD	Acute exacerbation of COPD
AF	Atrial fibrillation
AFL	Atrial flutter
AMI	Acute myocardial infarction
ATP	Adult treatment panel
GOLD	Global Initiative for Chronic Obstructive Pulmonary Disease
CC	Complete case
CoHb	Carboxyhemoglobin
COPD	Chronic obstructive pulmonary disease
cTnT	Cardiac troponin T
CAT	COPD assessment test
CRP	C-reactive protein
CV	Coefficient of variation
CVD	Cardiovascular disease
DSMB	Data safety and monitoring board
ECG	Electrocardiogram
ED	Endothelial dysfunction
FAB	Fragment antigen-binding
FEV1	Forced expiratory volume in one second
FMD	Flow mediated dilation
FVC	Forced vital capacity
GCP	Good clinical practice
HDL	High-density lipoprotein
HF	Heart failure
IHD	Ischemic heart disease
IL6	Interleukin 6
ITT	Intention to treat

LBBB	Left bundle branch block
LDL	Low-density lipoprotein
LoD	Limit of detection
LoB	Limit of blank
LLN	Lower limit of normal
LTOT	Long term oxygen therapy
LVH	Left ventricular hypertrophy
MMRC	Modified medical research council
6MWD	Six minute walking distance
NIV	Non-invasive ventilation
NO	Nitric oxide
NSVT	Non-sustained ventricular tachycardia
NT-proBNP	N-terminal B-type natriuretic peptide
PAH	Pulmonary arterial hypertension
PAT	Peripheral arterial tonometry
PP	Per protocol
PWV	Pulse wave velocity
RHI	Reactive hyperemia index
RCT	Randomized controlled trial
ROS	Reactive oxygen species
RVH	Right ventricular hypertrophy
RVSP	Right ventricular systolic pressure
URL	Upper reference limit
WHO	World Health Organization

LIST OF PAPERS

Paper I:

Elevated high-sensitivity cardiac troponin T is associated with increased mortality after acute exacerbation of chronic obstructive pulmonary disease.

Arne Didrik Høiseth, Anke Neukamm, Bo Daniel Karlsson, Torbjørn Omland, Pål Haugar Brekke, Vidar Søyseth.

Thorax 2011; 66:775-781.

Paper II:

High-sensitivity cardiac troponin T levels are increased in stable COPD.

Anke Neukamm, Arne Didrik Høiseth, Tor-Arne Hagve, Vidar Søyseth, Torbjørn Omland.

Heart 2013; 99:382-387.

Paper III:

Rosuvastatin treatment in stable chronic obstructive pulmonary disease (RODEO) – a randomized controlled trial.

Anke Neukamm, Arne Didrik Høiseth, Gunnar Einvik, Sverre Lehmann, Tor-Arne Hagve, Vidar Søyseth, Torbjørn Omland.

J Intern Med. 2015; 278(1):59-67.

WHAT THIS THESIS IS ABOUT

Chronic obstructive pulmonary disease (COPD) patients represent a major patient group worldwide. The incidence of the disease is increasing, resulting in growing morbidity and health care expenditures**.** COPD is a complex disease characterized by inflammatory components and frequent comorbidities, including cardiovascular disease, which may complicate diagnosis and limit treatment options. In addition, COPD, as well as its comorbidities, can be underdiagnosed and may subsequently be undertreated.

In order to assess the extent and potential implications of subclinical cardiac injury and statin effects on the immune, respiratory and cardiovascular systems in COPD, we tested the hypotheses that subclinical cardiac injury among patients with an exacerbation of COPD is associated with adverse outcome, that COPD is associated with elevation of cardiac troponins also in the stable state of the disease, and that statin treatment is associated with reduced inflammatory activity, improved pulmonary function and enhanced endothelium-dependent vasodilation in stable COPD patients without a clear indication for statin therapy.

We demonstrated that increased circulating troponin T concentrations are associated with worse prognosis in exacerbated COPD patients and that troponin T concentrations are higher in stable COPD patients than in randomly drawn subjects from the general population. Statin treatment in stable COPD patients did not have an effect on pulmonary function, but had anti-inflammatory actions and a beneficial effect on vascular function in the subgroup of patients with evidence of increased inflammatory activity.

INTRODUCTION

COPD

History

Terms describing voluminous lungs, enlarged airspaces (emphysema), as well as cough and hypersecretion have been used back to 1679, and there have been different names for COPD until the terms chronic bronchitis and emphysema were formally defined at the Ciba guest symposium of physicians in 1959 (1). The term COPD was first used in 1965 and has thereafter taken over as the preferred name for this disease. In 1998 the Global Initiative for Chronic Obstructive Lung Disease (GOLD) was formed in order to increase and spread knowledge and awareness of COPD and to help millions of COPD patients to cope with the disease, its comorbidities and complications.

During the last years the GOLD program has released several revised editions of a consensus report: "Global Strategy for the Diagnosis, Management, and Prevention of COPD" with the last revised edition published in 2011. Updated reports have been released in January 2013, January 2014, and January 2015. The 2015 update adds an appendix on Asthma COPD Overlap Syndrome. The GOLD guidelines include a classification system of COPD patients by airflow limitation, which is used as an important tool to guide therapy. Due to accumulating evidence that COPD is a complex disease and that airflow limitation is not closely correlated to many patient-related outcomes (2) the new classification from 2011 takes the patient's exacerbation history and symptoms into consideration, as further described below.

The GOLD document defines COPD as "a common and preventable and treatable disease, characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic pulmonary inflammatory response in the airways and the lungs to noxious particles or gases. Exacerbations and comorbidities contribute to the overall severity in individual patients" (3).

Epidemiology

About 65 million people have moderate to severe COPD worldwide. More than 3 million people died of COPD in 2012, which is equal to 6% of all deaths globally that year. More than 90% of COPD deaths occur in low- and middle-income countries. The primary cause of COPD is tobacco smoke (through tobacco use or second-hand smoke). With previous data pointing at greater prevalence and mortality of COPD in men, men and women seem now to be equally affected by the disease, possibly reflecting change in smoking patterns (www.goldcopd.org). In 2002 COPD was the fifth leading cause of death, and during the last years, COPD has become the third leading cause of death worldwide (Figure 1).

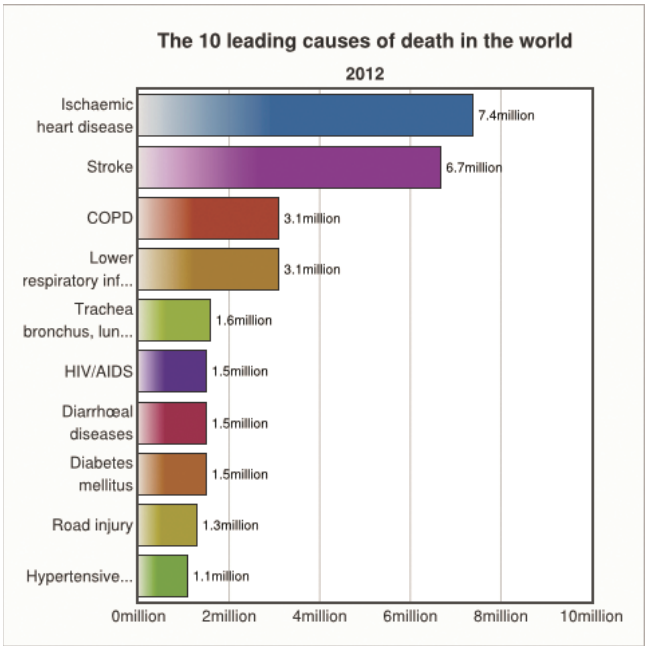

Figure 1: The 10 leading causes of deaths in the world. Reprinted with permission from the WHO media center, http://www.who.int/mediacentre/factsheets/fs310/en/ (updated May 2014).

Furthermore, one has to be aware of the fact that the disease still is relatively unknown in some areas and most likely often underdiagnosed (4-6). COPD is also associated with a tremendous economic burden. The total direct costs of respiratory disease are estimated to be about 6% of the total health care budget, with COPD accounting for 56% (38.6 Billion Euros). In the US the estimated direct costs are 29.5 Billion dollar (7). The annual direct COPD-related medical costs in Norway in 2005 were 141 million Euros for the population aged ≥40 years, and 284 Euros per COPD patient. The COPD prevalence is rising, and COPD exacerbations and hospitalizations account for the greatest proportion of the total COPD burden on the health care system (8).

COPD exacerbations

This thesis discusses papers including COPD patients in the exacerbated as well as in the stable state of the disease. A high percentage of COPD patients experience exacerbations. The current GOLD guideline definition of a COPD exacerbation is as follows: "An exacerbation of COPD is an acute event characterized by a worsening of the patient's respiratory symptoms that is beyond normal day-to-day variation and leads to a change in medication" (7). It remains under discussion, however, how COPD exacerbations should be defined in clinical practice, and we have reason to believe that exacerbations are not always diagnosed, documented and treated in the optimal way. An exacerbation can for example mimic symptoms caused by heart failure, but also symptoms caused by worsening anxiety or a pulmonary infection or even a combination of all of these. Having frequent exacerbations is now recognized as an important phenotype of COPD patients (9). This is important because COPD exacerbations are associated with hyperinflation and airway and systemic inflammation and patients with frequent exacerbations have been shown to have a worse prognosis (10-12). An early and correct diagnosis of a COPD exacerbation, the impact of eventual comorbidities as well as the phenotyping of the individual patients would be helpful to provide optimal treatment in COPD patients (7).

A stable COPD patient is a patient who is not suffering from an exacerbation, defined as mentioned above, but there is per today no clear and consistent definition in research as well as in clinical practice on the term “stable” in terms of duration or variation criteria of a stable period among COPD patients. Therefore, to be able to describe pathophysiological features in the stable as well as in the exacerbated state of the disease it is important to refine the definitions of these groups and to investigate biomarkers and clinical endpoints in both states of the disease.

Etiology

Smoking is the most important risk factors for the development of COPD, but today it is also emphasized that non-smokers can develop the disease. This fact has strengthened the interest in and knowledge of other important risk factors for COPD development and progression. Other contributing risk factors include genetic predisposition and environmental exposure (13) (particularly smoke, as well as exposure to particles in air such as outdoor and indoor air pollution or occupational exposures), age, impaired lung growth and development, socioeconomic status, asthma and/or bronchial hyperreactivity, as well as chronic bronchitis and infections (3). There are several pathophysiological aspects in disease development and progression:

A) Oxidative stress, mainly from cigarette smoke, but also released from otherwise activated inflammatory cells is a major mechanism in the development of COPD which is further increased in COPD exacerbations. There is a variety of inflammatory mediators that have been shown to be increased in COPD patients.

B) Imbalance between proteases, enzymes that break down connective tissue and antiproteases that protect against this break down is also believed to be an important feature of the development of damage and enlargement of the air sacs (alveoli) in the lungs, also known as emphysema, a major component of COPD.

C) COPD patients suffer from persistent air flow limitation and air trapping, gas change abnormalities, mucus hyper secretion, recurrent pulmonary infections and exacerbations and comorbidities. Exacerbation frequency and comorbidities are increasingly recognized as important predictive factors for disease progression and prognosis and have been included in the definition, grading and treatment approach of the disease.

Diagnosis

In order to diagnose COPD, spirometric measures are necessary. COPD is today defined by persistent airflow limitation, defined in the GOLD guideline as a forced expiratory volume in one second (FEV1) <80% of the predicted, and FEV1<70% of the forced vital capacity (FVC), even after administration of bronchodilatory agents (FEV_1/FVC).

Other studies use values below the lower limit of normal (LLN) which is defined as the FEV1/FVC ratio below the 5th percentile. According to prevalence studies FEV1/FVC <70% alone might overdiagnose and FEV1/FVC<LLN might underdiagnose COPD (14). In addition to spirometric measures one has to evaluate symptoms as dyspnea, chronic cough or sputum production, number of exacerbations and comorbidities in the patients. Standardized and validated questionnaires are used to evaluate subjective symptoms. These are the modified Medical Research Council questionnaire (mMRC) for the evaluation of dyspnea and the more recent COPD Assessment Test (CAT, (15, 16)) addressing primarily the evaluation of symptoms and functionality of the patient.

Following the recent GOLD guidelines (http://www.goldcopd.org) each patient can be categorized into one of four groups according to their symptoms, exacerbation history and spirometry as illustrated in Figure 2 and receive corresponding recommended treatment.

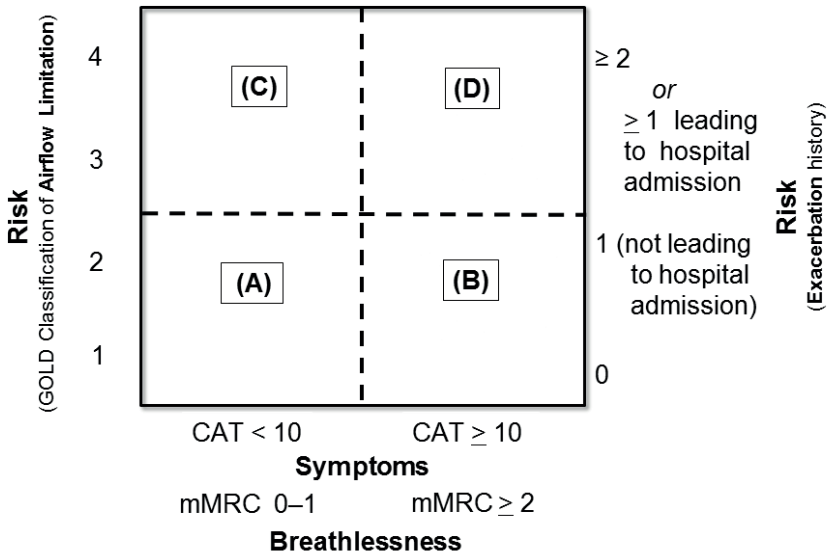

Figure 2: Combined COPD assessment (GOLD guidelines, updated 2014). From the Global Strategy for Diagnosis, Management and Prevention of COPD 2015, © Global Initiative for Chronic Obstructive Lung Disease (GOLD), all rights reserved. Available from http://www.goldcopd.org.

Treatment

There are several therapeutic options for COPD patients. For patients who still smoke, smoking cessation is critical to influence the natural history of the disease. Pulmonary rehabilitation plays an important part in improving the disease state and quality of life of COPD patients. Vaccinations are recommended to prevent pneumococcal and influenza infections. Pharmacological therapy for stable COPD includes bronchodilators, beta$_2$-agonists, anticholinergics, methylxanthines, corticosteroids and phophodiesterase-4 inhibitors. The treatment is supported by randomized controlled

trial data suggesting no harmful effect of classic bronchodilators and potentially benefits of an anti-inflammatory approach in COPD treatment (17-19). Patients with alpha-1 antitrypsin deficiency are treated with alpha 1-antitrypsin augmentation therapy. Overall, it is important that therapies are based on a risk/benefit assessment in the individual patient with the consideration of all comorbidities, symptoms and GOLD risk factors (lung function and exacerbation history). Non-invasive ventilation (NIV) is also increasingly used with stable severe COPD but there is insufficient evidence to formulate recommendations. Smoking cessation for those who are still smoking and long-term use of supplemental oxygen (LTOT) in those with severe resting hypoxemia are the only therapies that have clearly been shown to improve survival in patients with COPD. Almagro and colleagues suggested that management of co-morbidities contributes as much to improved survival as increased use of long-acting β_2 agonists and anticholinergics at hospital discharge (20). Comorbidities that occur frequently in COPD patients include cardiovascular disease, skeletal muscle disease, metabolic syndrome, osteoporosis, depression and lung cancer. Today, we know that comorbidities contribute significantly to disease severity and outcome. Comorbidities can occur in patients with mild, moderate or severe airflow limitation (2), influence mortality and hospitalizations independently (21) and deserve therefore increased attention and specific treatment. Comorbidities were therefore included in the GOLD definition of COPD in 2006 and the importance stated even more clearly in the revised statement from 2011. Today, we know that it is important to routinely look for comorbidities in COPD patients in order to treat them in time and appropriately.

COPD and inflammation

COPD is considered to be an inflammatory disease, and the major risk factor of COPD, tobacco smoking, is a potent stimulus for inflammation and leads to increased oxidative stress. Reactive oxygen species (ROS) and cytokines from the pulmonary system probably leak over to the systemic circulation and release pro-inflammatory cytokines

and acute phase proteins, which again lead to systemic inflammation and change in vasomotor and endothelial function and increased concentration of pro-coagulant factors. Still, systemic inflammation can persist after smoking cessation (22) and increases during exacerbation of the disease. The pathophysiological mechanisms of systemic inflammation in COPD are still not completely understood. The factors causing systemic inflammation in COPD patients are likely to be multifactorial. COPD often coexists with one or more systemic comorbid conditions, such as osteoporosis, cachexia, muscle weakness or cardiovascular disease. Complicating the picture, these conditions are often associated with systemic inflammation either as a consequence of or as a stimulus for the inflammatory process itself. Another stimulus for systemic inflammation can also be recurrent exacerbations with increased bacterial colonization and viral infections (9). A relation between COPD and markers of low-grade systemic inflammation has been demonstrated in numerous studies (23, 24). Inflammatory biomarkers such as C-reactive protein (CRP) and Interleukin 6 (IL6) have been shown to be increased among COPD patients (25) and are associated with poor clinical outcomes and improve clinical prediction of mortality in patients with COPD (26, 27). Various markers of systemic inflammation were investigated in the Framingham Heart Study (28) and showed a significant relation between increase in IL6 and CRP and decrease in FEV1 after adjusting for age, sex, BMI and smoking. Another study including stable COPD patients showed that subjects with elevated CRP levels had more severe airflow obstruction than subjects with normal CRP levels (29). In the Copenhagen City Heart Study (30) hospital admissions and mortality were highest among COPD patients with a CRP level >3 mg/l at inclusion. Additional analysis from the Copenhagen General Population Study showed that simultaneously elevated levels of CRP, fibrinogen, and leukocyte counts were associated with a two- to four-fold risk of major comorbidities in COPD (31). In the Lung Health Study (32), the association between the risk of both fatal and nonfatal cardiovascular events was increased two- to three-fold in patients with the highest quintile of CRP compared with those with the lowest quintile. However, another observational study found no association between CRP levels and mortality after a follow-up of three years of patients with moderate-to-severe COPD (33).

COPD and cardiovascular disease

Cardiovascular disease is a major comorbidity in COPD (34-37). General population studies and studies in patients with COPD indicate that COPD is an important risk factor for ischemic heart disease and sudden cardiac death. There is evidence of an association between COPD and cardiovascular disease, and although COPD and cardiovascular disease clearly share common risk factors such as smoking, COPD has been described as an independent risk factor for the development of cardiovascular disease (21, 38).

The Atherosclerosis Risk in Communities (ARIC) study, a longitudinal, population-based study with 15759 participants, 3434 COPD cases and a mean follow-up time of 9 years reported up to 27% cardiovascular deaths among patients with mild and moderate COPD (39). The TORCH study, a randomized, double-blind, placebo controlled trial including 6184 COPD patients and a three year follow-up time described that 27% of all deaths in COPD patients were related to cardiovascular causes (40). In a postmortem analysis, the leading cause of death was cardiac failure, accounting for 37% of all deaths among hospitalized COPD patients (41). In a large cohort of 384 888 patients with COPD admitted to a Veterans Administration hospital or clinic, the prevalence of coronary artery disease was 33.6%, significantly higher than the 27.1% prevalence seen in a matched cohort without COPD (42). A number of population studies have shown that airflow limitation as measured by FEV1 or FEV1/FVC ratio is a predictor of cardiovascular risk and describe FEV1 as an independent predictor of cardiovascular mortality in COPD (43). The Lung Health Study (44) reported that the 5-year mortality in 5 887 patients aged 35 to 46 years with COPD and mild to moderate airways obstruction was 2.5%, of whom 25% died of a cardiovascular event. For every 10% decrease in FEV1 there was an increase of about 28% in fatal coronary events and 20% in nonfatal coronary events among subjects with mild to moderate COPD. COPD also frequently co-exists with heart failure and the two diseases share common pathophysiological features, risk factors as well as similar symptoms. They also have the potential to camouflage the existence of each other in the individual patient (45).

Chronic heart failure has been found to be prevalent in more than 20% of COPD patients (46), and right heart failure is relatively frequent and related to the severity of hypoxia (47).

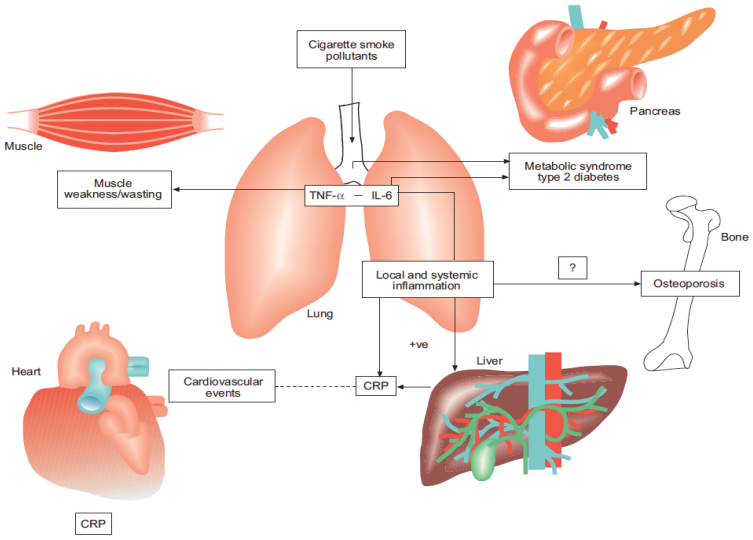

Figure 3: Inflammation and comorbidity in COPD, L.M. Fabbri et al. ERJ 2008: Reproduced with permission of the European Respiratory Society © Eur Respir J

It is a matter of debate whether COPD itself might act as an independent risk factor for the progression of the atherosclerotic process and development of cardiovascular disease. It has also been shown that the risk for cardiovascular events is increased following exacerbations of COPD (48). Inflammation has been proposed as a link between COPD and CVD, with a possible spillover of inflammatory mediators from the lungs to the peripheral blood (49) (Figure 3). Inflammatory mediators (CRP, IL6 and others) have been found to be increased in sputum and bronchioalveolar lavage samples as well as in circulating blood (23). In addition, arterial stiffness is increased in COPD patients, more in more severe COPD, which may be an effect of vascular inflammation (50, 51). Shared risk factors as smoking might explain the relation between COPD and

cardiovascular disease, but environmental and genetic risk factors might also influence the inflammatory state and the potential for the development of cardiovascular disease among COPD patients. However, the mechanisms responsible for the increased risk of cardiovascular disease in patients with COPD are still not fully understood.

CARDIOVASCULAR DISEASE

Epidemiology

Cardiovascular disease (CVD) is defined as a group of disorders of the heart and blood vessels and includes coronary heart disease (CHD), cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease and venous thromboembolism (WHO, Department of Cardiovascular Diseases. Fact sheet N°317, updated January 2015). CVD is a leading cause of morbidity and mortality throughout the world. According to the World Health Organization, about 17 million deaths per year can be attributed to CVD. This number is expected to rise with the shift in demographics to an aging population in the Western world (52) and an increase in traditional risk factors (smoking, hypertension, diabetes, dyslipidemia) in the developing world. By 2030 more than 23 million people are suspected to die annually from CVD.

Etiology

This thesis focuses on atherosclerosis as the major antecedent event in the development of CVD and the acute coronary syndrome. Atherosclerosis has during the past 2 decades been increasingly recognized to be an inflammatory disorder and not simply a cholesterol storage disease (53). Atherosclerotic lesions, also known as atheromas, are local thickenings of the intima consisting of inflammatory, endothelial and smooth muscle cells, lipids and cellular debris. Prior to atheroma development so-called fatty streaks, i.e. areas with accumulated macrophages and T-cells beneath the vascular

endothelium can be detected. An acute coronary event and subsequent myocardial infarction may occur when the atheromatous process prevents blood flow through the coronary artery, a process most commonly caused by the rupture or erosion of an atherosclerotic plaque resulting in thrombotic occlusion of the vessel (54).

Diagnosis

An important diagnostic tool in CVD is the clinical history of the patient and physical examination for signs of heart disease, such as for example shortness of breath, cyanosis, heart murmurs or edema. In addition, electrocardiograms at rest and during exercise may provide valuable information concerning cardiac function. Imaging techniques such as echocardiography, chest radiographs, computed tomography, coronary angiography and cardiac catheterization or cardiac magnetic resonance imaging are used when deemed necessary for a correct diagnosis of CVD in the individual patient. In addition, biomarkers such as cardiac troponins and B-natriuretic peptides play an important role as diagnostic tools.

Prevention and treatment

One has to differentiate between preventive treatment and treatment of established CVD. Primary prevention addresses individuals with risk factors who have not yet developed clinically manifest CVD. Secondary prevention addresses individuals with established CHD, cerebrovascular disease or peripheral vascular disease. (World Health Organization, Department of Cardiovascular Diseases. Fact sheet N°317, updated January 2015, (55, 56)). It is useful to estimate the total cardiovascular risk in the primary prevention group. The Framingham Heart study helped to identify major CVD risk factors - high blood pressure, high blood cholesterol, smoking, obesity, diabetes, and physical inactivity (57) and the concept of CVD risk classification has led to the development of effective treatment and prevention strategies in clinical practice. Such

strategies in primary prevention include lifestyle changes such as smoking cessation, dietary changes, increased physical activity, and weight control. Depending on risk scores and clinical measurements, treatment strategies may include anti-hypertensive drugs, lipid-lowering drugs such as statins or even antidiabetic or antiplatelet treatment. The major guidelines influencing clinical practice are the Adult Treatment panel (ATP) III guidelines developed by the National Cholesterol Education Program (NCEP) expert panel (58) , followed by the American College of Cardiology/American Heart Association (ACC/AHA) task force (59), and the European Society of Cardiology (ESC) (60). Individuals with proven CHD are at risk of developing recurrent cardiovascular events and there are currently no risk prediction tools to guide therapy. The treatment aim of secondary prevention in this group is to reduce symptoms and to slow the atherosclerotic process in order to prevent future cardiovascular events with the same treatment options as mentioned above. In addition, angiotensin receptor antagonists (ARB's), angiotensin converting enzyme inhibitors (ACE inhibitors) and beta-blockers are recommended in selected patients, depending on previous medical history.

Atherosclerosis and endothelial function

The endothelium plays a central role in the regulation of the vessel tone and defense against atherosclerosis. Endothelium-derived nitric oxide (NO) has been identified as a potent vasodilator and an important antiatherogenic agent. Additional actions of NO, include inhibition of platelet aggregation, monocyte adhesion to endothelial cells, and abnormal smooth muscle cell proliferation (61). Endothelial dysfunction (ED) occurs in the presence of vasoconstrictive, growth-promoting, procoagulant and proinflammatory factors (62). ED is often present long before CVD is clinically apparent (63) and the assessment of endothelial function is now widely recognized as an important index in the early assessment of CVD risk (64) (Figure 4).

There are several different invasive and noninvasive methods to assess endothelial function. Invasive methods include intracoronary or -brachial infusion of vasoactive

agents such as acetylcholine. Non-invasive methods include the measurement of endothelial cell adhesion molecule expression and release of key hemostatic regulatory molecules (65) as well as the measurement of endothelium-dependent vasodilatation by either ultrasound, gauge-strain plethysmography (66) or peripheral arterial tonometry. Flow-mediated vasodilation using ultrasound (FMD) and peripheral arterial tonometry (PAT) are further described below.

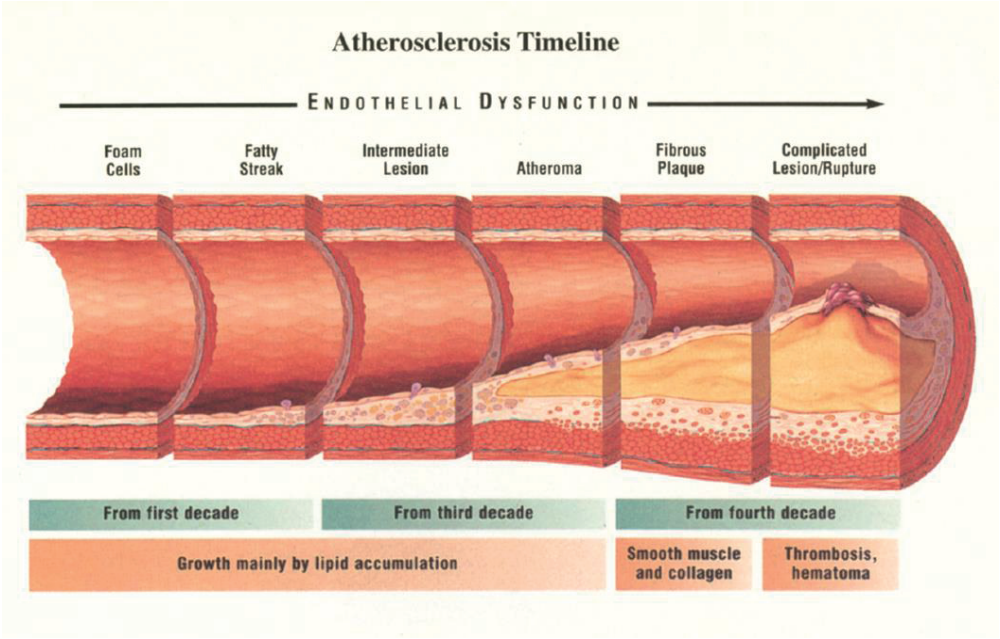

Figure 4: Atherosclerosis timeline demonstrating endothelial function as an early sign of atherosclerosis, Reprinted with permission from Elsevier from Pepine CJ, AM J Cardiol, 1998 (67), reproduced from Stary et al. Circulation, 1995 (68).

It is important to be aware of the fact that reports on "endothelial function" and "dysfunction" that are based on different measurements of endothelium-dependent dilatation provide only insights concerning one aspect of endothelial physiology. In this work endothelial function was assessed using PAT, a non-invasive method to quantify digital endothelium-dependent vasodilation.

Measurement of endothelium-dependent vasodilation using an ultrasound technique was first described in 1992 by Celermajer and colleagues (69). The test used involved noninvasive measurement of the diameter of the brachial artery by ultrasound before and after shear stress, a known stimulus for NO production, was induced by reactive hyperemia (70). This method is commonly known as the brachial artery ultrasound (BAUS) flow-mediated dilation (FMD) method (71). Another method of measuring endothelial vasomotor function after reactive hyperemia is by PAT (72) and involves quantifying arterial pulsatile volume at rest as well as during a condition of increased shear stress that results in the release of NO (and other mediators). It is performed with the use of a finger plethysmograph with the EndoPat™ method (Itamar Medical, Caesarea, Israel). Although the distal fingertip is not an intuitively obvious place to look for endothelial dysfunction as a marker of atherosclerosis risk, the peripheral vascular beds located at the distal part of the limbs are major sites of sympathetic alpha-adrenergic vasoconstrictor activity and therefore play an important role in circulatory regulation (73). It appears that endogenous NO-mediated vasoregulation is particularly prominent in the arteriovenuous anastomoses in the human fingertips (74) and it has been shown that approximately 60% of the PAT response is mediated by NO release (75). Two great advantages of the EndoPat™ method are that the method, in contrast to the FMD method, is operator independent, and measurements are normalized to the contralateral arm, adjusting for systemic effects that affect blood flow.

Endothelial function in COPD

Studies on endothelial function in COPD are limited. Barr and colleagues (76) found in 2007 that endothelial dysfunction measured by FMD was independently associated with FEV1 and the percentage of emphysema (measured with CT) in former smokers. These associations were linear across a spectrum of disease from normal pulmonary function and anatomy to moderate to severe COPD and emphysema. Eickhoff and colleagues (77) described a significant lower FMD in patients with moderate to severe COPD compared with smoking control subjects and a strong correlation of FMD and markers of systemic

inflammation and severity of airflow obstruction measured by spirometry independent of smoking history. It has also been shown that endothelial function measured by FMD is impaired during COPD exacerbations and improves after their resolution (78). Assessment of endothelial function with digital pulse wave tonometry is a relatively new validated method (64, 79) and only sparse data have been published on endothelial function in COPD patients with this method. Minet and colleagues (80) reported that endothelial dysfunction determined by EndoPat ™ occurred in half of the studied COPD patients and was amplified during COPD exacerbation. They also found that functional capacity assessed by the six-minute walking distance (6MWD) was a main predictor of endothelial dysfunction in COPD patients.

CVD – biomarkers, risk markers and risk factors

A *biomarker* has been defined as " a biological characteristic that is objectively measured and evaluated as an indicator of a normal or pathogenic biological process or a pharmacological response to a therapeutic intervention"(81). If this characteristic is quantitatively associated with a disease or other outcome, but does not necessarily contribute to disease progression it can be defined as a *risk marker*. A *risk factor* is any attribute, characteristic or exposure that increases the likelihood of developing a disease or an injury. A risk marker can be considered a risk factor if intervention to modulate this factor results in parallel modulation of risk, provided that the analysis demonstrating this risk modulation accounts for possible confounding factors. *Moderators* of risk factors provide information on who is susceptible to an exposure and improve our understanding of disease etiology and early diagnosis. This is important in identifying subpopulations with a possible beneficial treatment effect. Identification of *mediators* of risk factors, factors explaining how and why other risk factors affect the outcome, can lead to the design of interventions for the prevention or early detection of diseases. To be considered a *risk mediator*, the factor must show a strong correlation with an increased likelihood of a disease, occur after another relevant risk factor and must also contribute directly to the development of disease. A *clinical endpoint* is a

characteristic or variable that reflects how a patient feels, functions or survives. A *surrogate endpoint* is a biomarker intended to substitute for a clinical endpoint and is expected to predict clinical benefit or harm based on epidemiologic, therapeutic, pathophysiologic or other scientific evidence (81).

Smoking, obesity, arterial hypertension, diabetes mellitus, blood lipid composition, age, ethnicity and genetic predisposition are all examples of established risk factors for cardiovascular disease. This thesis focuses on inflammatory markers (CRP and IL6), troponin T and endothelial function measured by PAT as the main outcome measurements. Inflammation has been shown to play a central role in the development of atherosclerosis (82) and inflammatory biomarkers such as C-reactive protein (CRP) have been shown to be associated with the development of CVD (83). However it has been under debate if CRP should be declared a risk factor or a risk mediator for CVD (84, 85), but there is so far no clear evidence for CRP as a pathogenetic factor in cardiovascular disease (84, 86). Cardiac troponins are established biomarkers for myocardial injury and risk markers in acute coronary syndromes (ACS) as well as in populations without ACS as further described below. Endothelial function including non-invasive peripheral arterial tonometry (EndoPAT™) is an established biomarker in clinical research both as a surrogate marker for subclinical atherosclerosis and as a risk marker of future cardiovascular events (87). EndoPAT™ has been shown to predict cardiovascular events in low-risk subjects with unexplained chest pain (88) and in subjects with heart failure with preserved ejection fraction (89) as well as in high-risk populations (90).

CARDIAC TROPONINS

History

Today, cardiac troponin I (cTnI) and cardiac troponin T (cTnT) have received international endorsement as the standard biomarkers for detection of myocardial injury, for risk stratification in patients with a suspected acute coronary syndrome, and for the diagnosis of myocardial infarction (91, 92). After assays were developed in the 1980's, the diagnostic use of troponin T testing as a cardiac injury marker was incorporated in the definition of acute myocardial infarction (AMI) from the First Global MI Task force in the year 2000 (93). Later, the definition was refined in the Universal Definition of Myocardial Infarction Consensus Document in 2007 (91) and 2012 (94) emphasizing different conditions leading to a MI and the addition of cardiac troponins with a typical rise and gradual fall as the preferred diagnostic biomarker of cardiomyocyte necrosis. The Third Universal Definition of Myocardial Infarction from 2012 additionally emphasizes the differentiation between myocardial injury and infarction and discusses the challenges in interpretation of elevated troponin values measured by the new high sensitivity assays.

Over the last decades, several troponin assays have been developed with successive generations with increasing ability to detect low levels of troponin. In contrast to cTnI, there is only one manufacturer for the cTnT assay, allowing for the standardization across institutions. The first-generation assay for cTnT used bovine cTnT as the reference material with a non-specific binding to human skeletal muscle troponin. The second-generation assay for cardiac troponin T (cTnT) was completely specific for the cardiac isoform of TnT, utilizing two cardiac specific monoclonal antibodies, although still with a possible certain amount of cross-reactivity of skeletal muscle (95). With the third generation assay the use of recombinant human cTnT for standardization refined the specificity of the assay. The fourth-generation cTnT assay uses fragment antigen-binding (FAB) of two cTnT-specific mouse monoclonal antibodies in a sandwich format. The antibodies recognize epitopes located in the central part of the cTnT molecule without any cross-reactivity.

In 2012 experts agreed on a consensus on nomenclature for high-sensitivity assays that defined that they should have a coefficient of variance (CV) of <10% at the 99th percentile upper reference limit (URL) value in the population of interest. To be classified as high-sensitivity assays, concentrations below the 99th percentile should be detectable above the assay's limit of detection for >50% (ideally more than 95%) of healthy individuals in the population of interest (96, 97). The new methods have incrementally reduced the diagnostic cut-offs for the rule-out of MI from 0.5 μg/L in the first generation assays to the levels of 0.05–0.1 μg/L in the third generation and the level of 0.03 μg/L in the fourth generation assay. The fourth-generation cTnT assay has a limit of detection (LoD) of 0.01 ng/mL, a 99th percentile cut-off point of 0.01ng/mL, and a 10% coefficient of variation (CV) at 0.03 ng/mL. For the diagnosis of AMI, the fourth-generation cTnT assay is still considered the standard assay in the United States, whereas the new high-sensitivity (hs) cTnT assay is used in Europe and Asia.

The troponin complex

The troponin complex is found in striated muscle tissue and consists of three different polypeptides: troponin C, troponin T, and troponin I. Troponins are predominantly sarcomere-bound, but a small (<10% of total) cytoplasmic pool of troponins is present as illustrated in Figure 5. Whereas troponin C found in cardiomyocytes is identical to troponin C found in striated skeletal muscle, troponin I and T in the heart are genetically and immunologically distinct from the troponin I and T isoforms of the skeletal muscle. Accordingly, the isoforms of troponins I and T in the heart (cTnI, cTnT) are considered to be cardiac specific. Thus, the presence of cTnI and cTnT in peripheral blood indicates leakage from cardiomyocytes (98). When myocyte damage occurs, the cytoplasmic pool is released first, followed by a more prolonged release from myofilament bound troponin. Minimal myocardial necrosis can cause troponin elevations at a much lower level than after extensive myocardial necrosis and high-sensitive assays are essential to detect those concentrations (99).

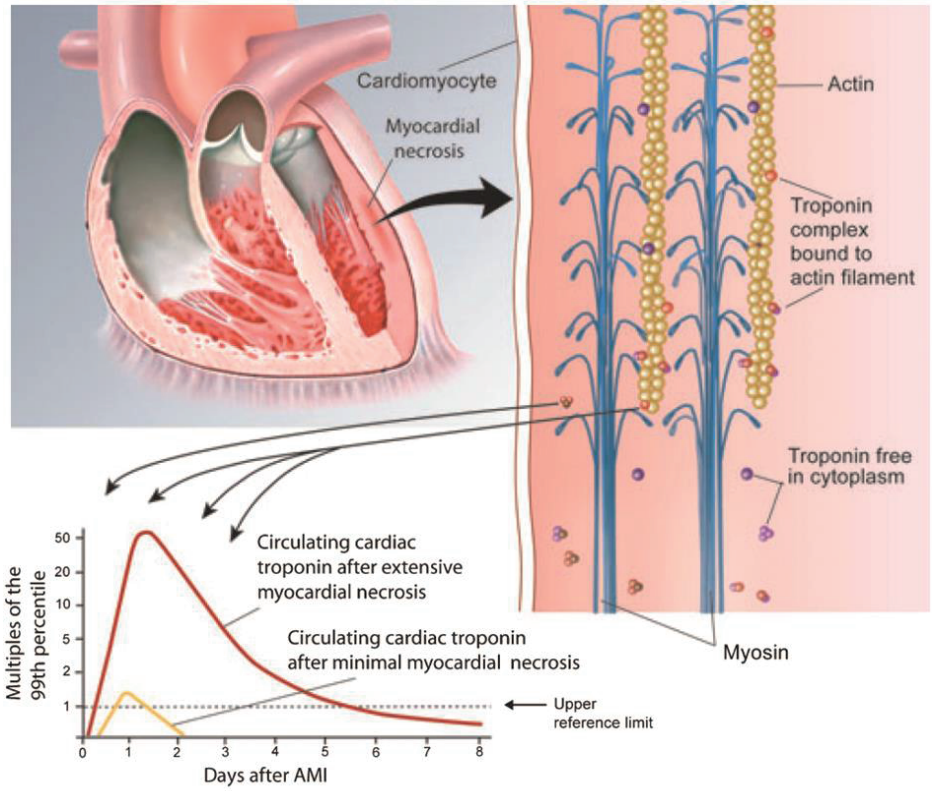

Figure 5: Release of cardiac troponins in acute myocardial infarction. With courtesy from Omland T, Journal of Internal Medicine, 2010 (100), reproduced with permission from Antman EM, N Engl J Med, 2002 (99). Copyright Massachusetts Medical Society.

High sensitivity troponin T assay

The new high-sensitive cTnT (hs-cTnT) assay is a modification of the fourth-generation cTnT assay. The detection antibody was genetically re-engineered to further reduce the susceptibility to interference by heterophilic antibodies. The variable region of the detection antibody is identical to that of the fourth-generation assay. The analytical sensitivity was improved by increasing the sample volume from 15 µL to 50 µL,

increasing the ruthenium concentration of the detection antibody, and lowering the background signal via buffer optimization. The development of high-sensitivity troponin T (hsTnT) assays (Roche Elecsys) can detect levels as low as 0.005 µg/L (5 ng/L) and has an upper limit of normal (99th centile) of 14 ng/L. Compared to conventional assays, which have a level of detection of 10 ng/L, and detectable levels in < 1% of subjects in the general population (101) the hs-cTnT assay measures concentrations that are tenfold lower than with the conventional assays, and 25-67% of the general population have been shown to have detectable levels (102, 103). 97.7% of patients with stable coronary artery disease (CAD) have been shown to have detectable levels of cTnT with this type of assay (104). hs-cTnT levels above the detection limit are associated with cardiac structure and impaired function and predictive of heart failure, cardiovascular death and all-cause mortality (103). But increased sensitivity leads also to reduced specificity, which means that low troponin concentrations are now also detectable among patients without symptomatic ischemic heart disease. It remains therefore a challenge to define the clinical importance of detectable cardiac troponin in patients without symptomatic heart disease.

Interpretation

Detection of cTnT or cTnI above the 99th percentile of a reference population is today a prerequisite for the diagnosis of AMI (94). As mentioned above, the highly sensitive tests improve AMI diagnostics but also challenge the interpretation and use of troponin values in clinical settings due to reduced specificity. We know today that elevations of cardiac troponins above the 99th percentile URL are not only found in patients with acute myocardial infarction and seem to be associated with structural changes of the myocardium (ventricular hypertrophy, remodeling or dysfunction), arrhythmia as well as with other conditions such as advanced age and male gender, impaired renal function, sepsis and severe pulmonary hypertension or embolism, as illustrated in Figure 6.

Cardiac Causes	Noncardiac Causes
Cardiac contusion resulting from trauma	Pulmonary embolism
Cardiac surgery	Severe pulmonary hypertension
Cardioversion	Renal failure
Endomyocardial biopsy	Stroke, subarachnoid hemorrhage
Acute and chronic heart failure	Infiltrative disease, e.g. amyloidosis
Aortic dissection	Cardiotoxic drugs
Aortic valve disease	Critical illness
Hypertrophic cardiomyopathy	Sepsis
Tachyarrythmia	Extensive burns
Bradyarrythmia, heart block	Extreme exertion
Apical ballooning syndrome	
Post-percutaneous coronary intervention	
Rhabdomyolysis with myocyte necrosis	
Myocarditis or endocarditis/pericarditis	

Figure 6: Cardiac and non-cardiac causes of troponin elevation. Reprinted from Mahajan et al. (105), reproduced from Jaffe AS (106) with permission from Elsevier.

Elevated troponin levels are commonly found among patients with structural heart disease, including stable coronary artery disease (107, 108) and heart failure (109), and in up to 2% of the general population (102, 103, 110). This is important because troponin elevations have been shown to be associated with adverse clinical outcomes in patients with acute myocardial infarction, as well as stable coronary artery disease (107), chronic heart failure (109), acute pulmonary embolism or chronic pulmonary arterial hypertension (111, 112). Some studies also suggest a possible cross reactivity of cTnT in skeletal muscular disease (113), but this needs to be confirmed in further investigations. It remains unclear how and to what extend cardiac troponin is released in conditions other than myocardial necrosis. The suggested pathophysiological mechanisms underlying troponin elevations are apoptosis, increased myocyte turnover, cellular release of proteolytic troponin degradation products, increased cellular wall permeability or formation and release of membranous blebs. Extreme exertion such as marathon running has also been associated with troponin release (114, 115).

Interestingly, the troponin half-life appears to be shorter than observed after AMI, suggesting a cytoplasmic rather than a structural origin of troponin in this context. (116). Whether cardiac troponins are released after reversible ischemia induced by stress-testing or rapid pacing is controversial (117, 118). Sabatine and colleagues detected changes in circulating cTnI that were associated with the degree of myocardial ischemia induced by transient stress-testing (119). Turer and colleagues measured troponin by the high-sensitivity assay after coronary pacing and their findings suggested that the heart may release troponin T also under conditions of moderate cardiac stress and in the absence of underlying CAD or apparent ischemia (120).

Troponin and COPD

Previous studies evaluating the association between COPD and cardiac troponin in general have mainly focused on patients with COPD exacerbations or have used COPD as a covariate for comorbidity. Retrospective data have shown elevations of cardiac troponin during COPD exacerbations (121) and that troponin elevation is associated with decreased survival in this patient group (122-125). One prospective study from 2003 describes a predictive value of Troponin I for in-hospital deaths among patients hospitalized for severe AECOPD (126). A summary of papers published previous to our first article is presented in Table 1. With mainly retrospective data present at that time, we wanted to provide prospective data on the association between cardiac troponin and survival in COPD patients hospitalized for an exacerbation. In addition there were no data on troponin levels in patients with COPD in the stable state of the disease and in paper II we wanted to expand the knowledge about myocardial affection measured by hs-cTnT in this particular group compared to the general population.

Table 1: Publications until the year 2009 addressing AECOPD and Troponin.

Year	Author	Population	troponin cut-off	Troponin positive	Mortality
2003	Baillard (126)	ICU patients, n=71 Severe AECOPD Prospective	cTnI >0.5µg/L	18%	OR 6.5, 24 hour in hospital mortality
2004	Harvey (121)	AECOPD , n=235 Retrospective	cTnI >0.4µg/L cTnT>0.03 µg/L	25%	Prevalence study
2008	Brekke (122)	AECOPD, n=396 Retrospective	cTnT ≥0.04µg/L	25%	HR 1.6, 1.9 years mean follow-up
2009	Fruchter (123)	AECOPD, n=182 Retrospective	cTnI >0.03 µg/L	46%	HR 1.3, 4.2 years mean follow-up
2009	Martins (124)	AECOPD, n=173 Retrospective	cTnI > 0.03 µg/L	70%	MRR 1.7, 1.5 years mean follow-up

AECOPD, acute exacerbation of COPD; cTnI, cardiac Troponin I; cTnT, cardiac Troponin T; HR, hazard ratio; ICU, intensive care unit; MRR, mortality rate ratio; OR, odds ratio.

STATINS

History

In the 1970s the Japanese microbiologist Akira Endo discovered a fermentation product (compactin) of *Penicillium citrinum* that had a strong inhibitory effect on Hydroxymethylglutaryl Coenzyme A (HMG-CoA) reductase. This subsequently led to the further development of other HMG-CoA reductase inhibitors, the statins. In 1987 lovastatin became available for prescription as the first statin of its class. Statins have subsequently been documented to have beneficial effects on mortality and morbidity in patients with established CVD. A clear reduction of mortality with simvastatin in the 4S trial (127) and clear reduction in coronary events with very few adverse effects resolved

the cholesterol controversy in the 1990s. Thereafter, statins have been found to reduce the incidence of cardiovascular events in individuals without known CVD from the general population (128, 129). The Heart Protection Study (HPS) and the JUPITER trial subsequently demonstrated a clinical benefit in a broad population, including those with low cholesterol levels (130, 131).

Types of statins

Statins vary in their potency to inhibit HMG-CoA reductase and can be differentiated according to how they dissolve in lipids or water into lipophilic and hydrophilic, respectively. Due to these properties, the different types of statins differ with regard to their lipid-lowering effects, their side effect profiles, as well as to the way they are eliminated. Rosuvastatin is the most potent inhibitor of HMG-CoA reductase on the market, followed by atorvastatin and simvastatin. Hydrophilic statins, pravastatin and rosuvastatin, have less tissue absorption, except for the liver, and have fewer side effects due to lower dependence on the cytochrome p450 enzyme (132). Lipophilic statins are considered more likely to enter vascular cells by passive diffusion than hydrophilic statins. Examples of statins from the lipophilic group are simvastatin, atorvastatin and fluvastatin. Clinical trials comparing lipophilic with hydrophilic statins have yielded varying results and potency and dosage seem to have greater impact on clinical outcomes. In the PROVE IT–TIMI 22 study intensive statin therapy (80 mg of atorvastatin) was superior to treatment with a moderate statin (40 mg of pravastatin) in terms of protection against death or major cardiovascular events in patients with a recent acute coronary syndrome (133).

Pleiotropic effects of statins

Statins have been proposed to exert important effects on the immune system that are independent of the effect on lipids, i.e. pleiotropic effects, which means that they are

producing more than one effect (134). The term "pleiotropic" is derived from the two ancient Greek words "pleio," meaning many, and "trepein," meaning influencing. Beside the cholesterol lowering effect, statins also inhibit the so-called isoprenoid synthesis, as illustrated in Figure 7, which results in antioxidant effects and the up-regulation of endothelial nitric oxide synthase, an enzyme involved in vascular endothelial function.

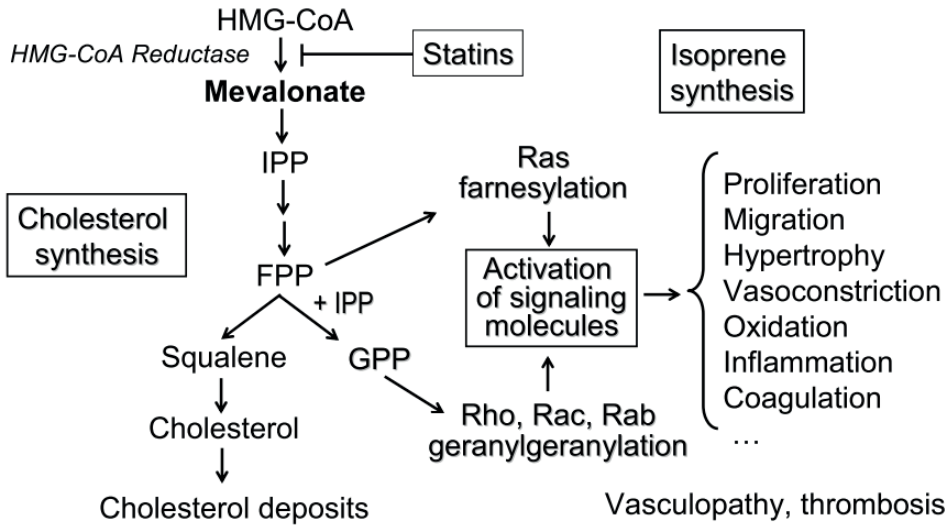

Figure 7: Cholesterol and isoprenoid synthesis influenced by statins (reprinted with permission from Davignon J et al, VHaRM 2005 (135)).

Additionally, several inflammatory markers, such as CRP and nuclear factor-kB, have been shown to be reduced by statins, leading to the hypothesis that statins possess anti-inflammatory properties (136-138). Other suggested mechanisms for pleiotropic effects of statins include immunomodulation, normalization of sympathetic outflow, plaque

stabilization, reduced activation of the blood coagulation cascade, and the inhibition of platelet aggregation (136). Anti-inflammatory actions seem to contribute to the beneficial cardiovascular effects, but controversy remains regarding the mode of action and impact of non-cardiovascular effects of statins. There are also clinical trials suggesting that statin benefits are independent of LDL-cholesterol reduction. As in the JUPITER trial, rosuvastatin was beneficial in the primary prevention of CVD in patients with elevated baseline inflammation (hsCRP >2 mg/L) but relatively low cholesterol levels (<130 mg/dl (3.4 mmol/L)), and other cardiovascular risks (131). In that trial, individuals with lower LDL-cholesterol and CRP after treatment benefitted particularly, which supports the importance of inflammatory components in the treatment of CVD and support the non-cholesterol-dependent effects of statins since the reduction of CRP by rosuvastatin was not related to LDL-cholesterol reduction. Another study measured the effect of rosuvastatin on coronary atherosclerosis in patients with ischemic heart disease and found that treatment with rosuvastatin significantly reduced intracoronary plaque volume and increased lumen volume without a significant correlation between LDL-cholesterol and plaque volume reduction (139).

Statins and COPD

In retrospective observational, case-control studies and population-based analyses, statin therapy has been associated with improved survival in patients with COPD (140, 141), a reduction in COPD exacerbations as well as in number of and time to COPD-related intubations and improved pulmonary function (142, 143). However, it remains unclear whether statins exert potential beneficial effects in COPD by primarily affecting vascular or respiratory function and whether a beneficial effect is mediated by attenuation of systemic inflammation or not. Experimental data suggest that statins may have beneficial effects on airway inflammation, matrix metalloproteinase activity, and mucin production (144-146). In rat models, simvastatin ameliorated the development of cigarette smoking–induced emphysema and pulmonary hypertension (147).

At the time when our project was initiated, two randomized placebo-controlled trials on statin treatment among patients with stable COPD had been published, as presented in Table 2. The first study (148) was double blind and included 125 patients with stable COPD. They reported a beneficial effect of pravastatin treatment on exercise time. The effect was more prominent among patients with decreasing levels of CRP during treatment. The second study (149) was open labelled, included 56 patients and failed to demonstrate any significant effect on circulating inflammatory biomarkers.

A summary on publications of randomized controlled trials (RCTs) on the effect of statins in COPD patients is presented in table 2. With only limited data on statin effect in COPD populations from double-blind RCT's and no data on the effect of rosuvastatin on endothelial function in stable COPD, we wanted to add knowledge about the effect of statin treatment on the vasculature, pulmonary function and systemic inflammation.

Table 2: Publications of RCTs addressing statins and COPD until the year 2010.

Year	Author	Population	Study Type	Statin	Outcome	Follow up
2008	Lee (93)	Stable COPD. n=125	RCT Double blinded	Pravastatin	Exercise time, CRP	6 months
2010	Kaczmarek (94)	Stable COPD, n=56	RCT Open labelled	Simvastatin	CRP, IL6, MMP, TNFα, fibrinogen	3 months

CRP, C-reactive protein; COPD, chronic obstructive pulmonary disease; IL6, interleukin 6; MMP, matrix metalloproteinase; TNFα, tumor necrosis factor alpha.

RATIONALE AND AIMS OF THE PRESENT STUDY

Rationale

The introduction demonstrates the importance of understanding the relationship between COPD and cardiovascular disease, including possible pathophysiological mechanisms, diagnostic methods, predictive factors, as well as treatment options.

On initiation of this project, previous studies describing markers of myocardial injury were mainly of retrospective design, did not use the newest high sensitivity cTnT assay and had focused on patients with chronic obstructive pulmonary disease in the exacerbated state. Further, there were several observational studies describing a beneficial cholesterol-independent effect of statin treatment in COPD patients, but randomized controlled trials were sparse.

With the new high sensitivity cTnT assay available, we wanted to evaluate subclinical myocardial injury and its prognostic value in patients with AECOPD in a prospective cohort study. However, with most COPD patients being in the stable state of the disease we also wanted to expand our investigations and describe the prevalence of subclinical myocardial injury in stable COPD patients compared with the general population.

Finally, with the promising data from several previous retrospective studies, clearly suggesting a beneficial effect of statins in COPD, we wanted to shed light on a potentially beneficial treatment effect of statin therapy on vascular function, lung function and biomarkers in stable COPD patients in a randomized double-blind controlled clinical trial.

General aim

The general aim was to assess the prevalence and prognostic value of subclinical myocardial injury measured by cardiac troponin T and to evaluate the effect of statin treatment on vascular function, pulmonary function and systemic inflammation in patients with COPD.

Specific aims of the papers

1) To prospectively assess the prognostic value of cardiac troponin T measured with a high-sensitivity assay in patients hospitalized with an exacerbation of COPD (paper I).

2) To assess the levels of cardiac troponin T measured with a high-sensitivity assay in COPD patients in the stable state of the disease compared to a sample from the general population (paper II).

3) To test the hypothesis that treatment with rosuvastatin for 3 months is associated with improved endothelial function assessed by peripheral arterial tonometry, improved pulmonary function assessed by spirometry and reduced systemic inflammation assessed by measurement of interleukin 6 and C-reactive protein in stable COPD patients (paper III).

MATERIALS AND METHODS

"No one does anything right in life, until they realize that they are making a mistake"
(Albert Einstein)

Material

Study design and subjects

Paper I is based on the results of a prospective cohort study of 99 patients who were included from January 3rd 2005 through to November 30th 2006. All data were manually entered into a Microsoft Access 2000 database. Mortality status through December 31st 2008 was retrieved from the hospital's electronic records, which are annually updated with the National Population Registry. All patients in paper I were eligible for preliminary inclusion if admitted through the emergency department of the Akershus University Hospital with assumed AECOPD. The recruiting physicians had no knowledge of any laboratory results. The attending physicians and nurses in the emergency department were instructed to record clinical information on a one-page form and blood samples were drawn and subsequently stored in a bio bank. The completed form was collected by the recruiting physician and stored in a designated box at the emergency department. An assigned physician collected the submitted forms the following morning and contacted the patients on the ward to retrieve written informed consent and medical history. Exclusion criteria were: age<50 years, metastatic cancer and Eastern Cooperative Oncology Group (ECOG) performance status ≥2, neuromuscular disease with respiratory failure, or anticipated non-cooperability.

Paper II is based on the results of a prospective cohort study in which 101 patients were included from June 23rd 2009 through October 11th 2011. Patients were recruited from the outpatient department and were eligible if they had an established diagnosis of COPD stage I to IV after GOLD criteria and were within the age range between 40 and 80 years. Patients received study information and consent form per mail and were

contacted 1 week later per telephone and asked if they were willing to participate in the trial. Patients were classified as stable if they did not have worsening of their respiratory symptoms beyond normal day-to-day variation or change in medication at least three weeks prior to inclusion. Exclusion criteria included diagnosed lung disease other than COPD, except asthma, prior history of cardiovascular disease, congestive heart failure, valvular heart disease, significant arrhythmias or conduction delays, uncontrolled arterial hypertension, body mass index >40 kg/m^2 and significant neurological or haematological disorder. The subjects serving as references in paper II consisted of 120 individuals, male or female, living in ten municipalities in the hospital's catchment area. They were invited after random selection from the National Population Registry between 2006 and 2008. Data were manually registered into a Microsoft Excel database and exported into a SPSS - database.

Paper III is based on a randomized, controlled, double-blind, clinical trial (Effect of ROsuvastatin Therapy on Peripheral VasoDilator Function, Inflammatory Markers and Pulmonary Function in Patients with StablE Chronic Obstructive Pulmonary Disease (RODEO)) in which 99 patients were included from March 22nd 2010 until July 10th 2013. Eligible patients were recruited from the outpatient clinic of Akershus University Hospital and from the outpatient clinic from Haukeland University Hospital. Patients were eligible if they had a verified diagnosis of COPD stage I to IV after GOLD criteria and were between 40 and 80 years of age. Exclusion criteria included any other diagnosed lung disease except chronic asthmatic bronchitis and mild bronchiectasis, any history of or active coronary artery disease, cerebrovascular or peripheral vascular disease, history of or clinically significant congestive heart failure, valvular heart disease, clinically significant arrhythmias or conduction delays, uncontrolled arterial hypertension (defined as blood pressure above 180/110 mmHg with or without the use of antihypertensive medication), body mass index >40 kg/m^2, history of diabetes mellitus or measured fasting glucose >11 mmol/L, history of hypercholesterolaemia or measured total cholesterol >8 mmol/L, known poliomyelitis, motor neuron disease, cranial or temporal arteritis, stroke or myopathy, neutropenia or aneamia (Hb <8 g/dL), history of chronic renal failure, serum creatinine >176 μmol/L (2.0 mg/dL) or creatine

kinase >3 times the upper limit of normal (ULN), acute or chronic liver disease (serum transaminases >3 times the ULN), pregnancy (self-reported and blood test prior to inclusion) or active abuse of drugs or alcohol or poor compliance anticipated.

There was no run-in period in the trial and participants were randomly assigned to either rosuvastatin 10 mg or placebo once daily for 12 weeks and there was one control investigation after the treatment-period. Due to slow recruitment, patient inclusion was extended to the Haukeland University Hospital, Bergen, Norway, where 8 patients of the total of 99 patients were recruited from the pulmonary outpatient clinic and investigated after the same criteria. The complete trial was monitored by the department of Good Clinical Practice (GCP) from the Oslo University Hospital, Oslo and by Innovest AS, Bergen with a total of 6 and 3 monitoring visits, respectively. Data were recorded electronically and on a paper case report form (CRF). They were manually entered into a Microsoft Excel database and exported into a SPSS-database.

Sample size determination

Sample size calculation for paper I was based on a pilot study on 29 consecutively hospitalized patients with AECOPD by Brekke, Omland and Søyseth. They found, that seven patients (24%) had cTnT above normal levels (≥0.04), using a fourth generation assay. Six of these (86%) died during a 10 month follow-up, compared with two (9%) of the patients in the group with normal cTnT levels. Assuming a 50% increased mortality in the group with elevated hs-cTnT, α=0.05 and a prevalence of elevated hs-cTnT of 25%, 104 patients were necessary to achieve 90% power to detect a significant association between hs-cTnT and mortality.

Sample size calculation for the study population of paper II and III was based on the measurement of peripheral vasodilator function. Based on previous small, unpublished studies of healthy individuals at the Akershus University Hospital (night shift work and endothelial function, n= 12) and from the literature, the standard deviation for the difference between two repeated Endo-Pat™ measurements used was 0.59 (150, 151).

We assumed initially that statin treatment is associated with a 30% increase in the reactive hyperemia index (RHI) in the statin group, and calculated that the study would have 80% power (α=0.05) with 61 patients per arm. Including an expected cross-over rate of 10% in both groups we initially planned to include 70 patients per arm. Due to additional information and literature on test-retest reliability during the study period (152) and reduced number of available patients the power calculations in the protocol were revised in 2013. According to the publication of McCrea and colleagues (152), considering an absolute change in the PAT hyperaemia ratio of 0.25 units (corresponding to a 10% increase in RHI from pre- to post-treatment, based on RHI measured in the population with stable COPD from our cross-sectional study), 72 patients (36 per arm) were required to obtain a power (1- ß) 0.80 at an α of 0.05. Given an expected cross-over rate of 10%, the study was considered to be sufficiently powered with 50 patients per arm as by October 2013. There was no separate sample size calculation for the cross-sectional part of the study.

Methods

Endothelial function

Endothelium-dependent vascular function was assessed by peripheral arterial tonometry (PAT) (EndoPAT™, Itamar Medical,Caesarea, Israel). Digital pulse wave amplitude (PWA) was recorded with a finger probe of the PAT device prior to and during reactive hyperaemia, induced by occluding the arterial blood supply to the non-dominant forearm for 5 minutes by a blood pressure cuff. The occlusion pressure was at least 60 mmHg above systolic blood pressure (minimally 200 mmHg, and maximally 300 mmHg). Forearm occlusion has been shown to be less uncomfortable for the patient and to yield the same results as upper arm occlusion (153). An inflatable rubber balloon on the inside of the finger probe is filled with air after being placed on the finger and puts a counter pressure of 70 mmHg on the finger to avoid venous pooling of blood and veno-arteriolar reflexes. Each recording consisted of 7 minutes of baseline

measurement, 5 minutes of occlusion measurement, and 7 minutes post occlusion measurement (hyperemic period). The reactive hyperemia index (RHI), a measure of peripheral vasodilator function, was calculated using a computerized automated algorithm (software version 3.4.4). The RHI is the ratio of the average PWA during the 1-minute period beginning after exactly 60 seconds of reactive hyperaemia compared with the average PWA during a 210 second pre-occlusion baseline period. Hamburg and colleagues described earlier that the relation between cardiovascular risk factors in the Framingham cohort and PAT hyperemic response was strongest in the 90- to 120-second interval after fingertip flow was restored (154). To decrease the effect of confounding factors, the ratio is normalized to the concurrent pulse wave amplitude signal of the contra-lateral arm.

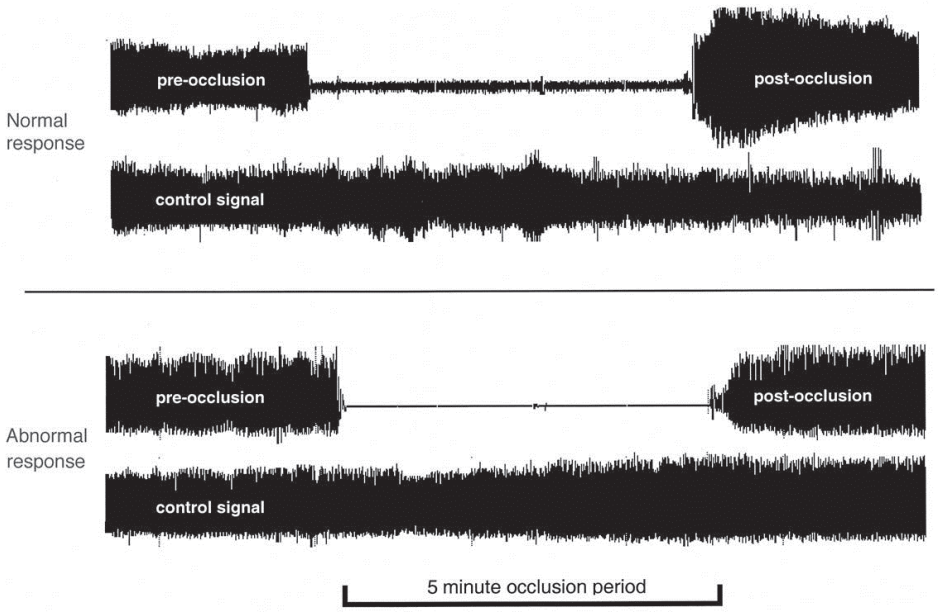

Figure 8: EndoPAT™ recordings of subjects with normal and abnormal reactive hyperemic response, reprinted with permission from Bonetti et al., JACC 2004 (64).

This method has been shown to correlate well with the vasodilator response of coronary vessels following infusion of the endothelium-dependent vasodilator acetylcholine (155) and to provide prognostic information concerning the future incidence of cardiovascular events in the general population (156, 157).

The subjects were in supine position for a minimum of 20 minutes before measurements, in a quiet, temperature-controlled (21–24°C) room with dimmed lights. All measurements were performed in the morning and the subjects were asked to remain as calm and silent as possible during the entire measurement period. Cyclooxygenase inhibitors, cardio active drugs, alcohol, food, sweetened beverages, caffeine and smoking were prohibited the last 12 hours prior to the study. All measurements were performed according to the manufacturer's instructions. An example of a result of EndoPat™ measurement is presented in Figure 8 with a normal response above and an abnormal response below.

Blood samples

cTnT: The hs-cTnT concentration in serum was measured with the Elecsys 2010 Troponin T hs STAT assay (Roche Diagnostics, Mannheim, Germany). The 99th percentile for hs-cTnT in a healthy reference population was 14ng/L, 95% confidence interval 12.7-24.9ng/L. The lowest concentration with a coefficient of variation (CV) less than or equal to 10% with the Elecsys Troponin T hs STAT assay was 13 ng/L. The Elecsys 2010 Troponin T hs STAT assay has a detection limit (LoD) of 5.0 ng/L and a limit of blank LoB) of 3.0ng/L. LoB is the highest apparent analyte concentration expected to be found when replicates of a blank sample containing no analyte are tested. LoD is the lowest analyst concentration likely to be reliably distinguished from the LoB and at which detection is feasible.

NT-proBNP: and IL6: N-terminal pro B-type natriuretic peptide (NT-proBNP) and interleukin-6 (IL6) concentrations in serum/plasma were measured with the Cobas e602 (Roche Diagnostics, Mannheim, Germany). NT-proBNP has a LoD of 0.6 pmol/L

and IL6 has a LoD of 1.5 pg/ml. The analytical repeatability of the NT-proBNP method was 1.6% (level 14.6 pmol/L) and for the IL6 determinations the repeatability was 3.1% (level 12.1 pg/mL) (Roche Diagnostics, Mannheim, Germany).

CRP: The CRP concentrations in serum were measured with a high sensitive method using the Cobas c 311/501 analyzer (Roche Diagnostics, Mannheim, Germany). hsCRP has a LoD of 0.15 mg/L (1.43 nmol/L, 0.015 mg/dL) and the analytical repeatability of the hsCRP method was 1.0% (level 4.34 mg/L).

Spirometry

Spirometry with reversibility testing with salbutamol was performed at the outpatient clinic as recommended by Miller (158). COPD patients were classified into four categories (A-D) in accordance with the combined COPD assessment of the revised GOLD strategy document, including associations between symptoms (MMRC scale), spirometric classifications and future risk of exacerbations (7). Since the questionnaire about dyspnea did not exactly reveal the number of exacerbations within the last year prior to inclusion (appendix), patients with GOLD I and II were contacted one more time about previous exacerbations, and hospital records were checked for hospitalizations in this period. Some patients had a FEV1/FVC>70 with a previously secured COPD diagnosis. By evaluating the spirometric time volume curve we identified patients who did not exhale completely 6 seconds and are therefore expected to have a lower FEV1/FVC than recorded. Those patients were kept in the trial and the others were excluded.

Six-Minute Walk Test

The Six-Minute Walk Test (6MWT) was performed after the ATS Statement Guidelines (159). The tests were performed by the nurses at the pulmonary outpatient clinic in the

morning. Some of the patient performance was curtailed in addition to dyspnea due to local pain in the hip, feet or legs and documented accordingly.

Dyspnea scale

All subjects completed a questionnaire on respiratory symptoms according to the modified Medical Research Council (mMRC) dyspnea scale) (160). The mMRC scale was used as an objective measure of stable-state breathlessness, which is associated with level of disability. The questions concerning the mMRC scale were a part of a composite questionnaire on smoking status and history, other symptoms such as cough and wheezing and use of antibiotic therapy prior to inclusion. After inclusion, patients filled out the questionnaire by themselves, but they were checked for completeness by the investigating physician and completed in cooperation with the patient if necessary.

Electrocardiogram

An electrocardiogram (ECG) was recorded with CardioSoft PC Based 12 Lead Interpretive ECG System (Version V6.51) (GE Medical Systems, Milwaukee WI 53223 U.S.A.). ECGs were evaluated by two independent physicians in paper III, and by one physician in paper II. ECGs were scored with regard to the following measures: rhythm, left ventricular hypertrophy (LVH, Sokolow-Lyon criteria), right ventricular hypertrophy (RVH), current ischemia (ST-segment depression), and prior MI (pathological Q wave, T-wave inversion or left bundle branch block (LBBB). ECGs were evaluated independently and blinded for all other data.

Chest radiographs

Chest radiographs were acquired routinely in almost all patients admitted with worsening of dyspnea had chest radiographs taken on admission. After May 2005, these were electronically processed and stored, whereas the older radiographs were on conventional X-ray films. During 2010, two experienced physicians (one radiologist and one pulmonologist) analyzed the chest radiographs taken on admission. It was also noted whether the radiograph was taken in the erect or supine position and the investigators systematically determined the presence or absence of the following variables: cephalization of the lung veins (a composite of Kerley B-lines, enlarged vessels in the lung apex, peribronchial cuffing, perihilar haze, and interstitial or perihilar edema), pneumonic infiltrates, pleural effusion, and the heart/thorax ratio.

Ethics

The study protocol for paper I and the RODEO protocol for paper II and III were approved by the Regional Committee for Medical Ethics (S-09266c 2009/5732 (RODEO)), and all participants received written and oral information before providing a written consent for participation. The privacy protection and establishment of database and bio bank were approved by the Norwegian Data Inspectorate and Norwegian Health Inspectorate, respectively. The interventional part of the RODEO study described in paper III was conducted in compliance with Good Clinical Practice (GCP) regulatory requirements and with approval from the Norwegian Medicines Agency and the independent ethics committee in accordance with the Declaration of Helsinki, with the informed, written consent of all participants.

Statistical analysis

Univariate analysis

All variables were investigated for distribution and outliers by plotting the data. This confirmed that the distributions of hs-cTnT, IL6 and hsCRP were skewed, with long right tails. hs-cTnT was categorized with cut-offs at 14ng/L and 39ng/L in paper I, and measurements of hs-TnT below the limit of blank (LoB) and between the limit of blank and the LoD [15] were replaced with LoB/2 and LoD/2, respectively in paper II. Baseline data are presented in the first table in all papers. Baseline characteristics of the study population were summarized within each randomization group in paper III. We used Chi-square and Fisher's exact test on categorical variables, Student's t-test on continuous variables, and univariate linear regression analyses to evaluate the relationship between two continuous variables. Performing these initial analyses to select variables for further analyses, a p-value <0.20 was considered the entry criterion.

Survival analysis paper I

In paper I the primary outcome variable was survival time. The final analysis included repeat admissions in extended survival regression models. When examining univariate associations between covariables and mortality, we calculated mortality rates (MR) and compared them using age-adjusted log-rank tests. All recorded admissions were used in this analysis. In the multivariable survival analysis all admissions were used in extended Cox regression analysis with time-dependent covariables.

Regression analysis paper II

We used least square univariate linear regression for continuous covariates categorized in quartiles with log-transformed hs-cTnT (ln[hs-cTnT]) as the dependent variable to detect linear trends. Additionally, Pearson's correlation analyses were performed in

order to investigate associations between relevant continuous determinants for hs-cTnT. For each category of the covariate hs-cTnT was expressed as the geometric mean because of its skewed distribution. The association between hs-cTnT and COPD was investigated using multiple ordinary least square regression adjusting for covariates that were associated with COPD as well as hs-cTnT with corresponding p-values less than 0.2. The initial full model was reduced by backward elimination if the p-value for association between ln[hs-cTnT] and the covariate was ≥0.05 and if removal of the covariate did not change the association between ln[hs-cTnT] and COPD > 20%.

ANCOVA analysis paper III

For rosuvastatin vs. placebo, the difference in change between the treatment groups from baseline to 12 weeks for primary and secondary end points and feasibility measures was estimated using analysis of covariance (ANCOVA) with the posttest as outcome and pretest as covariate (161). The primary analysis of efficacy endpoints used an Intention to Treat (ITT) population, which included a Complete Case (CC) analysis. Linear regression analysis was predefined to be adjusted for variables with a possible impact on the primary endpoint endothelial function. The primary analysis of efficacy endpoints also used a sensitivity analysis of the ITT population (all patients as randomized) with simple imputation techniques for missing values, using three imputation models with no change and a major positive (50%) and a major negative (50%) change each for the missing values. Another analysis of efficacy endpoints used a per-protocol (PP) population. The analysis of safety endpoints used a safety population. The per-protocol population excluded individuals who discontinued the study or who had low study compliance with more than 40 unused tablets at study termination (n=8). The safety population included all participants in the group, based on treatment actually received. Additionally, treatment effects in predefined subgroups with elevated circulating pro-inflammatory markers, defined as hsCRP above median value and IL6 above median value were evaluated. Two posthoc sensitivity analyses were performed,

excluding patients with a) increased cardiovascular risk according to the Adult Treatment Panel III (ATP-III criteria) and b) a history of asthma.

All statistical analyses were performed using SPSS Statistics software (IBM, Version 20) in paper II and III and STATA 10 (StataCorp LP, TX, USA) and SAS software (SAS Institute, Inc., Cary, North Carolina, USA, Version 9.1.3) in paper I.

Literature search

The main database source of literature in this work has been Medline. Literature searches in PubMed and Ovid have used MeSH and self-selected search terms. Other database search has included Embase, Media center of World Health Organization, as well as search from reference lists from relevant publications. The literature has been limited to the English language.

SUMMARY OF PAPERS AND RESULTS

Paper I

Prevalence and prognostic value of cardiac Troponin T in COPD patients hospitalized for acute exacerbation of COPD.

In this prospective study 99 patients with acute exacerbation of chronic obstructive pulmonary disease were followed until death or termination of the study. During a median follow-up time of 1.9 years, 57 patients (58%) died. 97 patients (98%) had measurable levels of hs-cTnT and 73 (74%) had hs-cTnT above the normal range (≥ 14.0 ng/l). The crude mortality rates in patients having hs-cTnT <14.0, 14.0-39.9 and ≥ 40 ng/l were 4.6, 30.2 and 58.3 per 100 patient-years, respectively. Adjusting for relevant time dependent variables using an extended Cox regression analysis, the HRs (95% CI) for death were 4.5 (1.2 to 16) and 8.9 (2.4 to 32) among patients having hs-cTnT 14.0-39.9 and >= 40 ng/l, respectively, compared with patients with hs-cTnT < 14.0 ng/l. The association between mortality and hs-cTnT was strongly modified by heart rate at admission (p<0.001) in the way that the association between hs-cTnT and mortality was stronger among patients with tachycardia.

Paper II

Prevalence of Troponin T in COPD patients in the stable state and its association with pulmonary function, inflammatory markers and comorbidity.

In this cross-sectional study 101 stable COPD patients from the hospital's outpatient clinic and 120 individuals derived from a random general population sample were examined and compared with regard to levels of cardiac Troponin T and its association with pulmonary function, inflammatory markers as well as cardiovascular comorbidity. The crude geometric means of circulating hs-cTnT in the cases and the references were 7.75 and 3.01 ng/l, respectively (p <0.001); that is, a relative ratio of 2.57 (95% CI 2.05 to 3.23). After adjustment for relevant confounders, this ratio was moderately

attenuated to 1.65 (1.31-2.08). In the total study cohort, as well as among stable COPD patients, we found a significant positive association between hs-cTnT and IL6 concentrations (p <0.001) and the presence of pathologic Q waves (p=0.023). Among stable COPD patients, one quartile increase in forced expiratory volume in one second (FEV1) was associated with a 39% decrease in hs-cTnT and patient category (Global Initiative of Obstructive Lung Disease classification 2011) was positively associated with hs-cTnT (p trend <0.001) after multivariate adjustment.

Paper III

Randomized, placebo-controlled, double-blind, parallel trial on the effect of short-term treatment of rosuvastatin on endothelial function, pulmonary function and inflammatory markers in patients with stable COPD.

In this randomized, placebo-controlled, double blind, parallel trial 99 patients with stable COPD were assigned to receive rosuvastatin 10 mg (n=49) or matching placebo (n=50) once daily for 12 weeks. The primary outcome measure was change in endothelium-dependent vascular function measured with peripheral arterial tonometry and expressed as the reactive hyperaemia index (RHI). Secondary end points were change in pulmonary function, as assessed by FEV1 and FEV1/forced vital capacity (FVC), and change in the circulating inflammatory markers interleukin-6 (IL6) and high-sensitivity C-reactive protein (hsCRP). In the overall study population, no significant between-group difference in change in endothelium-dependent vascular or pulmonary function was observed. Rosuvastatin therapy was associated with a reduction of hsCRP (–20% vs. 11%, p=0.017) and an attenuation of the rise in IL6 (8% vs. 30%, p=0.028) compared with placebo. In a prespecified subgroup analysis of patients with supra-median circulating hsCRP concentrations (>1.7 mg/L), rosuvastatin was associated with improved endothelium-dependent vascular function (13% vs. 2%, p=0.026).

GENERAL DISCUSSION

"If we knew what it was we were doing, it would not be called research, would it?"
(Albert Einstein)

Methodological considerations

Internal validity

Internal validity is the extent to which a conclusion based on a study is possible and is improved by the extent to which a study minimizes error (or 'bias'). Major threats to internal validity can be random error and systematic error.

Random error

Troponin measurement

In paper I and II we measured TnT using a high-sensitive assay (hs-cTnT). The hs-cTnT analysis was introduced in clinical routine at the Akershus University Hospital laboratory in the year 2009. The laboratory staff at Akershus University Hospital performing the hs-cTnT analyses was trained, experienced and familiar with the equipment by the time of the analysis, and we have no reason to suspect errors related to the equipment or the use of the essays. hs-cTnT analyses in paper I were all performed in a single run and by the same person from thawed samples. One third of the hs-cTnT analyses from paper II were analyzed continuously as a part of routine diagnostics at the Akershus University Hospital laboratory and two thirds were performed in a single run from thawed samples. One major challenge with the hs-cTnT assay is the level of analytic precision in the low concentration range. According to the manufacturer, the LoD is 5ng/L, the LoB is 3ng/L, the 99th percentile is 13ng/L, and the

concentration with a coefficient of variation of ≤10% is 14ng/L. The thawed samples had never been thawed before, were not transported long distances and all samples had been handled in the exact same way. We can however not completely rule out the possibility of random errors related to the handling, storage and thawing of samples in the study. According to the manufacturer, the specimens are stable for 24 hours at 2-8 degrees Celsius and for 12 months at minus 20 degrees Celsius. The hs-cTnT assay has high tissue specificity with the assay antibodies binding to specific antigens that are exclusively found on cTnT proteins. There are no known reports of cross-reactions with other proteins. There is though still the possible bias of interference of hemolysis, heterophilic antibodies, human anti-animal antibodies, autoimmune antibodies, anti-cTn antibodies or fibrin clots (162-164).

EndoPat measurement

The digital PAT assessment is operator-independent due to control measurement of the contralateral arm and adjusting for systemic effects, it is easy to perform, and a strict written protocol was followed under all investigations. All measurements were performed at the same time of the day, in a fasting and smoke abstinent state, without intake of vasoactive medication and using standardized measurement procedures. We based the data on medication, food intake and smoking on oral information from the patient with smoking data supported by non-specific measurement of carboxyhemoglobin (CoHb). A discrepancy between recorded and real patient data is therefore still possible. All EndoPat™ measurements were performed after thorough training of the staff. The risk of a significant impact from random error related to the user is therefore low. The reliability and reproducibility of the digital PAT seem to be acceptable, although there is relatively few data on this subject with most studies published after the initiation of our study (152, 165-167). In comparison with FMD, the within-day variability for EndoPat™ measurements was higher (18% versus 10%, respectively, $p<0.05$), and the between day variability was similar (165). In larger

cohorts, the coefficient of variation for within-day variability (0.5-4 hours) was between 15% and 22% with lower inter-day reproducibility (166, 167).

Randomization and blinding

Randomization is defined as the random allocation of a treatment between the treatment groups. Systemic differences can lead to the over- or underestimation of the difference between the treatment groups and can be avoided by this method. Random allocation means that each patient has a known chance, usually an equal chance of being given each treatment and that the treatment arm is unpredictable. Mostly, a table of random numbers or a random number generator on a computer is used for simple randomization. Other methods include block randomization which ensures closely similar numbers of patients in each group, and stratified randomization in order to keep the groups balanced for certain relevant patient characteristics (168, 169). We chose block randomization due to a relatively small sample size and slow recruitment pace in order to ensure a balanced number in the groups. Subjects were randomized 1:1 in a double-blind fashion to either rosuvastatin (active) or placebo using a computer-generated code of random permuted blocks of four by a member of the data safety and monitoring board (DSMB). The sequence was stored in the pharmacy and concealed from investigators and participants. Treatment allocation was performed by an independent pharmacist. The active treatment and placebo had an identical capsule appearance. We did not stratify for certain patient characteristics, but predefined a multivariate model in the analysis to adjust for variables associated with the primary endpoint in case of an unpredicted misdistribution between the groups.

Sample size

The impact of random error, imprecision, can be minimized with large sample sizes. The size of the samples in this thesis were pre-defined by power calculations based on a

pilot study for the prospective study of paper I and on previous data on endothelial function for paper II and III as described previously. Sufficiently large samples are necessary to distinguish a small association from no association, so this is a relevant issue, in particular in cases where no statistically significant associations were found. The sample sizes were moderate in all papers and sample size could be an issue especially for paper III (risk of type II error) and is discussed in the relevant section.

Systematic error

Selection bias

The study population in paper I was approximately 14% of the sample population. Many AECOPD patients were admitted without being considered for inclusion in the study due to variable execution of tasks by the staff upon admission, which can represent a selection bias in this population. Inclusion rate might have varied due to the varying enthusiasm to inclusion of the staff, the general workload in the emergency department, and additional workload with a more severely ill patient or the failure to initiate practical procedures such as the gathering of relevant blood samples. Failure to include more severely ill patients might introduce systemic bias. However, comparing the data with other studies does not indicate that we have included particularly healthy patients. With a two-group design, selection bias might be of greater importance in paper II. Initially, a recruitment strategy of COPD patients in the stable state to paper II and III based on recruitment from general practitioners in the area was attempted, a method that proved ineffective. In the end, the study population consisted of patients recruited from the outpatient clinic at the Akershus University Hospital and Haukeland University Hospital. The fact that patients were recruited from the outpatient clinic may have introduced a possible selection bias as these patients may have more severe disease with a possible higher number of symptoms, comorbidities and exacerbations than the average COPD patient attending the general practitioner. The study population from paper II was approximately 35% of the sample population. The control sample from the

general population in paper II was collected prior to the inclusion of the COPD population. When comparing the population with the source population accessed from the Norwegian Central Statistics Office (table 07459, July 2011) we found that the sample population seems to be representative of the source population in the same period, that is, from 2006 to 2010. Patient selection to paper III is based on the same procedures as paper II, but with additional exclusion criteria due to the interventional design of the trial such as diabetes mellitus, previous or clear indication for statin use, and use of medications interacting with rosuvastatin. The study population from paper III was approximately 21% of the sample population and patients were mainly excluded because they did not wish to participate in a clinical trial, they were current statin users or because they had a history of cardiovascular disease or diabetes. The controlled, double blinded randomized design of paper III should prevent systematic differences between baseline characteristics of the groups. However, given the relatively moderate sample size, stratification during recruitment with regard e.g. to smoking status would have ensured a more balanced distribution between the groups.

Confounding factors

When evaluating an effect and an association between an exposure and an outcome one has to take confounding, effect modification and interaction into consideration. Confounding is a distortion in the estimated measure of association that occurs when the primary exposure of interest is mixed up with some other factor that is associated with the outcome. Effect modification is when the association between an exposure and the outcome depends on the level of a third variable, the effect modifier. Interaction is described as the heterogeneity of an observed effect (170) in terms of effect modification by other variables in more than one direction. In paper I we identified heart rate and redistribution of pulmonary flow from the bases to the apices of the lung due to pulmonary congestion (cephalization) as a possible confounder of the association between troponin T elevation and outcome. However, the mortality rates did not change noteworthy in adjusted analyses. No ECG measurements were included in the analyses

of paper I but were evaluated in a post-hoc analysis. In paper II important possible confounding factors were history of comorbidities such as inflammatory diseases, undiagnosed heart disease or heart failure and of course age, gender or reduced renal function. Adjusting for those factors in the multivariate model did though not alter the results. We did however not include other measurements of cardiovascular comorbidity in the analyses beside ECG measurements and the biomarker NT-proBNP. In the first two papers, sample sizes were too small to adjust for all possible confounders, which may have resulted in possible statistical instability and overfitting of the multivariate models. Through the randomization procedure we minimized the impact of confounding factors in the interventional study of paper III. Due to chance, a skewed distribution of factors is still possible. As this could possibly have influenced the primary endpoint result, vascular function, we predefined an analysis adjusting for age, active smoking and hypertension. Indeed, there was a significant difference in the number of patients with current smoking status in the placebo vs. statin group. However, adjusted analysis yielded similar results as the unadjusted analysis. We could of course also have stratified patients according to these important variables in order to ensure an equal distribution between the groups. Concerning age, we had a middle aged population and a quite small age range in the study population and a well-balanced gender distribution in all populations.

Possible bias in the statistical analysis

All data were plotted carefully and explored graphically in the initial exploratory analysis. Baseline analyses of distributions and univariable associations in paper I were performed on the 99 baseline admissions only in order to keep observations independent from each other. We used a non-parametric test (Kruskal-Wallis) for normally distributed variables due to simplicity reasons. Applying non-parametric tests to normally distributed data does not violate any assumptions, although p-values may be less conservative using a parametric test, like a t-test. In survival analysis the model of fit of a regular Cox model and an extended Cox model was tested by comparing the two

models' log-likelihood criteria. The extended model based on 219 admissions in the 99 patients had a better fit. When using the extended model, the time from an observation until the end-point or censoring may be much shorter than the observation time for any individual. Therefore, it can be misleading to refer to the results as "long-term survival" or similar. In survival analysis of paper I most variables were dichotomized. This may have resulted in some information being lost as compared to using continuous variables, but at the same time permits assessment of effect modification in relatively comprehensible tables. In paper II we used quartiles of the continuous covariates, which could have caused some loss of information. However, by using quartiles we were able to better demonstrate a significant trend of hs-cTnT values for each covariate. In paper III we adhered strictly to the predefined analysis plan related to the primary and secondary endpoint and the pre-specified subgroup analysis in order to prevent multiple hypothesis testing and outcome-report bias. ANCOVA analysis was chosen for the analysis of the primary and secondary endpoint as it is a recommended and commonly used method in double-blind randomized clinical trial evaluation.

External validity

External validity is the extent to which the results of a study are applicable and generalizable to other situations and across populations. Recruiting COPD patients admitted for AECOPD might lead to a higher percentage of COPD patients of the frequent exacerbation phenotype. This cohort is therefore not representative for COPD patients in general, but was neither intended to be so. The aim was to assess troponin T status on admission for AECOPD and to gather subsequent mortality data. The study populations in paper II and III excluded patients with previously known cardiovascular disease. Cardiovascular disease is a common comorbidity in COPD patients and exclusion of these patients therefore influences the generalizability of the results in this trial. However, the introduction of such patients would have made the interpretation of results problematic. The population in paper II is not representative of a general COPD population, but can be generalized to a subgroup of COPD patients from a hospital

outpatient clinic in a presumably stable state and without previously known cardiovascular disease. The study population in paper III represents the same COPD patients from the outpatient clinic as in paper II, but with patients who are even more selected due to the strict exclusion criteria from the interventional part of the study. This population is therefore only generalizable to stable COPD outpatient clinic patients without clear indications for statin use and a generally lower cardiovascular risk profile.

Summary of methodological considerations

Taking the limitations in design and statistical analysis into consideration, the results from paper I and II seem suitable to describe prevalence and predictive value of troponin T as a marker of cardiac injury in COPD patients in the stable and the exacerbated state of the disease. Unrecognized subclinical heart disease in the source population could have been a source of type I error and supplemental information on cardiovascular comorbidity in the adjusted analysis would have been desirable. Paper III provides data of a well performed double-blind, randomized controlled clinical trial, but results should be viewed in relation to possible type II errors of weak associations between the intervention and the primary and secondary endpoints and need confirmation in larger clinical trials. The results of all papers are based on selected groups of COPD patients and are therefore not generalizable to a general COPD population.

Discussion of the results

General findings

The general findings of our studies are that patients with COPD show elevated levels of cardiac troponin T in the exacerbated as well as in the stable state of the disease and that cardiac troponin T predicts total mortality in AECOPD. In addition, statin treatment seems to primarily have a beneficial impact on the cardiovascular system in stable COPD patients with elevated biomarkers of systemic inflammation.

Troponin T in exacerbated COPD patients

Prior to the work with this thesis, retrospective studies have reported elevated troponin T levels in acute exacerbation of COPD measured with the conventional assay (121, 122, 125). We confirmed these findings in our prospective study with a high sensitivity assay. We also observed that hs-TnT elevation was associated with increased risk of mortality in AECOPD patients.

This is in agreement with the results from another prospective study from Chang and colleagues which was published only three months after the publication of paper I. They included 244 AECOPD patients without known CAD and showed that elevated levels of troponin T measured by the conventional assay strongly predicted 30-day mortality independently of other markers of severity and prognosis, but not long-term mortality (171). Marcun and colleagues prospectively investigated 127 AECOPD patients and did not find an association between troponin T and mortality but observed an association with rehospitalization in a 6-months follow-up period (172). However, causes for rehospitalization were not defined and with only 28% of the patients with elevated cTnT at admission and 19% at discharge the follow-up time might have been too short to give significant results with regards to mortality. They also used a fourth generation assay with a cut-off just above the detection limit and might therefore have missed low-grade troponin elevations. Kelly and colleagues recently found that troponin elevation together with severe acidosis and requirement for non-invasive ventilation were

associated with increased in-hospital mortality in patients attending the emergency department with an acute exacerbation of COPD (173).

The prognostic value of hs-cTnT in our study from paper I was clearly modified by the heart rate of the patients in the way that hs-cTnT was a stronger predictor for all-cause mortality among patients with tachycardia. Although our study was not designed to look into causative mechanisms, tachycardia or arrhythmias might reflect cardiac strain in our trial resulting in elevated levels of cTnT. McAllister et al. (174) published a study in 2012 describing that one in 12 COPD patients hospitalized for AECOPD met the criteria for myocardial infarction, including patients with common cardiovascular risk factors. Over half of the patients included had tachycardia. In an earlier study on exacerbated COPD patients from our hospital, raised troponin was found to be associated with lower hemoglobin and tachycardia (175). Tachycardia possibly represents underlying pathological mechanisms worsening the patient's prognosis. Together with increased ventricular filling pressure as seen in heart failure, pulmonary hypertension or in pulmonary embolism, tachycardia might contribute to cTnT release from cardiomyocytes without myocardial cell death. Tachycardia can be triggered by AECOPD-related hypoxia, leading to pulmonary vasoconstriction with increased right ventricular afterload, potentially increasing right ventricular strain and dysfunction. Tachycardia, together with hypoxemia and increased afterload increases myocardial oxygen demand without meeting it, thereby inducing a type 2 myocardial infarction. In our study population we did however not find an association between hypoxemia and troponin elevation, but the use of supplementary oxygen on the way to the hospital might have influenced the results in the way that the level of oxygen was higher at the time of measurement in some patients. The association between slightly increased cardiac troponin values with tachycardia and other arrhythmias is generally well known in clinical practice. Troponin has for example been shown to be elevated among patients with atrial fibrillation and to improve risk prediction in this patient group (176). COPD has been shown to be a significant predictor of atrial fibrillation (AF) and atrial flutter (AFL) and non-sustained ventricular tachycardia (NSVT) (177). However, in our study only 7% had known atrial fibrillation. Pulmonary hypertension or embolism might be

additional reasons for cardiac strain, but we did not include imaging methods to investigate for these conditions in our study. Respiratory acidosis, reflecting decompensated respiratory failure can also lead to tachycardia and troponin release, but in our study, we did not observe such an association with the blood gas analyses being mainly in the normal range. Treatment with adrenergic agents is contributing to tachycardia in COPD patients, which can affect the myocardium with negative consequences, especially for patients with unrecognized heart disease. Patients with more advanced COPD with frequent exacerbations use higher amounts of adrenergic agents and are more likely to have underlying heart disease. Adrenergic medications should therefore have been included in the analysis. Post hoc analyses were therefore performed in 2013: the use of inhaled beta-adrenergic agents (short- or long-acting or a composite of the two) was associated neither with tachycardia nor with hs-TnT category.

Other likely factors to explain the rise of troponin in AECOPD with subsequent increased risk include underlying coronary heart disease, the influence of ventricular hypertrophy, undetected ventricular dysfunction and systemic inflammation. Underlying coronary heart disease is likely to be underdiagnosed in this patient group due to similar symptoms (178). Campo and colleagues recently published a paper with the results from a prospective study including 694 AECOPD patients showing that cTnT actually failed to predict all-cause death. Only age, creatinine and NT-proBNP emerged as predictors for all-cause mortality in this population. However, cTnT was an independent predictor of cardiac death (CD), nonfatal AMI and the composite endpoint including CD and nonfatal AMI, with a stronger predictive value among patients without previously known IHD, suggesting subclinical IHD (179). Although a history of CHD was not associated with troponin in the population in paper I, we did not assess cardiac features of ischemic heart disease with electrocardiographic recordings or echocardiography, raising the possibility for an underestimation of the results. In post hoc analyses, ECGs from admission were therefore analyzed by two independent physicians. There was no association between higher hs-cTnT concentrations and electrocardiographic signs of prior MI, defined as pathological Q, loss of precordial R-wave progression or T-wave

inversion, (median values 29.8 vs 22.2 ng/L, p=0.15) or left bundle branch block (54.3 vs. 24.9 ng/L, p=0.20).

There is evidence that ventricular dysfunction is frequent during COPD exacerbations. Natriuretic peptides represent the gold standard for biomarkers in HF and have also been shown to be useful in excluding HF and to predict outcome (180). In our first study, we did not analyze biomarkers of myocardial strain such as BNP, but we used cephalization (upper lobe vascular redistribution) on the chest radiographs as a measure of left ventricular failure. NT-proBNP was later analyzed in the paper I cohort, and there was only a weak-to-moderate correlation between hs-cTnT and NT-proBNP (r= 0.34) (181). NT-proBNP was, however, elevated in the patients hospitalized with AECOPD and a significant predictor of long-term mortality in this population, independent of hs-cTnT. There was no association between NT-proBNP and ECG markers of right ventricular hypertrophy (RVH) or indices of prior MI, but an association was observed with peripheral edema. Taking the low sensitivity of the measurements used to detect heart failure in these patients into consideration, there might still be structural changes in the heart or diastolic dysfunction with impact on cTnT values and prognosis. Recently, Høiseth in our group compared a stable to a dynamic rise/fall pattern of hs-cTnT in AECOPD patients (182). Stable, moderately elevated hs-cTnT during AECOPD was associated with a poor long-term prognosis, independently of NT-proBNP concentration and a history of heart failure, suggesting possible subclinical structural changes in the heart, possible diastolic dysfunction and other additional possible factors contributing to cTnT elevation and its predictive value in AECOPD. However, in this study the study population was quite small (a subgroup of the cohort of paper I, n=65), with only 12 individuals presenting stable and moderately elevated hs-cTnT.

The question of other possible factors contributing to the relationship between hs-cTnT and mortality in this patient group, such as chronic anemia and systemic inflammation has been raised earlier (183). AECOPD is associated with an increased inflammatory state, possibly contributing to increased cardiovascular risk due to endothelial

dysfunction, atherosclerosis, an increased pro-coagulant state and risk of plaque ruptures (184). Recently, Harrison and colleagues could show that thrombocytosis was associated with both 1-year mortality and in-hospital mortality in patients with AECOPD (185) suggesting an inflammatory role of platelets and a possible beneficial effect of antiplatelet therapy. Unfortunately, we did not perform comparable measurements in our study. Recently, it has been demonstrated that COPD patients with frequent exacerbations have increased arterial stiffness as a measure of atherosclerosis. Arterial stiffness was also was related to inflammation (186). Kim and colleagues showed that untreated COPD patients had a higher level of CRP and carotid intima thickness than subjects without COPD (187). Seifarth and colleagues recently demonstrated that troponin T and high sensitivity CRP were related to plaque progression measured by CT coronary angiography, although not in a COPD population (188). However, in our first study we found no strong association between CRP levels or leucocyte count and hs-cTnT, neither was the leukocyte count identified as an effect modifier in this population, which might be due to the limited number of biomarkers or patient number in our study.

Finally, a recent hypothesis-generating study describes an interesting new aspect of a possible reason for myocardial damage and subsequent troponin release. Brown and colleagues describe the formation of cardiac microlesions and elevated troponin in relation with invasive infections with streptococcus pneumonia in mice (189). COPD and AECOPD is strongly associated with bacterial and viral infections (190). The pulmonary microbiome in COPD patients and infections in AECOPD are likely to play an important role in the inflammatory state of the disease and the development of structural changes in the lung and vasculature (191) as well as the myocardium, thereby influencing comorbidities and prognosis of these patients. There has been increasing interest in this field within the last years and further investigations are needed to shed light on the influence of the lung microbiome on cardiovascular comorbidity in COPD patients.

Troponin T in stable COPD patients

With paper II we wanted to focus on stable COPD patients and compare troponin levels among patients in the stable state with a reference sample from the general population without COPD. We could demonstrate that troponin T also is elevated in patients with COPD in the stable phase of the disease compared to the general population.

This is important because we believe that COPD patients hospitalized with an AECOPD present with aggravated disease related symptoms and comorbidities as a possible trigger for troponin release. Just before the publication of paper II, Søyseth et al. from our study group found that cTnT is elevated in another population with stable COPD patients and that the values differed significantly from patients hospitalized with AECOPD (192). This suggested that troponin elevation also is related to the exacerbation itself. This publication includes a slightly bigger sample (n=124) of patients in the stable state of COPD than paper II (n=101), but with similar inclusion criteria, methods and baseline values. Patel and colleagues (186) showed that troponin T levels increase during moderate COPD exacerbations compared to the stable state, that they do not recover for several weeks and that they relate to increased sputum purulence and exacerbation length. Patel and colleagues studied a population of 98 stable COPD patients, including some with ischemic heart disease (IHD). Our stable COPD patients had a higher prevalence of troponin T above the upper reference limit (URL) at 14ng/L with 29% compared to 17% and 16.4% in the other populations. Hattori and colleagues describe a prevalence of 9% with hs-cTnT above the URL, but they included patients with a life-long smoking history and COPD symptoms, of these were 63% diagnosed with COPD the other classified as being at risk of the disease, which is an interesting approach as COPD is likely to be underdiagnosed (193).

The cross-sectional design of the study of paper II does not allow us any causative conclusions of the results, but it is hypothesis-generating about possible pathophysiological mechanisms underlying hs-cTnT elevation in the stable state of the disease. Troponin release could be partially mediated by an inflammatory process, e.g.

by increasing the permeability of the cell wall of the cardiomyocyte or by inducing cardiomyocyte apoptosis. In paper II, elevated IL6 was a significant predictor of cardiac troponin T in stable COPD patients. Troponin release has previously been shown to be elevated and related to inflammatory markers in marathon runners (194). Inflammatory markers such as leukocytes, CRP and IL6 have been shown to be elevated in COPD patients compared to smokers with normal lung function and nonsmokers in the ECLIPSE study (27). COPD patients with signs of a persistently increased systemic inflammation had a higher rate of exacerbations and worse survival. The result of troponin measurement and association with inflammatory markers in the same population is eagerly awaited. Søyseth et al described an association between leucocyte count and troponin T levels, an association that was apparently not mediated by the levels of IL6 or CRP. Hattori and colleagues described CRP as an independent predictor of hs-cTnT elevation in stable COPD. We identified an association between IL6 and troponin T levels in paper II. However, in contrast to Søyseth et al we found no association between hs-cTnT and neutrophil count. By only evaluating neutrophils as one subtype of leukocytes, we might have missed valuable information from other inflammatory cell types and components. In addition, the discrepancy of the results might also reflect the complexity of immunological action in COPD and maybe other markers of pulmonary or systemic inflammation might be more useful to describe the inflammatory state in COPD.

Unrecognized ischemic heart disease could be another reason for cTnT elevation in our population. In paper II we excluded patients with previously known cardiovascular disease. We could identify four patients with a pathological Q-wave on the ECG and included this variable in the multivariate analysis of the paper. We can, however, not rule out the possibility of subclinical and undiagnosed ischemic heart disease in these patients without having performed invasive cardiovascular investigations such as coronary angiography. Patel and colleagues described a population with stable COPD with 20% ischemic heart disease (IHD) comprising stable angina, previous MI, and previous coronary artery intervention, such as bypass grafting, angioplasty and stenting (186). They confirmed our observation that myocardial injury measured by hs-cTnT is

common among stable COPD patients and extended our findings by demonstrating that hs-cTnT is commonly increased among patients with comorbid IHD. They also reported that arterial stiffness, another marker of cardiovascular risk, was related to serum troponin T (rho=0.350; p=0.001) but not to NT-proBNP(r= 0.161; p=0.146), which was within the normal range. In our population we also measured PAT as a marker of endothelial dysfunction and cardiovascular risk. We found no association between the reactive hyperemia index (RHI) measured by PAT and hs-cTnT (rho=0.158, p=0.114).

Hattori and colleagues excluded all patients with a history and ECG signs of cardiovascular disease from their study. They reported in their paper on patients with early stage/stable COPD that, in addition to CRP, hs-cTnT values were independently affected by age and right ventricular systolic pressure (RVSP) assessed by echocardiography (193). Increased right ventricular pressure in stable COPD patients might represent pulmonary vascular remodeling, which has been shown in mild COPD and even in smokers with normal lung function (195). RVSP assessed with echocardiography, however, cannot define pulmonary arterial hypertension (PAH) alone, and the most direct and exact way of measuring pulmonary artery pressure is by right heart catheterization (196). Hilde and colleagues demonstrated nicely with right heart catheterization that stable COPD patients without previously known pulmonary hypertension have signs of subclinical reduced right ventricular function (197). To our knowledge there are so far no published studies directly relating pulmonary arterial pressure values to high-sensitivity cardiac troponin levels in patients with stable or exacerbated COPD. Therefore it is not quite clear to what degree pulmonary hypertension contributes to troponin elevation in stable COPD.

Heart failure has been shown to be underdiagnosed among COPD patients, and about 20% of COPD patients seem to have unrecognized heart failure (198, 199). The presence of heart failure signifies ominous prognosis in this patient group (200). It has been previously shown that BNP levels are increased during AECOPD and fall during recovery (201). Patel and colleagues demonstrated that stable COPD patients with previously known ischemic heart disease (IHD) as assessed by the Charlson Comorbidity index,

have higher levels of NT-proBNP than stable COPD patients without IHD, but NT-proBNP levels and IHD were not predictive of the frequency of exacerbations but of exacerbation severity (202). In our population of stable COPD patients we found only modestly elevated levels of NT-proBNP, suggesting that congestive heart failure was not present. We evaluated the electrocardiographic measurements with the Sokolow-Lyon electrocardiographic voltage criteria, recognizing that these criteria have a low sensitivity for the detection of anatomic left ventricular hypertrophy and have previously been shown to vary according to the underlying heart disease (203).

Most of the previously described studies on cTnT and cTnT have shown an inverse association between pulmonary function and troponin measurement among stable COPD patients (except for the study of Patel and colleagues where this association was not presented). In paper II we also described an inverse association between the new GOLD classification adding exacerbation risk and symptoms and hs-cTnT. We did not though observe a significant difference in the magnitude of thecorrelation coefficients between the old and the new GOLD classification and hs-cTnT (rho=0.48, p=0.001 versus rho=0.47, p=0.001, respectively) in our population. This may be due to the fact that the new classification not sufficiently captures subclinical cardiovascular comorbidity and inflammation. COPD severity has been shown to be independently associated with the occurrence of atrial fibrillation and non-sustained ventricular tachycardia (177), both arrhythmias that can cause troponin release within this particular patient group. Patients with documented atrial fibrillation or other arrhythmias during the ECG recording were not included in the analyses of paper II, and there is thus no reason to suspect arrhythmic activity at the time of investigation as a reason for troponin elevation. However, since our patients did not perform a long-term Holter registration there is still the possibility of undetected arrhythmias that may have caused troponin release prior to investigations. Heart rate had a fairly narrow range in the stable COPD population and was not associated with troponin T in the population of paper II.

To the best of my knowledge there are at the time of writing this thesis no further publications investigating prevalence, determinants or the prognostic value of troponins in COPD patients in the stable state, and paper II provides valuable information comparing stable COPD patients with a presumably healthy reference population in terms of cardiac troponin values. Subclinical heart failure, myocardial strain, arrhythmias and systemic inflammation may represent direct and indirect stimuli for cardiac troponin release in the stable state of the disease, which might aggravate during a COPD exacerbation. Longitudinal and interventional studies are needed to document a causal relationship between these factors and hs-cTnT elevation in stable COPD as well as to investigate the prognostic value of cTnT measurement in this particular population.

Statin treatment in COPD patients

Observational studies have described a beneficial effect of statin treatment in COPD. This was recently confirmed in another observational study by Fruchter and colleagues, emphasizing the lipid-independent effect of statins in patients with COPD (204). With our interventional study we provided evidence that short-term treatment with rosuvastatin has a beneficial effect by improving vascular function in a subgroup of stable COPD patients without indication for statin therapy, but with elevated baseline CRP measured by a high sensitive assay.

We did not find an effect of rosuvastatin treatment on our primary endpoint, endothelial function as assessed by EndoPAT, in the overall population. John and colleagues recently showed in a double-blind, placebo controlled randomized trial that statin treatment in stable COPD had no effect on pulse wave velocity as a measure of arterial stiffness and cardiovascular risk in the total population, which might be due to short treatment duration, low dosage of simvastatin or insufficient sample size using a short-term treatment of 6 weeks with simvastatin 20mg in 70 patients (205). In paper III, sample size might also be an issue, but we considered the sample size to be sufficient for reliable

assessment of our continuous variable primary endpoint. However, the dosage of the study medication and the duration of the the treatment period may potentially have contributed to the lack of significant results. In addition, most patients in our population did not demonstrate endothelial dysfunction at baseline, which could have reduced the likelihood of observing an improvement in the primary outcome variable, although a cutoff for a definition of endothelial dysfunction with EndoPat measurement has to be seen as arbitrary. In a prespecified subgroup analysis, John and colleagues found that treatment with simvastatin reduced aortic pulse wave velocity (PWV) in those with a high baseline aortic PWV. They suggest a beneficial statin effect among stable COPD patients in terms of reduced cardiovascular risk in those not primarily targeted for primary prevention, which corresponds to our own results from paper III. The fact that we could identify a subgroup of patients with evidence of systemic baseline inflammation fits well with the established knowledge describing certain COPD phenotypes with elevated inflammatory markers and worse prognosis (206). However, there was no significant correlation between the lowering in CRP and the improvement in endothelial function. Neither was there a significant association between baseline CRP-levels and enothelial function in our study. These observations are in accordance with previous large epidemiological studies that found no clear association between hs-CRP and RHI (156, 207). This suggests that although markers of systemic inflammation identify a subgroup of stable COPD patients that seem to benefit from statin therapy, these markers are not necessarily suitable as tools for monitoring the effect on endothelial function. It is also still unclear to what extend therapeutic improvement in endothelial function translates into lower CV morbidity and mortality in this patient group. Endothelial function is not considered a primary therapeutic target in the prevention of atherosclerotic disease (208). However, Modena and colleagues demonstrate that improvement in endothelial function measured by FMD in hypertensive post-menopausal women was associated with an improvement in cardiovascular prognosis (209). Kitta and colleagues also assessed endothelial function by FMD and showed that persistent impairment of endothelial dysfunction despite optimized medical therapy was a strong independent predictor of adverse CV events

among patients with coronary artery disease (210). These results can however not be extrapolated to a COPD population. In contrast to FMD , there is no clear evidence on the impact of therapeutic changes in RHI over time. Further studies should investigate if RHI might serve as a stable surrogate endpoint or possibly a risk factor for CVD in COPD patients as well as in other populations (211, 212).

In the total study population we observed an anti-inflammatory effect of statin therapy with a significant difference in change in hs-CRP and IL6 between the treatment groups. The effect on IL6 seems to be influenced by a rise of IL6 in the placebo group. This rise might represent progression of inflammation and disease in the placebo group, however, AECOPD during follow-up and IL6 was not significantly correlated (r=0.023, p=0.825). There was a significantly higher number of current smokers in the placebo group in paper III, which could have influenced the effect on inflammatory markers. However, we could not find a correlation between current smoking status and IL6 and CRP values in our population (r=-0.131, p=0.198 and r=0.037, p=0.713, respectively) and patients had abstained from smoking at least 12 hours prior to investigation. Most patients did not change their smoking status under the treatment period. Three patients (two in the placebo and one in the rosuvastatin group) mentioned that they had quitted smoking 1-2 weeks before follow-up, but this time period is considered too short as to declare them as non-smokers. Excluding these patients from the analysis did not alter the results. Therefore, we suspect no major impact of current smoking status in this context. Previous interventional studies have found conflicting results regarding an anti-inflammatory effect of statin treatment in patients with COPD. Lee and colleagues described a significant effect of a six months treatment with Pravastatin 40mg in stable COPD (n=125) on CRP and IL6 (148). Kaczmarek and colleagues did not find a significant effect of a three months treatment with simvastatin 40 mg in stable COPD on five different inflammatory markers including CRP and IL6 (149), which might be due to small sample size (n=56) and short treatment period. John and colleagues did neither find an effect of treatment with simvastatin 20mg on inflammatory markers including CRP after a period of six weeks (205). Again, treatment period and study size might have been too small to provide significant results. Mroz and colleagues described the results

from a randomized, controlled, double-group pilot-study of 12-week treatment with 40mg atorvastatin. They observed a significant decrease in neutrophils and eosinophils in sputum and a significant decrease in CRP in the blood samples from the treatment group compared to placebo. In lung biopsies, inflammatory cell numbers ($CD45^+$ cells) decreased significantly in the placebo group. They performed transcriptome profiling in lung biopsies and also described a downregulation of immunological response genes in the atorvastatin group. However, the study was not blinded and the included number was small, with 5 patients in the placebo and 12 patients in the treatment group (213). Further randomized clinical studies with larger sample sizes are needed to confirm an anti-inflammatory effect of statins in stable COPD patients and the results from a large multi-center trial with simvastatin in regard to the anti-inflammatory effect are awaited as further described below (214).

In our study, treatment with rosuvastatin did not improve pulmonary function. Potential explanations for the lack of effect on pulmonary function include the short treatment period of 12 weeks, the relatively low dose of rosuvastatin with 10 mg and the lack of increased inflammatory activation at baseline in a substantial proportion of patients. As mentioned above, the recently published large multi-center trial (STATCOPE) also reported no effect of long-term treatment with simvastatin on pulmonary function. The primary outcome in the STATCOPE trial was the COPD exacerbation rate and no effect of simvastatin was observed. However, STATCOPE and our study from paper III included a high percentage (>70%) of patients using inhaled corticosteroids, which might have affected and attenuated an additional effect of statin treatment in these groups due to the anti-inflammatory effects of the steroids. Additionally, the STATCOPE study did not limit inclusion to patients with elevated baseline inflammation and it remains to be seen if such a subgroup has a greater treatment effect in terms of increased pulmonary function or less exacerbations in this and other COPD populations.

In a recently published observational study with 5794 unselected individuals from the general population statin use was associated with less COPD exacerbations but not in patients without cardiovascular comorbidity, which suggests that statins only have a

beneficial effect in COPD patients with coexisting cardiovascular disease (215). It remains unresolved what preventive effects there might be. A small triple-blinded, randomized clinical trial with atorvastatin for 6 months in COPD patients with secondary pulmonary hypertension reported no significant decrease in systolic pulmonary arterial hypertension and no significant improvement in six-minute walking distance (6MWD) (216). This is in accordance with our results, as we did not find an effect of rosuvastatin treatment in 6MWD.

To be able to distinguish between a possible pulmonary or only cardiovascular benefit of statin treatment in COPD patients, large scale interventional studies with long-term follow-up over several years and hard cardiovascular outcomes are needed, including COPD patients with well characterized symptoms, risk profiles and subclinical cardiovascular comorbidity.

CONCLUSIONS AND PERSPECTIVES

"If you can't explain it to a six year old, you don't understand it yourself"
(Albert Einstein)

Conclusions

In our studies we have assessed the prevalence and prognostic value of cardiovascular markers in stable and exacerbated COPD, respectively, and the effect of statin treatment on pulmonary and cardiovascular function as well as systemic inflammation in patients with COPD. We have shown that cardiac troponin T, a marker of cardiomyocyte injury, is associated with outcome in patients hospitalized for COPD exacerbation. We have also shown that hs-cTnT is elevated among patients with COPD in the stable state compared to presumably healthy persons from the general population. Statin treatment in stable COPD patients does not seem to improve pulmonary function, but attenuates biomarkers of inflammation and improves endothelial function in a subgroup of patients with evidence of increased systemic inflammation at baseline. Cardiovascular comorbidity might be underdiagnosed in COPD patients, and statin treatment seems to have impact mainly on cardiovascular function in this patient group.

Clinical implications

Many COPD patients may have undiagnosed cardiovascular disease and more investigations are necessary to identify these patients in order to give them adequate treatment. It remains a question though, which diagnostic measures should be used how and when in order to define the optimal treatment. Treatment should include a personalized medicine strategy, which should take the patients baseline inflammatory state into consideration and carefully assess possible underlying cardiovascular disease. Patients with a low risk profile but elevated systemic inflammation may still profit from

treatment even though there is no published evidence showing a clear beneficial effect of statin therapy in COPD patients with low cardiovascular risk beyond that of recommended primary prevention.

Suggestions for future research

Future trials in COPD patients should investigate COPD subgroups and phenotypes and focus on detailed mapping of COPD features such as genetics, symptoms, pulmonary infections and lung microbiome, medications and comorbidities with validated and specific methods. In order to do so it might be useful to use contemporary imaging techniques in trial populations such as echocardiography or MRI for cardiac evaluation and for example CT thorax for the evaluation of emphysema or bronchiectasis and coronary artery calcification. Sputum investigation and bronchoscopy provide valuable insight in intra-pulmonary state and features and should be considered further in future trials. The predictive value of troponins in stable COPD patients should be assessed with a clear definition of the stable state of disease. Randomized clinical trials should investigate the effect of statins against the effect of corticosteroids among COPD patients, especially in subgroups with evidence of baseline inflammation. Study populations should include patients at an early and stable stage of COPD and with low cardiovascular risk, as assessed by different risk calculators. Future studies should include long-term follow-up of cardiac and inflammatory biomarkers and use hard cardiovascular endpoints.

REFERENCES

1. Scadding JG. Principles of definition in medicine with special reference to chronic bronchitis and emphysema. Lancet. 1959;1(7068):323-5.

2. Agusti A, Calverley PM, Celli B, Coxson HO, Edwards LD, Lomas DA, et al. Characterisation of COPD heterogeneity in the ECLIPSE cohort. Respir Res. 2010;11(1):122.

3. Vestbo J, Hurd SS, Rodriguez-Roisin R. The 2011 revision of the global strategy for the diagnosis, management and prevention of COPD (GOLD)--why and what? The clinical respiratory journal. 2012;6(4):208-14.

4. Menezes AM, Perez-Padilla R, Jardim JR, Muino A, Lopez MV, Valdivia G, et al. Chronic obstructive pulmonary disease in five Latin American cities (the PLATINO study): a prevalence study. Lancet. 2005;366(9500):1875-81.

5. Bednarek M, Maciejewski J, Wozniak M, Kuca P, Zielinski J. Prevalence, severity and underdiagnosis of COPD in the primary care setting. Thorax. 2008;63(5):402-7.

6. Soriano JB, Rigo F, Guerrero D, Yanez A, Forteza JF, Frontera G, et al. High prevalence of undiagnosed airflow limitation in patients with cardiovascular disease. Chest. 2010;137(2):333-40.

7. Vestbo J, Hurd SS, Agusti AG, Jones PW, Vogelmeier C, Anzueto A, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med. 2013;187(4):347-65.

8. Nielsen R, Johannessen A, Benediktsdottir B, Gislason T, Buist AS, Gulsvik A, et al. Present and future costs of COPD in Iceland and Norway: results from the BOLD study. Eur Respir J. 2009;34(4):850-7.

9. Wedzicha JA, Brill SE, Allinson JP, Donaldson GC. Mechanisms and impact of the frequent exacerbator phenotype in chronic obstructive pulmonary disease. BMC medicine. 2013;11:181.

10. Wedzicha JA, Seemungal TA. COPD exacerbations: defining their cause and prevention. Lancet. 2007;370(9589):786-96.

11. O'Donnell DE, Parker CM. COPD exacerbations . 3: Pathophysiology. Thorax. 2006;61(4):354-61.

12. Laratta CR, van Eeden S. Acute exacerbation of chronic obstructive pulmonary disease: cardiovascular links. BioMed research international. 2014;2014:528789.

13. Berndt A, Leme AS, Shapiro SD. Emerging genetics of COPD. EMBO Molecular Medicine. 2012;4(11):1144-55.

14. Mohamed Hoesein FA, Zanen P, Lammers JW. Lower limit of normal or FEV1/FVC < 0.70 in diagnosing COPD: an evidence-based review. Respir Med. 2011;105(6):907-15.

15. Jones PW, Harding G, Berry P, Wiklund I, Chen WH, Kline Leidy N. Development and first validation of the COPD Assessment Test. Eur Respir J. 2009;34(3):648-54.

16. Gupta N, Pinto LM, Morogan A, Bourbeau J. The COPD assessment test: a systematic review. European Respiratory Journal. 2014.

17. White WB, Cooke GE, Kowey PR, Calverley PM, Bredenbroker D, Goehring UM, et al. Cardiovascular safety in patients receiving roflumilast for the treatment of COPD. Chest. 2013;144(3):758-65.

18. Rodrigo GJ, Castro-Rodriguez JA, Nannini LJ, Plaza Moral V, Schiavi EA. Tiotropium and risk for fatal and nonfatal cardiovascular events in patients with chronic obstructive pulmonary disease: systematic review with meta-analysis. Respir Med. 2009;103(10):1421-9.

19. Donohue JF, Singh D, Kornmann O, Lawrence D, Lassen C, Kramer B. Safety of indacaterol in the treatment of patients with COPD. International journal of chronic obstructive pulmonary disease. 2011;6:477-92.

20. Almagro P, Salvado M, Garcia-Vidal C, Rodriguez-Carballeira M, Delgado M, Barreiro B, et al. Recent improvement in long-term survival after a COPD hospitalisation. Thorax. 2010;65(4):298-302.

21. Mannino DM, Thorn D, Swensen A, Holguin F. Prevalence and outcomes of diabetes, hypertension and cardiovascular disease in COPD. Eur Respir J. 2008;32(4):962-9.

22. Sin DD, Man SF. Systemic inflammation and mortality in chronic obstructive pulmonary disease. Canadian journal of physiology and pharmacology. 2007;85(1):141-7.

23. Gan WQ, Man SF, Senthilselvan A, Sin DD. Association between chronic obstructive pulmonary disease and systemic inflammation: a systematic review and a meta-analysis. Thorax. 2004;59(7):574-80.

24. Karadag F, Karul AB, Cildag O, Yilmaz M, Ozcan H. Biomarkers of systemic inflammation in stable and exacerbation phases of COPD. Lung. 2008;186(6):403-9.

25. Fabbri LM, Rabe KF. From COPD to chronic systemic inflammatory syndrome? Lancet. 2007;370(9589):797-9.

26. Celli B, Locantore N, Yates J. Inflammatory biomarkers improve clinical prediction of mortality in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2012;185:1065 - 72.

27. Agusti A, Edwards LD, Rennard SI, MacNee W, Tal-Singer R, Miller BE, et al. Persistent systemic inflammation is associated with poor clinical outcomes in COPD: a novel phenotype. PLoS One. 2012;7(5):e37483.

28. Walter RE, Wilk JB, Larson MG, Vasan RS, Keaney JF, Jr., Lipinska I, et al. Systemic inflammation and COPD: the Framingham Heart Study. Chest. 2008;133(1):19-25.

29. Broekhuizen R, Wouters EF, Creutzberg EC, Schols AM. Raised CRP levels mark metabolic and functional impairment in advanced COPD. Thorax. 2006;61(1):17-22.

30. Dahl M, Vestbo J, Lange P. C-reactive protein as a predictor of prognosis in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2007;175:250 - 5.

31. Thomsen M, Dahl M, Lange P, Vestbo J, Nordestgaard BG. Inflammatory biomarkers and comorbidities in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2012;186(10):982-8.

32. Man S, Connett J, Anthonisen N. C-reactive protein and mortality in mild to moderate chronic obstructive pulmonary disease. Thorax. 2006;61:849 - 53.

33. de Torres JP, Pinto-Plata V, Casanova C, Mullerova H, Cordoba-Lanus E, Muros de Fuentes M, et al. C-reactive protein levels and survival in patients with moderate to very severe COPD. Chest. 2008;133(6):1336-43.

34. Maclay JD, McAllister DA, Macnee W. Cardiovascular risk in chronic obstructive pulmonary disease. Respirology. 2007;12(5):634-41.

35. Maclay JD, MacNee W. Cardiovascular disease in COPD: mechanisms. Chest. 2013;143(3):798-807.

36. Curkendall SM, DeLuise C, Jones JK, Lanes S, Stang MR, Goehring E, Jr., et al. Cardiovascular disease in patients with chronic obstructive pulmonary disease, Saskatchewan Canada cardiovascular disease in COPD patients. Annals of epidemiology. 2006;16(1):63-70.

37. Curkendall SM, Lanes S, de Luise C, Stang MR, Jones JK, She D, et al. Chronic obstructive pulmonary disease severity and cardiovascular outcomes. European journal of epidemiology. 2006;21(11):803-13.

38. Finkelstein J, Cha E, Scharf SM. Chronic obstructive pulmonary disease as an independent risk factor for cardiovascular morbidity. International journal of chronic obstructive pulmonary disease. 2009;4:337-49.

39. Mannino DM, Doherty DE, Sonia Buist A. Global Initiative on Obstructive Lung Disease (GOLD) classification of lung disease and mortality: findings from the Atherosclerosis Risk in Communities (ARIC) study. Respir Med. 2006;100(1):115-22.

40. Calverley PM, Anderson JA, Celli B, Ferguson GT, Jenkins C, Jones PW, et al. Salmeterol and fluticasone propionate and survival in chronic obstructive pulmonary disease. N Engl J Med. 2007;356(8):775-89.

41. Zvezdin B, Milutinov S, Kojicic M, Hadnadjev M, Hromis S, Markovic M, et al. A postmortem analysis of major causes of early death in patients hospitalized with COPD exacerbation. Chest. 2009;136(2):376-80.

42. Mapel DW, Dedrick D, Davis K. Trends and cardiovascular co-morbidities of COPD patients in the Veterans Administration Medical System, 1991-1999. COPD. 2005;2(1):35-41.

43. Sin DD, Man SF. Chronic obstructive pulmonary disease as a risk factor for cardiovascular morbidity and mortality. Proc Am Thorac Soc. 2005;2(1):8-11.

44. Anthonisen NR, Connett JE, Enright PL, Manfreda J, Lung Health Study Research G. Hospitalizations and mortality in the Lung Health Study. Am J Respir Crit Care Med. 2002;166(3):333-9.

45. Güder G, Rutten F. Comorbidity of Heart Failure and Chronic Obstructive Pulmonary Disease: More than Coincidence. Curr Heart Fail Rep. 2014;11(3):337-46.

46. Rutten FH, Cramer MJ, Lammers JW, Grobbee DE, Hoes AW. Heart failure and chronic obstructive pulmonary disease: An ignored combination? Eur J Heart Fail. 2006;8(7):706-11.

47. Haddad F, Doyle R, Murphy DJ, Hunt SA. Right ventricular function in cardiovascular disease, part II: pathophysiology, clinical importance, and management of right ventricular failure. Circulation. 2008;117(13):1717-31.

48. Donaldson GC, Hurst JR, Smith CJ, Hubbard RB, Wedzicha JA. Increased risk of myocardial infarction and stroke following exacerbation of COPD. Chest. 2010;137(5):1091-7.

49. Sinden NJ, Stockley RA. Systemic inflammation and comorbidity in COPD: a result of 'overspill' of inflammatory mediators from the lungs? Review of the evidence. Thorax. 2010;65(10):930-6.

50. Sabit R, Shale DJ. Vascular structure and function in chronic obstructive pulmonary disease: a chicken and egg issue? Am J Respir Crit Care Med. 2007;176(12):1175-6.

51. Sabit R, Bolton CE, Edwards PH, Pettit RJ, Evans WD, McEniery CM, et al. Arterial stiffness and osteoporosis in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2007;175(12):1259-65.

52. Jelani A, Jugdutt BI. STEMI and heart failure in the elderly: role of adverse remodeling. Heart failure reviews. 2010;15(5):513-21.

53. Libby P, Theroux P. Pathophysiology of coronary artery disease. Circulation. 2005;111(25):3481-8.

54. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med. 2005;352(16):1685-95.

55. Fletcher GF, Bufalino V, Costa F, Goldstein LB, Jones D, Smaha L, et al. Efficacy of Drug Therapy in the Secondary Prevention of Cardiovascular Disease and Stroke. The American Journal of Cardiology. 2007;99(6, Supplement 3):S1-S35.

56. Perk J, De Backer G, Gohlke H, Graham I, Reiner Ž, Verschuren M, et al. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). European Heart Journal. 2012;33(13):1635-701.

57. Kannel WB, Dawber TR, Kagan A, Revotskie N, Stokes IJ. Factors of Risk in the Development of Coronary Heart Disease—Six-Year Follow-up ExperienceThe Framingham Study. Annals of internal medicine. 1961;55(1):33-50.

58. Expert Panel on D, Evaluation, and Treatment of High Blood Cholesterol in A. EXecutive summary of the third report of the national cholesterol education program (ncep) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel iii). JAMA. 2001;285(19):2486-97.

59. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, et al. 2013 ACC/AHA Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation. 2014;129(25 suppl 2):S1-S45.

60. Reiner Ž, Catapano AL, De Backer G, Graham I, Taskinen M-R, Wiklund O, et al. ESC/EAS Guidelines for the management of dyslipidaemias2011 2011-07-01 00:00:00. 1769-818 p.

61. Cooke JP, Tsao PS. Is NO an endogenous antiatherogenic molecule? Arteriosclerosis and thrombosis : a journal of vascular biology / American Heart Association. 1994;14(5):653-5.

62. Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med. 1999;340(2):115-26.

63. McVeigh GE, Cohn JN. Endothelial dysfunction and the metabolic syndrome. Current diabetes reports. 2003;3(1):87-92.

64. Bonetti PO, Pumper GM, Higano ST, Holmes DR, Jr., Kuvin JT, Lerman A. Noninvasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. J Am Coll Cardiol. 2004;44(11):2137-41.

65. Lekakis J, Abraham P, Balbarini A, Blann A, Boulanger CM, Cockcroft J, et al. Methods for evaluating endothelial function: a position statement from the European Society of Cardiology Working Group on Peripheral Circulation. European journal of cardiovascular prevention and rehabilitation : official journal of the European Society of Cardiology, Working Groups on Epidemiology & Prevention and Cardiac Rehabilitation and Exercise Physiology. 2011;18(6):775-89.

66. Tousoulis D, Antoniades C, Stefanadis C. Evaluating endothelial function in humans: a guide to invasive and non-invasive techniques. Heart. 2005;91(4):553-8.

67. Pepine CJ. The effects of angiotensin-converting enzyme inhibition on endothelial dysfunction: potential role in myocardial ischemia. The American Journal of Cardiology. 1998;82(9, Supplement 2):23S-7S.

68. Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W, et al. A Definition of Advanced Types of Atherosclerotic Lesions and a Histological Classification of Atherosclerosis: A Report From the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation. 1995;92(5):1355-74.

69. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet. 1992;340(8828):1111-5.

70. Joannides R, Haefeli WE, Linder L, Richard V, Bakkali EH, Thuillez C, et al. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. Circulation. 1995;91(5):1314-9.

71. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol. 2002;39(2):257-65.

72. Hamburg NM, Benjamin EJ. Assessment of endothelial function using digital pulse amplitude tonometry. Trends in cardiovascular medicine. 2009;19(1):6-11.

73. Burton AC, Patel DJ. Reactive hyperemia in the human finger. Circ Res. 1956;4(6):710-2.

74. Noon JP, Haynes WG, Webb DJ, Shore AC. Local inhibition of nitric oxide generation in man reduces blood flow in finger pulp but not in hand dorsum skin. The Journal of physiology. 1996;490 (Pt 2):501-8.

75. Nohria A, Gerhard-Herman M, Creager MA, Hurley S, Mitra D, Ganz P. Role of nitric oxide in the regulation of digital pulse volume amplitude in humans. Journal of applied physiology. 2006;101(2):545-8.

76. Barr RG, Mesia-Vela S, Austin JH, Basner RC, Keller BM, Reeves AP, et al. Impaired flow-mediated dilation is associated with low pulmonary function and emphysema in ex-smokers: the Emphysema and Cancer Action Project (EMCAP) Study. Am J Respir Crit Care Med. 2007;176(12):1200-7.

77. Eickhoff P, Valipour A, Kiss D, Schreder M, Cekici L, Geyer K, et al. Determinants of systemic vascular function in patients with stable chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2008;178(12):1211-8.

78. Marchetti N, Ciccolella DE, Jacobs MR, Crookshank A, Gaughan JP, Kashem MA, et al. Hospitalized acute exacerbation of COPD impairs flow and nitroglycerin-mediated peripheral vascular dilation. COPD. 2011;8(2):60-5.

79. Kuvin JT, Patel AR, Sliney KA, Pandian NG, Sheffy J, Schnall RP, et al. Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. AmHeart J. 2003;146(1):168-74.

80. Minet C, Vivodtzev I, Tamisier R, Arbib F, Wuyam B, Timsit JF, et al. Reduced six-minute walking distance, high fat-free-mass index and hypercapnia are associated with endothelial dysfunction in COPD. Respir Physiol Neurobiol. 2012;183(2):128-34.

81. Biomarkers Definitions Working G. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. Clinical Pharmacology & Therapeutics. 2001;69(3):89-95.

82. Hansson GK. Inflammation, Atherosclerosis, and Coronary Artery Disease. The New England Journal of Medicine. 2005;352(16):1685-95.

83. Ridker PM, Silvertown JD. Inflammation, C-reactive protein, and atherothrombosis. J Periodontol. 2008;79(8 Suppl):1544-51.

84. F. Wensley PG, S. Burgess et al. Association between C reactive protein and coronary heart disease: mendelian randomisation analysis based on individual participant data2011 2011-02-15 23:34:41.

85. Bisoendial RJ, Boekholdt SM, Vergeer M, Stroes ESG, Kastelein JJP. C-reactive protein is a mediator of cardiovascular disease2010 2010-09-01 00:00:00. 2087-91 p.

86. Zacho J, Tybjærg-Hansen A, Jensen JS, Grande P, Sillesen H, Nordestgaard BG. Genetically Elevated C-Reactive Protein and Ischemic Vascular Disease. New England Journal of Medicine. 2008;359(18):1897-908.

87. Flammer AJ, Anderson T, Celermajer DS, Creager MA, Deanfield J, Ganz P, et al. The Assessment of Endothelial Function: From Research Into Clinical Practice. Circulation. 2012;126(6):753-67.

88. Rubinshtein R, Kuvin JT, Soffler M, Lennon RJ, Lavi S, Nelson RE, et al. Assessment of endothelial function by non-invasive peripheral arterial tonometry predicts late cardiovascular adverse events. European Heart Journal. 2010;31(9):1142-8.

89. Akiyama E, Sugiyama S, Matsuzawa Y, Konishi M, Suzuki H, Nozaki T, et al. Incremental prognostic significance of peripheral endothelial dysfunction in patients with heart failure with normal left ventricular ejection fraction. J Am Coll Cardiol. 2012;60(18):1778-86.

90. Matsuzawa Y, Sugiyama S, Sumida H, Sugamura K, Nozaki T, Ohba K, et al. Peripheral Endothelial Function and Cardiovascular Events in High-Risk Patients. Journal of the American Heart Association. 2013;2(6).

91. Thygesen K, Alpert JS, White HD. Universal Definition of Myocardial Infarction. Journal of the American College of Cardiology. 2007;50(22):2173-95.

92. Morrow DA, Antman EM. Evaluation of high-sensitivity assays for cardiac troponin. ClinChem. 2009;55(1):5-8.

93. Antman E, Bassand J-P, Klein W, Ohman M, Lopez Sendon JL, Rydén L, et al. Myocardial infarction redefined—a consensus document of The Joint European Society of Cardiology/American College of Cardiology committee for the redefinition of myocardial infarction: The Joint European Society of Cardiology/ American College of Cardiology Committee∗. Journal of the American College of Cardiology. 2000;36(3):959-69.

94. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD. Third universal definition of myocardial infarction. J Am CollCardiol. 2012;60(16):1581-98.

95. Baum H, Braun S, Gerhardt W, Gilson G, Hafner G, Müller-Bardorff M, et al. Multicenter evaluation of a second-generation assay for cardiac troponin T. Clinical Chemistry. 1997;43(10):1877-84.

96. Apple FS, Collinson PO, Biomarkers ITFoCAoC. Analytical characteristics of high-sensitivity cardiac troponin assays. Clin Chem. 2012;58(1):54-61.

97. Thygesen K, Mair J, Giannitsis E, Mueller C, Lindahl B, Blankenberg S, et al. How to use high-sensitivity cardiac troponins in acute cardiac care. Eur Heart J. 2012;33(18):2252-7.

98. Jaffe AS, Ravkilde J, Roberts R, Naslund U, Apple FS, Galvani M, et al. It's time for a change to a troponin standard. Circulation. 2000;102(11):1216-20.

99. Antman EM. Decision making with cardiac troponin tests. N Engl J Med. 2002;346(26):2079-82.

100. Omland T. New features of troponin testing in different clinical settings. Journal of internal medicine. 2010;268(3):207-17.

101. Wallace TW, Abdullah SM, Drazner MH, Das SR, Khera A, McGuire DK, et al. Prevalence and determinants of troponin T elevation in the general population. Circulation. 2006;113(16):1958-65.

102. de Lemos JA, Drazner MH, Omland T, Ayers CR, Khera A, Rohatgi A, et al. Association of troponin T detected with a highly sensitive assay and cardiac structure and mortality risk in the general population. JAMA. 2010;304(22):2503-12.

103. Saunders JT, Nambi V, de Lemos JA, Chambless LE, Virani SS, Boerwinkle E, et al. Cardiac troponin T measured by a highly sensitive assay predicts coronary heart disease, heart failure, and mortality in the Atherosclerosis Risk in Communities Study. Circulation. 2011;123(13):1367-76.

104. Omland T, de Lemos JA, Sabatine MS, Christophi CA, Rice MM, Jablonski KA, et al. A sensitive cardiac troponin T assay in stable coronary artery disease. N Engl J Med. 2009;361(26):2538-47.

105. Mahajan VS, Jarolim P. How to interpret elevated cardiac troponin levels. Circulation. 2011;124(21):2350-4.

106. Jaffe AS, Babuin L, Apple FS. Biomarkers in acute cardiac disease: the present and the future. J Am Coll Cardiol. 2006;48(1):1-11.

107. Korosoglou G, Lehrke S, Mueller D, Hosch W, Kauczor HU, Humpert PM, et al. Determinants of troponin release in patients with stable coronary artery disease:

insights from CT angiography characteristics of atherosclerotic plaque. Heart. 2011;97(10):823-31.

108. Omland T, de Lemos JA, Sabatine MS, Christophi CA, Rice MM, Jablonski KA, et al. A sensitive cardiac troponin T assay in stable coronary artery disease. NEnglJMed. 2009;361(26):2538-47.

109. Latini R, Masson S, Anand IS, Missov E, Carlson M, Vago T, et al. Prognostic value of very low plasma concentrations of troponin T in patients with stable chronic heart failure. Circulation. 2007;116(11):1242-9.

110. deFilippi CR, de Lemos JA, Christenson RH, Gottdiener JS, Kop WJ, Zhan M, et al. Association of serial measures of cardiac troponin T using a sensitive assay with incident heart failure and cardiovascular mortality in older adults. JAMA. 2010;304(22):2494-502.

111. Lankeit M, Friesen D, Aschoff J, Dellas C, Hasenfuss G, Katus H, et al. Highly sensitive troponin T assay in normotensive patients with acute pulmonary embolism. Eur Heart J. 2010;31(15):1836-44.

112. Filusch A, Giannitsis E, Katus HA, Meyer FJ. High-sensitive troponin T: a novel biomarker for prognosis and disease severity in patients with pulmonary arterial hypertension. Clin Sci (Lond). 2010;119(5):207-13.

113. Jaffe AS, Vasile VC, Milone M, Saenger AK, Olson KN, Apple FS. Diseased Skeletal Muscle: A Noncardiac Source of Increased Circulating Concentrations of Cardiac Troponin T. Journal of the American College of Cardiology. 2011;58(17):1819-24.

114. Shave R, Baggish A, George K, Wood M, Scharhag J, Whyte G, et al. Exercise-induced cardiac troponin elevation: evidence, mechanisms, and implications. J Am Coll Cardiol. 2010;56(3):169-76.

115. Shave R, Oxborough D. Exercise-induced cardiac injury: evidence from novel imaging techniques and highly sensitive cardiac troponin assays. Progress in cardiovascular diseases. 2012;54(5):407-15.

116. White HD. Pathobiology of troponin elevations: do elevations occur with myocardial ischemia as well as necrosis? JAmCollCardiol. 2011;57(24):2406-8.

117. Kurz K, Giannitsis E, Zehelein J, Katus HA. Highly sensitive cardiac troponin T values remain constant after brief exercise- or pharmacologic-induced reversible myocardial ischemia. Clin Chem. 2008;54(7):1234-8.

118. Roysland R, Kravdal G, Hoiseth AD, Nygard S, Badr P, Hagve TA, et al. Cardiac troponin T levels and exercise stress testing in patients with suspected coronary artery

disease: the Akershus Cardiac Examination (ACE) 1 study. Clinical Science. 2012;122(11-12):599-606.

119. Sabatine MS, Morrow DA, de Lemos JA, Jarolim P, Braunwald E. Detection of acute changes in circulating troponin in the setting of transient stress test-induced myocardial ischaemia using an ultrasensitive assay: results from TIMI 35. European Heart Journal. 2009;30(2):162-9.

120. Turer AT, Addo TA, Martin JL, Sabatine MS, Lewis GD, Gerszten RE, et al. Myocardial Ischemia Induced by Rapid Atrial Pacing Causes Troponin T Release Detectable by a High-Sensitivity Assay: Insights from a Coronary Sinus Sampling Study. Journal of the American College of Cardiology. 2011;57(24):2398-405.

121. Harvey MG, Hancox RJ. Elevation of cardiac troponins in exacerbation of chronic obstructive pulmonary disease. Emergency medicine Australasia : EMA. 2004;16(3):212-5.

122. Brekke PH, Omland T, Holmedal SH, Smith P, Soyseth V. Troponin T elevation and long-term mortality after chronic obstructive pulmonary disease exacerbation. EurRespirJ. 2008;31(3):563-70.

123. Fruchter O, Yigla M. Cardiac troponin-I predicts long-term mortality in chronic obstructive pulmonary disease. COPD. 2009;6(3):155-61.

124. Martins CS, Rodrigues MJ, Miranda VP, Nunes JP. Prognostic value of cardiac troponin I in patients with COPD acute exacerbation. The Netherlands journal of medicine. 2009;67(10):341-9.

125. Pavasini R, d'Ascenzo F, Campo G, Biscaglia S, Ferri A, Contoli M, et al. Cardiac troponin elevation predicts all-cause mortality in patients with acute exacerbation of chronic obstructive pulmonary disease: Systematic review and meta-analysis. Int J Cardiol. 2015;191(0):187-93.

126. Baillard C, Boussarsar M, Fosse JP, Girou E, Le TP, Cracco C, et al. Cardiac troponin I in patients with severe exacerbation of chronic obstructive pulmonary disease. Intensive Care Med. 2003;29(4):584-9.

127. Pedersen TK, J;Berg, K;Haghfelt, T;et al. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet. 1994;344(8934):1383-9.

128. Pignone M, Phillips C, Mulrow C. Use of lipid lowering drugs for primary prevention of coronary heart disease: meta-analysis of randomised trials2000 2000-10-21 07:00:00. 983 p.

129. Sacks FM, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, Cole TG, et al. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. N Engl J Med. 1996;335(14):1001-9.

130. Group MBHPSC. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20 536 high-risk individuals: a randomised placebocontrolled trial. The Lancet. 2002;360(9326):7-22.

131. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM, Jr., Kastelein JJ, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med. 2008;359(21):2195-207.

132. McKenney JM. Pharmacologic characteristics of statins. Clin Cardiol. 2003;26(4 Suppl 3):III32-8.

133. Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R, et al. Intensive versus Moderate Lipid Lowering with Statins after Acute Coronary Syndromes. New England Journal of Medicine. 2004;350(15):1495-504.

134. Mihos CG, Salas MJ, Santana O. The pleiotropic effects of the hydroxy-methyl-glutaryl-CoA reductase inhibitors in cardiovascular disease: a comprehensive review. Cardiol Rev. 2010;18(6):298-304.

135. Davignon J, Leiter LA. Ongoing clinical trials of the pleiotropic effects of statins. Vascular health and risk management. 2005;1(1):29-40.

136. Zhou Q, Liao JK. Pleiotropic effects of statins. - Basic research and clinical perspectives. Circulation journal : official journal of the Japanese Circulation Society. 2010;74(5):818-26.

137. Arnaud C, Burger F, Steffens S, Veillard NR, Nguyen TH, Trono D, et al. Statins reduce interleukin-6-induced C-reactive protein in human hepatocytes: new evidence for direct antiinflammatory effects of statins. Arteriosclerosis, thrombosis, and vascular biology. 2005;25(6):1231-6.

138. Arnaud C, Veillard NR, Mach F. Cholesterol-independent effects of statins in inflammation, immunomodulation and atherosclerosis. Current drug targets Cardiovascular & haematological disorders. 2005;5(2):127-34.

139. Takayama T, Hiro T, Yamagishi M, Daida H, Hirayama A, Saito S, et al. Effect of rosuvastatin on coronary atheroma in stable coronary artery disease: multicenter coronary atherosclerosis study measuring effects of rosuvastatin using intravascular ultrasound in Japanese subjects (COSMOS). Circulation journal : official journal of the Japanese Circulation Society. 2009;73(11):2110-7.

140. Soyseth V, Brekke PH, Smith P, Omland T. Statin use is associated with reduced mortality in COPD. Eur Respir J. 2007;29(2):279-83.

141. Mancini GB, Etminan M, Zhang B, Levesque LE, FitzGerald JM, Brophy JM. Reduction of morbidity and mortality by statins, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers in patients with chronic obstructive pulmonary disease. J Am Coll Cardiol. 2006;47(12):2554-60.

142. Dobler CC, Wong KK, Marks GB. Associations between statins and COPD: a systematic review. [Review] [39 refs]. BMC Pulmonary Medicine. 2009;9:32.

143. Janda S, Park K, FitzGerald JM, Etminan M, Swiston J. Statins in COPD: a systematic review. [Review] [34 refs]. Chest. 2009;136(3):734-43.

144. Chen YJ, Chen P, Wang HX, Wang T, Chen L, Wang X, et al. Simvastatin attenuates acrolein-induced mucin production in rats: involvement of the Ras/extracellular signal-regulated kinase pathway. International immunopharmacology. 2010;10(6):685-93.

145. Kim SE, Thanh Thuy TT, Lee JH, Ro JY, Bae YA, Kong Y, et al. Simvastatin inhibits induction of matrix metalloproteinase-9 in rat alveolar macrophages exposed to cigarette smoke extract. Experimental & molecular medicine. 2009;41(4):277-87.

146. Zeki AA, Franzi L, Last J, Kenyon NJ. Simvastatin inhibits airway hyperreactivity: implications for the mevalonate pathway and beyond. Am J Respir Crit Care Med. 2009;180(8):731-40.

147. Lee J-H, Lee D-S, Kim E-K, Choe K-H, Oh Y-M, Shim T-S, et al. Simvastatin Inhibits Cigarette Smoking–induced Emphysema and Pulmonary Hypertension in Rat Lungs. American Journal of Respiratory and Critical Care Medicine. 2005;172(8):987-93.

148. Lee TM, Lin MS, Chang NC. Usefulness of C-reactive protein and interleukin-6 as predictors of outcomes in patients with chronic obstructive pulmonary disease receiving pravastatin. American Journal of Cardiology. 2008;101(4):530-5.

149. Kaczmarek P, Sladek K, Skucha W, Rzeszutko M, Iwaniec T, Dziedzina S, et al. The influence of simvastatin on selected inflammatory markers in patients with chronic obstructive pulmonary disease. Polskie Archiwum Medycyny Wewnetrznej. 2010;120(1-2):11-7.

150. Bonetti PO, Barsness GW, Keelan PC, Schnell TI, Pumper GM, Kuvin JT, et al. Enhanced external counterpulsation improves endothelial function in patients with symptomatic coronary artery disease. J Am Coll Cardiol. 2003;41:1761-8.

151. Tomfohr LM, Martin TM, Miller GE. Symptoms of depression and impaired endothelial function in healthy adolescent women. JBehavMed. 2008;31(2):137-43.

152. McCrea CE, Skulas-Ray AC, Chow M, West SG. Test-retest reliability of pulse amplitude tonometry measures of vascular endothelial function: implications for clinical trial design. Vasc Med. 2012;17(1):29-36.

153. Faizi AK, Kornmo DW, Agewall S. Evaluation of endothelial function using finger plethysmography. Clinical physiology and functional imaging. 2009;29(5):372-5.

154. Hamburg NM, Keyes MJ, Larson MG, Vasan RS, Schnabel R, Pryde MM, et al. Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. Circulation. 2008;117(19):2467-74.

155. Moerland M, Kales AJ, Schrier L, van Dongen MG, Bradnock D, Burggraaf J. Evaluation of the EndoPAT as a Tool to Assess Endothelial Function. International journal of vascular medicine. 2012;2012:904141.

156. Hamburg NM, Keyes MJ, Larson MG, Vasan RS, Schnabel R, Pryde MM, et al. Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. Circulation. 2008;117(19):2467-74.

157. Yeboah J, Crouse JR, Hsu FC, Burke GL, Herrington DM. Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: the Cardiovascular Health Study. Circulation. 2007;115(18):2390-7.

158. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J. 2005;26(2):319-38.

159. ATS Statement: Guidelines for the Six-Minute Walk Test. Am J Respir Crit Care Med. 2002;166:111 - 7.

160. Bestall JC, Paul EA, Garrod R, Garnham R, Jones PW, Wedzicha JA. Usefulness of the Medical Research Council (MRC) dyspnoea scale as a measure of disability in patients with chronic obstructive pulmonary disease. Thorax. 1999;54(7):581-6.

161. Van Breukelen GJ. ANCOVA versus change from baseline: more power in randomized studies, more bias in nonrandomized studies [corrected]. Journal of clinical epidemiology. 2006;59(9):920-5.

162. Korley FK, Jaffe AS. High-sensitivity troponin: where are we now and where do we go from here? Biomark Med. 2014;8(8):1021-32.

163. Hof D, Klingenberg R, von Eckardstein A. Sensible use of high-sensitivity troponin assays. Methods in molecular biology. 2013;963:385-406.

164. Vafaie M, Biener M, Mueller M, Schnabel PA, André F, Steen H, et al. Analytically false or true positive elevations of high sensitivity cardiac troponin: a systematic approach. Heart. 2014;100(6):508-14.

165. Onkelinx S, Cornelissen V, Goetschalckx K, Thomaes T, Verhamme P, Vanhees L. Reproducibility of different methods to measure the endothelial function. Vasc Med. 2012;17(2):79-84.

166. Brant LC, Barreto SM, Passos VM, Ribeiro AL. Reproducibility of peripheral arterial tonometry for the assessment of endothelial function in adults. Journal of hypertension. 2013;31(10):1984-90.

167. Liu J, Wang J, Jin Y, Roethig HJ, Unverdorben M. Variability of peripheral arterial tonometry in the measurement of endothelial function in healthy men. Clin Cardiol. 2009;32(12):700-4.

168. Altman DG, Bland JM. Statistics notes. Treatment allocation in controlled trials: why randomise? BMJ. 1999;318(7192):1209.

169. Altman DG, Bland JM. How to randomise. BMJ. 1999;319(7211):703-4.

170. Altman DG, Matthews JNS. Statistics Notes: Interaction 1: heterogeneity of effects1996 1996-08-24 00:00:00. 486 p.

171. Chang CL, Robinson SC, Mills GD, Sullivan GD, Karalus NC, McLachlan JD, et al. Biochemical markers of cardiac dysfunction predict mortality in acute exacerbations of COPD. Thorax. 2011;66(9):764-8.

172. Marcun R, Sustic A, Brguljan PM, Kadivec S, Farkas J, Kosnik M, et al. Cardiac biomarkers predict outcome after hospitalisation for an acute exacerbation of chronic obstructive pulmonary disease. Int J Cardiol. 2012;161(3):156-9.

173. Kelly AM, Klim S. Is elevated troponin associated with in-hospital mortality in emergency department patients admitted with chronic obstructive pulmonary disease? European journal of emergency medicine : official journal of the European Society for Emergency Medicine. 2013;20(1):54-7.

174. McAllister DA, Maclay JD, Mills NL, Leitch A, Reid P, Carruthers R, et al. Diagnosis of myocardial infarction following hospitalisation for exacerbation of COPD. European Respiratory Journal. 2012;39(5):1097-103.

175. Brekke PH, Omland T, Holmedal SH, Smith P, Soyseth V. Determinants of cardiac troponin T elevation in COPD exacerbation - a cross-sectional study. BMC Pulm Med. 2009;9(1):35.

176. Hijazi Z, Wallentin L, Siegbahn A, Andersson U, Alexander JH, Atar D, et al. High-sensitivity troponin T and risk stratification in patients with atrial fibrillation during treatment with apixaban or warfarin. J Am Coll Cardiol. 2014;63(1):52-61.

177. Konecny T, Park JY, Somers KR, Konecny D, Orban M, Soucek F, et al. Relation of Chronic Obstructive Pulmonary Disease to Atrial and Ventricular Arrhythmias. The American Journal of Cardiology. 2014;114(2):272-7.

178. Brekke PH, Omland T, Smith P, Soyseth V. Underdiagnosis of myocardial infarction in C. RespirMed. 2008;102(9):1243-7.

179. Campo G, Pavasini R, Malagu M, Punzetti S, Napoli N, Guerzoni F, et al. Relationship between Troponin Elevation, Cardiovascular History and Adverse Events in Patients with acute exacerbation of COPD. COPD. 2015;0(0):null.

180. Gaggin HK, Januzzi Jr JL. Biomarkers and diagnostics in heart failure. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease. 2013;1832(12):2442-50.

181. Hoiseth AD, Omland T, Hagve TA, Brekke PH, Soyseth V. NT-proBNP independently predicts long term mortality after acute exacerbation of COPD - a prospective cohort study. Respir Res. 2012;13:97.

182. Hoiseth AD, Neukamm A, Hagve TA, Omland T, Brekke PH, Soyseth V. The clinical value of serial measurement of high-sensitivity cardiac troponin T in acute exacerbations ofchronic obstructive pulmonary disease. Open heart. 2014;1(1):e000001.

183. Barnes P, Celli B. Systemic manifestations and comorbidities of COPD. Eur Respir J. 2009;33:1165 - 85.

184. Man SFP, Van Eeden S, Sin DD. Vascular Risk in Chronic Obstructive Pulmonary Disease: Role of Inflammation and Other Mediators. Canadian Journal of Cardiology. 2012;28(6):653-61.

185. Harrison MT, Short P, Williamson PA, Singanayagam A, Chalmers JD, Schembri S. Thrombocytosis is associated with increased short and long term mortality after exacerbation of chronic obstructive pulmonary disease: a role for antiplatelet therapy? Thorax. 2014;69(7):609-15.

186. Patel AR, Kowlessar BS, Donaldson GC, Mackay AJ, Singh R, George SN, et al. Cardiovascular risk, myocardial injury, and exacerbations of chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2013;188(9):1091-9.

187. Kim SJ, Yoon DW, Lee EJ, Hur GY, Jung KH, Lee SY, et al. Carotid atherosclerosis in patients with untreated chronic obstructive pulmonary disease. The international

journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease. 2011;15(9):1265-70, i.

188. Seifarth H, Schlett CL, Lehman SJ, Bamberg F, Donnelly P, Januzzi JL, et al. Correlation of concentrations of high-sensitivity troponin T and high-sensitivity C-reactive protein with plaque progression as measured by CT coronary angiography. Journal of Cardiovascular Computed Tomography. 2014;8(6):452-8.

189. Brown AO, Mann B, Gao G, Hankins JS, Humann J, Giardina J, et al. Streptococcus pneumoniae translocates into the myocardium and forms unique microlesions that disrupt cardiac function. PLoS pathogens. 2014;10(9):e1004383.

190. Zwaans WAR, Mallia P, van Winden MEC, Rohde GGU. The relevance of respiratory viral infections in the exacerbations of chronic obstructive pulmonary disease—A systematic review. Journal of Clinical Virology. 2014;61(2):181-8.

191. Malhotra R, Olsson H. Immunology, genetics and microbiota in the COPD pathophysiology: potential scope for patient stratification. Expert Rev Respir Med. 2015;0(0):1-7.

192. Søyseth V, Bhatnagar R, Holmedahl NH, Neukamm A, Høiseth AD, Hagve T-A, et al. Acute exacerbation of COPD is associated with fourfold elevation of cardiac troponin T. Heart. 2013;99(2):122-6.

193. Hattori K, Ishii T, Motegi T, Kusunoki Y, Gemma A, Kida K. Relationship between serum cardiac troponin T level and cardiopulmonary function in stable chronic obstructive pulmonary disease. International journal of chronic obstructive pulmonary disease. 2015;10:309-20.

194. Saravia SG, Knebel F, Schroeckh S, Ziebig R, Lun A, Weimann A, et al. Cardiac troponin T release and inflammation demonstrated in marathon runners. Clinical laboratory. 2010;56(1-2):51-8.

195. Peinado VI, Pizarro S, Barbera JA. Pulmonary vascular involvement in COPD. Chest. 2008;134(4):808-14.

196. Galie N, Hoeper MM, Humbert M, Torbicki A, Vachiery JL, Barbera JA, et al. Guidelines for the diagnosis and treatment of pulmonary hypertension: the Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS), endorsed by the International Society of Heart and Lung Transplantation (ISHLT). Eur Heart J. 2009;30(20):2493-537.

197. Hilde JM, Skjørten I, Grøtta OJ, Hansteen V, Melsom MN, Hisdal J, et al. Right Ventricular Dysfunction and Remodeling in Chronic Obstructive Pulmonary Disease

Without Pulmonary Hypertension. Journal of the American College of Cardiology. 2013;62(12):1103-11.

198. Rutten FH, Cramer MJ, Grobbee DE, Sachs AP, Kirkels JH, Lammers JW, et al. Unrecognized heart failure in elderly patients with stable chronic obstructive pulmonary disease. Eur Heart J. 2005;26(18):1887-94.

199. Macchia A, Rodriguez Moncalvo JJ, Kleinert M, Comignani PD, Gimeno G, Arakaki D, et al. Unrecognised ventricular dysfunction in COPD. Eur Respir J. 2012;39(1):51-8.

200. Boudestein LCM, Rutten FH, Cramer MJ, Lammers JWJ, Hoes AW. The impact of concurrent heart failure on prognosis in patients with chronic obstructive pulmonary disease. European Journal of Heart Failure. 2009;11(12):1182-8.

201. Stolz D, Breidthardt T, Christ-Crain M, Bingisser R, Miedinger D, Leuppi J, et al. Use of B-type natriuretic peptide in the risk stratification of acute exacerbations of COPD. Chest. 2008;133(5):1088-94.

202. Patel ARC, Donaldson GC, Mackay AJ, Wedzicha JA, Hurst JR. THe impact of ischemic heart disease on symptoms, health status, and exacerbations in patients with copd. Chest. 2012;141(4):851-7.

203. Murphy ML, Thenabadu PN, de Soyza N, Meade J, Doherty JE, Baker BJ. Sensitivity of electrocardiographic criteria for left ventricular hypertrophy according to type of cardiac disease. The American Journal of Cardiology. 1985;55(5):545-9.

204. Fruchter O, Yigla M, Kramer MR. Lipid profile and statin use: the paradox of survival after acute exacerbation of chronic obstructive pulmonary disease. The American journal of the medical sciences. 2015;349(4):338-43.

205. John ME, Cockcroft JR, McKeever TM, Coward WR, Shale DJ, Johnson SR, et al. Cardiovascular and inflammatory effects of simvastatin therapy in patients with COPD: a randomized controlled trial. International journal of chronic obstructive pulmonary disease. 2015;10:211-21.

206. Vestbo J. COPD: Definition and Phenotypes. Clinics in Chest Medicine. 2014;35(1):1-6.

207. Konttinen J, Lindholm H, Sinisalo J, Kuosma E, Halonen J, Hopsu L, et al. Association between lowered endothelial function measured by peripheral arterial tonometry and cardio-metabolic risk factors - a cross-sectional study of Finnish municipal workers at risk of diabetes and cardiovascular disease. BMC Cardiovascular Disorders. 2013;13(1):83.

208. Matsuzawa Y, Guddeti RR, Kwon TG, Lerman LO, Lerman A. Secondary Prevention Strategy of Cardiovascular Disease Using Endothelial Function Testing. Circulation journal : official journal of the Japanese Circulation Society. 2015.

209. Modena MG, Bonetti L, Coppi F, Bursi F, Rossi R. Prognostic role of reversible endothelial dysfunction in hypertensive postmenopausal women. J Am Coll Cardiol. 2002;40(3):505-10.

210. Kitta Y, Obata JE, Nakamura T, Hirano M, Kodama Y, Fujioka D, et al. Persistent impairment of endothelial vasomotor function has a negative impact on outcome in patients with coronary artery disease. J Am Coll Cardiol. 2009;53(4):323-30.

211. Frick M, Weidinger F. Endothelial Function: A Surrogate Endpoint in Cardiovascular Studies? Current pharmaceutical design. 2007;13(17):1741-50.

212. Bruno RM, Gori T, Ghiadoni L. Endothelial function testing and cardiovascular disease: focus on peripheral arterial tonometry. Vascular health and risk management. 2014;10:577-84.

213. Mroz RM, Lisowski P, Tycinska A, Bierla J, Trzeciak PZ, Minarowski L, et al. Anti-inflammatory effects of atorvastatin treatment in chronic obstructive pulmonary disease. A controlled pilot study. Journal of physiology and pharmacology. 2015;66(1):111-28.

214. Criner GJ, Connett JE, Aaron SD, Albert RK, Bailey WC, Casaburi R, et al. Simvastatin for the prevention of exacerbations in moderate-to-severe COPD. N Engl J Med. 2014;370(23):2201-10.

215. Ingebrigtsen TS, Marott JL, Nordestgaard BG, Lange P, Hallas J, Vestbo J. Statin use and exacerbations in individuals with chronic obstructive pulmonary disease. Thorax. 2015;70(1):33-40.

216. Moosavi SAJ, Raji H, Faghankhani M, Yazdani R, Esmaeili M. Evaluation of the Effects of Atorvastatin on the Treatment of Secondary Pulmonary Hypertension due to Chronic Obstructive Pulmonary Diseases: A Randomized Controlled Trial. Iran Red Crescent Med J. 2013;15(8):649-54.