Chronic Obstructive Pulmonary Disease

Early detection and prevention in primary care

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Om du tänker för länge på nästa steg kommer du att tillbringa resten av livet på ett ben.

To my family, Therese, Philip, Alexander and David.
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Abstract

ABSTRACT

Background and aims. Early detection of Chronic Obstructive Pulmonary Disease (COPD) and secondary prevention by means of smoking cessation are the only available methods of stopping the progression of the disease. The overall aim was to examine the possibilities of early detection and prevention of COPD in General Practice. The specific aims were to evaluate a method of detecting COPD at its early stages, to investigate the rate of emphysema in smokers with normal lung function and smokers defined as preclinical COPD, to investigate the effects of performed spirometries and brief smoking cessation advice on smoking habits and to test if concentrations of certain biomarkers in blood, saliva and exhaled breath condensate (EBC) could identify subjects with COPD or non-COPD subjects supposed to be at risk of developing COPD.

Methods. The first study evaluated an invitational method, which offered voluntary screening spirometry to a targeted population of smokers 40-55 years old. In the second follow-up study, all smokers with COPD and half of the smokers with normal lung function (NLF) were annually invited for spirometry and brief smoking cessation advice for a duration of 3 years, with half of the smokers with NLF being tested only last year. In the third study, 54 smokers with NLF were examined with High Resolution Computed Tomography (HRCT), with blood samples also being collected from each subject. In study four, 19 subjects categorised as having COPD, 30 non-COPD subjects and 15 healthy non-smoking volunteers were studied by means of spirometry, DLCO, and analysis of biomarkers in EBC, saliva and serum.

Results. A total of 512 smokers responded. The prevalence of COPD was 27.5% and was classified as mild in 85% of the sufferers, moderate in 13% and severe in 2%. At year 1, 10% of the smokers with COPD had been continuously abstinent from smoking, compared to 2% of smokers with NLF. The prolonged abstinence rate increased yearly, and at year 3 the smoking cessation rates in smokers with COPD was 25% compared to 7% in smokers with NLF. By visual analysis, HRCT showed signs of emphysema in 43% of the subjects. Emphysema was also associated with low BMI. Higher serum concentrations of lysozyme and lower DLCO were recorded in those with COPD compared to non-COPD subjects. With the exception of chlorine, none of the remaining biomarkers were detected in EBC.

Conclusions. By invitational targeted screening, COPD can be easily detected in its mild stages by using spirometry. By becoming diagnosed with COPD, smokers seem to be more motivated to stop smoking, and COPD patients should repeatedly be offered spirometry and smoking cessation advice which may prevent the progression of the disease to a severe disabling form. HRCT may detect smoke related parenchymal lung damage (i.e. emphysema) in symptom-free smokers with normal spirometry. Serum lysozyme and DLCO appeared to be the strongest discriminator between COPD and non-COPD subjects. The use of EBC as a tool to measure exhaled inflammatory biomarkers involved in COPD is as yet uncertain.
LIST OF PAPERS

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


### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AUC&lt;sub&gt;ROC&lt;/sub&gt;</td>
<td>Area under the curve (receiver operating characteristic)</td>
</tr>
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<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CLE</td>
<td>Centrilobular emphysema</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>DLCO</td>
<td>Diffusing capacity of the lung for carbon monoxide</td>
</tr>
<tr>
<td>EBC</td>
<td>Exhaled breath condensate</td>
</tr>
<tr>
<td>ECP</td>
<td>Eosinophil Cationic Protein</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>ERS</td>
<td>European Respiratory Society</td>
</tr>
<tr>
<td>FEF&lt;sub&gt;50&lt;/sub&gt; (MEF&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>Forced expiratory flow at 50% of FVC</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Forced expiratory volume in one second</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;%&lt;/sub&gt;</td>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;/FVC ratio in per cent</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced vital capacity</td>
</tr>
<tr>
<td>GOLD</td>
<td>Global Initiative for Chronic Obstructive Lung Disease</td>
</tr>
<tr>
<td>HRCT</td>
<td>High Resolution Computed Tomography</td>
</tr>
<tr>
<td>Hs-CRP</td>
<td>High-sensitive C-reactive protein</td>
</tr>
<tr>
<td>HU</td>
<td>Hounsfield unit</td>
</tr>
<tr>
<td>ICS</td>
<td>Inhaled corticosteroids</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>MPO</td>
<td>Myeloperoxidase</td>
</tr>
<tr>
<td>PFT</td>
<td>Pulmonary Function Test</td>
</tr>
<tr>
<td>PSE</td>
<td>Paraseptal emphysema</td>
</tr>
<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver operating characteristic</td>
</tr>
<tr>
<td>VC (SVC)</td>
<td>Vital capacity (Slow vital capacity)</td>
</tr>
<tr>
<td>VC&lt;sub&gt;max&lt;/sub&gt;</td>
<td>The best of SVC or FVC</td>
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DEFINITIONS

Pack-years (Paper I and III)

A measurement of smoking exposure equivalent to smoking one packet of cigarettes a day for one year. One pack-year is defined as 20 manufactured cigarettes (1 pack) smoked per day for one year. Formula: Number of years of smoking x average number of cigarettes smoked per day/20.

Lung function assessment

Vital Capacity (VC)
Maximum volume of air exhaled slowly from full inspiration to maximum expiration. Not time dependent. The values are expressed as a percentage of the normal predicted value for a person.

Forced vital capacity (FVC)
Maximum volume of air exhaled from full inspiration to forced maximum expiration. The values are expressed as a percentage of the normal predicted value for a person.

Forced expiratory volume in one second (FEV1)
Volume of exhaled air in the first second of a forced expiration. Expressed as a percentage of the predicted normal value for a person.

Forced expiratory flow at 50% of FVC (FEF50 (MEF50))
Expressed as a percentage of the predicted normal value for a person.

Rapid decliner
In this thesis, a fall of 350 mL in FEV1 based on a five year period was defined as the cut off level for rapid decline.
**Spirometric definition of COPD**

The following guidelines were used as the spirometric criteria for COPD and classifications of its severity:

**A) European Respiratory Society 1995¹ (Paper I, II and III)**

**Definition**

FEV₁/VC or FEV₁/FVC ratio (FEV%) < 88% of predicted for males and FEV₁/VC or FEV₁/FVC ratio (FEV%) < 89% for females.

**Severity of impairment**

Mild if FEV₁% of predicted ≥ 70
Moderate if FEV₁% of predicted 50-69
Severe if FEV₁% of predicted <50%.

**B) Global Initiative for Chronic Obstructive Lung Disease (GOLD)² (Paper IV)**

**Definition**

FEV₁/FVC or FEV₁/VC < 0.70% (post-bronchodilator)

**Severity of impairment**

Stage I (Mild): FEV₁≥80% of predicted
Stage II (Moderate): 50% ≤ FEV₁<80% of predicted
Stage III (Severe): 30% ≤ FEV₁<50% of predicted
Stage IV (Very severe): FEV₁<30% of predicted

All subjects in the studies performed spirometry test with the use of a nose clip.
INTRODUCTION

Historical remarks

Chronic obstructive pulmonary disease (COPD) is a disease consisting of several components. Historically, the bronchitis component which we today label as chronic bronchitis (chronic cough and mucus secretion) was first described by Badham in 1814. Some years later Laënnec in 1821 also described the chronic bronchitis aspect, but he in addition also portrayed the emphysema component of the disease.

In more recent times, the awareness and research into the COPD field and chronic bronchitis started after the fog catastrophe in London in 1952. During a week in December 1952 the smog killed approximately 4000 people, with the mortality rate for people suffering from respiratory and cardiac diseases being especially high. In Great Britain, by initiation of the British Medical Research Council’s (MRC) committee, research into chronic bronchitis began to seriously take off after the 1952 catastrophe. Tobacco smoke was now recognized as a risk factor for developing chronic bronchitis and airflow obstruction. Post mortem studies into the morphology of the airways and lung parenchyma revealed an association between chronic bronchitis and emphysema.

During this period there was a lack of a set of clear terms and definitions used to describe respiratory symptoms and airflow obstruction, and none of the terms used at the time took into consideration the physiological and functional criterias. Diagnostic labels used during the 1950’s and 1960’s were ‘chronic bronchitis’, ‘chronic airflow obstruction’, ‘chronic obstructive lung disease’ or ‘non-specific chronic pulmonary disease’. It was at the CIBA guest symposium in 1959 and at the American Thoracic Society Committee meeting in 1962, that clear definitions regarding asthma, chronic bronchitis and emphysema were first made. The common term for what we today call COPD was ‘chronic bronchitis with emphysema’. The term Chronic Obstructive Pulmonary Disease (COPD) is a recent one, and became commonly accepted and used during the early 1990’s.
Theories concerning the etiology of COPD

There are two hypotheses concerning the etiology of COPD, the British and the Dutch hypothesis. According to the British hypothesis, presented at the CIBA guest symposium in 1959, the pathogenesis for chronic bronchitis was based on host and exogenous factors, such as repeated chest infections, air pollution and smoking. According to this hypothesis it was suggested that the exogenous factors caused hypersecretion of mucus which inhibited the host defence, causing repeated acute or chronic respiratory tract infections and eventually to a decline in lung function.

In contrast to the British theory, the Dutch hypothesis proposed that genetically determined host factors (such as genetic predisposition to atopy and bronchial hyperresponsiveness), combined with environmental factors (such as smoking) could predict the hosts response to the exogenous factors. According to this theory asthma, chronic bronchitis and emphysema are different expressions of a primary abnormality in the airways, and an interaction between genetic predispositions and exogenous factors determines which manifestation a subject develops. In the COPD field this postulated concept of genetically determined host factors provides an explanation as to why subjects exposed to identical exogenous factors (tobacco smoke and environmental pollution), developed different symptoms and manifestations i.e. chronic bronchitis on its own, or in addition to airflow obstruction. According to this hypothesis, asthma and COPD have a single genotype with two phenotypes.

The modern day view concerning the etiology of COPD began with Fletcher and his co-workers, and was later developed by others, and resembles the Dutch hypothesis. Fletcher revealed that in susceptible smokers (comparable with the host factors), tobacco smoking is strongly related to chronic bronchitis and airflow obstruction, and that these were two different diseases. One of the two diseases was chronic bronchitis without airflow obstruction and the other was airflow obstruction which in some individuals could co-exist with chronic bronchitis. For the first time Fletcher and his colleagues were able to show that tobacco smoking accelerated the decline of FEV₁, and that smoking cessation could halt this rapid decline. It was demonstrated that different populations of smokers, i.e. susceptible and non-susceptible smokers, showed different trends in their lung function decline. Cigarette smoking is recognised as the cause of COPD in the vast
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majority of patients. Although not fully understood, it is widely accepted that an abnormal inflammatory response of the lungs to noxious particles and gases beyond the normal protective inflammatory response is involved in the development of COPD.\textsuperscript{14,15}

Risk factors of COPD

Risk factors for COPD can be divided into environmental exposures and genetic host factors with the disease arising from an interaction between the two factors.

Environmental exposures

Environmental exposures such as cigarette smoke, dust, fumes and chemicals have shown to induce an inflammatory response in the lungs which leads to the pathological lesions found in smokers with COPD.\textsuperscript{15} Cigarette smoking is recognised as the cause of COPD in the vast majority of patients.\textsuperscript{1,4,15-18} The second most important risk factor related to COPD is chronic exposure to occupational dusts, fumes and chemicals.\textsuperscript{19-21} When the exposures are sufficiently intense or prolonged, occupational dusts and chemicals can cause COPD independent of cigarette smoking. The risk of developing COPD is greater with concurrent cigarette smoking compared to chronic exposure of harmful fumes, dust and chemicals on their own.\textsuperscript{20,15} The most important substances of chronic exposures are grain, isocyanates, cadmium, coal and mineral dusts. Professions which carry a significant risk of developing COPD are mining, quarry and construction work, and work in the textile, wood and paper industries.\textsuperscript{22}

In the Swedish study by Bergdahl et al. the fraction of COPD attributable to airborne exposure among 300 000 construction workers was estimated as 10.7%.\textsuperscript{23} The study by Trupin et al conducted in the United States estimated the proportion of COPD prevalence attributable to occupational exposures as 20%.\textsuperscript{24} In the consensus statement of 2003 from the American Thoracic Society (ATS), based on several large scale general population studies, it was calculated that the attributable fraction of occupational exposures to COPD was 15%.\textsuperscript{25}

Other environmental risk factors that can cause COPD are still unclear. Low birth weight, history of childhood respiratory infections, indoor and outdoor
pollutions are discussed as possible contributors, but appear to be less significant when compared to cigarette smoke, occupational dust and fume exposures.\textsuperscript{15} Socio-economic status in terms of social class, educational level, income and occupation has also been associated with the risk of developing COPD.\textsuperscript{26-28,27} It is however not clear whether this pattern reflects exposures to indoor and outdoor air pollutants, crowding, poor nutrition, or other factors that are related to socio-economic status.

**Genetic host factors**

Not all smokers develop clinically significant COPD. Although it is yet to be proven that there is an individual susceptibility to the exogenous environmental risk factors due to genetic predisposition, it has been suggested that genetically determined factors modifies each individual’s risk for COPD. The genetic risk factor that is best documented is a hereditary deficiency of alpha-1 antitrypsin, but studies have suggested that genetic factors other than alfa-1-antitrypsin deficiency may be involved in the susceptibility of cigarette smokers.\textsuperscript{29,30}

**Inflammation in COPD**

Chronic obstructive pulmonary disease is defined as a disease associated with an abnormal inflammatory response of the lung to noxious particles or gases, with some significant extrapulmonary effects that may contribute to the severity of the disease in individual patients.\textsuperscript{15} The pathologic hallmarks accountable for the functional consequence of COPD are inflammation of the central airways, inflammation of the peripheral small airways and destruction of the lung parenchyma. The most important factor leading to COPD is cigarette smoking. There is evidence of local inflammatory activity in the whole pulmonary compartment in smokers with COPD compared with non-smokers. Biopsy studies from central and peripheral airways in smokers with COPD have shown an increased number of T-lymphocytes, predominantly cytotoxic T-cells (CD8\textsuperscript{+}), macrophages and neutrophils in the mucosal epithelium and subepithelium in the peripheral airways and lung parenchyma.\textsuperscript{14,31-34} The number of CD8\textsuperscript{+} cells was found to be negatively correlated to pulmonary function assessed on the basis of FEV\textsubscript{1}.
% predicted\textsuperscript{35}. Other evidence of inflammatory activity include an increased number of activated neutrophils and macrophages in bronchoalveolar lavage fluid and induced sputum\textsuperscript{36,37}.

The small airways (bronchioles with a diameter < 2mm) and the surrounding parenchyma (alveolar walls) are the key sites of inflammation. When the normal repair processes are hampered, aberrant tissue responses in the lung can occur, resulting in the development of several features of the characteristic pathology seen in COPD, e.g. alveolar destruction (emphysema), loss of elastic recoil and peribronchial fibrosis\textsuperscript{38,39}. Retamales et al. showed that there was a greater increase in the number of polymorphonuclear neutrophils and macrophages in the parenchyma and alveolar spaces in smokers with severe COPD compared with normal smokers\textsuperscript{40}.

Smoking is thought to create an imbalance between oxidative and antioxidative factors in the lung, causing oxidative stress. Oxidative stress, defined as an increased exposure to oxidants and/or decreased antioxidant capacities, is widely recognized as a key event in the pathogenesis of COPD\textsuperscript{39}. Cigarette smoke contains a high concentration of reactive oxygen compounds, which can induce oxidative stress and result in processes such as inactivation of antiproteases\textsuperscript{41}. Cigarette smoke can also directly deplete antioxidants, thereby shifting the balance towards oxidant burden\textsuperscript{42,43}. Macrophages can be directly activated by cigarette smoke, and are therefore thought to play an important role in maintaining the chronic inflammation in the pulmonary tissue of COPD patients\textsuperscript{44}. Polymorphonuclear neutrophils can participate by responding to chemotactic factors released by macrophages and epithelial cells\textsuperscript{45}. Activated neutrophils and macrophages contribute to the development of tissue damage by release of reactive oxygen species, i.e. free radicals and proteases\textsuperscript{39,46}. The released neutrophil proteinases are capable of degrading most components of the extracellular matrix, an event that is normally inhibited by antiproteinases such as alpha-1-antitrypsin (\(\alpha\)-1-AT).

As chronic inflammation is an important process in COPD, pro-inflammatory mediators such as chemokines and cytokines will play an important role in the pathogenesis of COPD. There is now increasing recognition of COPD as a multi-component disease with manifested systemic complications\textsuperscript{47}. The disease is not restricted to just the airways, as emphysema and airflow limitation, but can also often present with significant extrapulmonary abnormalities. The identification of these cytokines in the plasma of patients with COPD strongly suggests that the local inflammatory response communicates with the systemic circulation via these mediators. The local inflammatory process in the lungs may spill over into the systemic circulation.
to produce systemic changes either by direct effects of released chemokines and cytokines, or indirectly by modification and activation of peripheral inflammatory cells. 39, 48 The intensity of the inflammatory process correlates with the severity of COPD, and there is also evidence that patients with high values of inflammatory markers when stable had a more rapid decline of lung function over time. 33, 49-51

Markers in the blood of the resulting inflammation in smokers include C-reactive protein (CRP), fibrinogen, interleukin-6 (IL-6), interleukin-8 (IL-8), lysozyme, tumour necrosis factor-α (TNF-α (cachexin)), hydrogen peroxid, myeloperoxidase (MPO) and isoprostanes. C-reactive protein is an acute phase reactant protein present in plasma, and is synthesised by the liver in response to inflammation. CRP is elevated in patients with stable COPD. 52 Lysozyme and myeloperoxidase are enzymes present in cytoplasmic granules of the polymorphonuclear neutrophils. During oxidative stress MPO produces hypochlorous acid from hydrogen peroxide and chloride anions. 53 Fibrinogen is synthesised by hepatocytes and is an acute phase reactant and a clotting factor. It is released into the circulation in response to the cytokine IL-6. 54 Smokers with COPD have elevated plasma levels of fibrinogen, particularly during exacerbations. 55, 56, 57 Another inflammatory marker is tumour necrosis factor-alpha (TNF-α), produced by alveolar macrophages, alveolar epithelial cells and activated neutrophiles. It has been observed in the chronic inflammatory process and has been associated with COPD patients suffering from involuntary weight loss, which suggests that it may play a role in the cachexia seen in patients with severe COPD. 58-60 Potentially all these markers can signify early changes, or even act as indications of susceptibility in subjects with normal lung function. 61, 62

**BMI**

COPD has been recognised as a disease of a complex nature that does not just affect the lungs and airways, thereby making it a systemic disease. 53, 64 Besides inflammation which causes the pulmonary pathology in COPD as a result of tobacco smoke, malnutrition and unexplained weight loss are also known and clinically relevant problems in patients with more advanced COPD. 65, 66 Data from the Copenhagen City Heart Study showed that the severity of COPD tended to be greater in patients with low BMI (with low BMI
defined as a BMI less than 20 kg.m\(^{-2}\)). In subjects with mild airflow limitation (FEV\(_1\) > 70\% of predicted value) 3.5\% of males and 12.5\% of females also had low BMI.\(^6\) Studies have also shown that loss of skeletal muscle may also occur in COPD patients with normal weight.\(^6\)

Weight loss in patients with COPD was an independent risk factor shown to increase the risk of exacerbations and all-cause mortality, independent of the degree of airflow limitation.\(^6\),\(^7\),\(^8\)-\(^13\) Weight gain on the other hand, seemed to have a protective effect in normal and underweight patients with severe COPD.\(^7\)

The loss of weight is most likely multifactorial in origin. Established explanations for weight loss in COPD include increased basal metabolic rate due to the increased energy cost of breathing, as well as physical inactivity and malnutrition due to eating difficulties.\(^7\),\(^8\)-\(^13\) Studies have also indicated that COPD patients tend to expend more energy during physical activities compared to healthy subjects.\(^8\),\(^2\),\(^3\),\(^8\) One study however, which included fourteen stable COPD patients in a rehabilitation programme showed an unexpected decrease in energy expenditure during the physiotherapy programme in most of the patients. The authors’ explanation for the unexpected results was that the patients had a compensatory decline in physical activity during the remainder of the day.\(^8\)

Atrophy of skeletal muscle is generally the main cause of weight loss in advanced COPD.\(^8\),\(^2\) The cellular and molecular mechanisms leading to skeletal muscle atrophy are as yet unclear, but systemic inflammation present in COPD could be a potential pathogenic factor that could explain some of the weight loss.\(^4\)

Systemic inflammation and hypoxia are particularly prevalent among COPD patients with low body weight.\(^8\) There is increasing evidence that the immune system, in particular inflammatory cytokines, play an important role in the development of weight loss and cachexia. The central cytokine in the loss of muscle mass is TNF-\(\alpha\). TNF-\(\alpha\), which in laboratory animals is associated with accelerated metabolism and protein turnover, was shown to be elevated in the blood of COPD patients suffering from involuntary weight loss.\(^8\),\(^9\) One study however, demonstrated that low BMI was on its own a risk for developing COPD.\(^8\)
Emphysema

Emphysema is defined in anatomic terms as abnormal permanent enlargement of the airspaces distal to the terminal bronchioles, accompanied by the destruction of their walls without obvious fibrosis. Cigarette smoking has several pathological effects on the lungs, including large airway disease (chronic bronchitis), small airway disease (bronchiolitis and peribronchial fibrosis) and parenchymal destruction (alveolitis, emphysema). Pathologically, emphysema can be divided into three subtypes: centrilobular emphysema (CLE), panacinar emphysema, and paraseptal emphysema (PSE), based on the portion of the primary acinus involved. Radiologically, emphysematous lesions decrease the attenuation (low density) of X-rays passing through the thorax, thereby allowing emphysema to be detected on thin-section CT scans. By visual analysis of high-resolution computed tomography scans (HRCT), two subtypes of emphysema can be distinguished, centrilobular- and paraseptal emphysema.

High Resolution Computed Tomography (HRCT)

HRCT highlights areas of abnormally low attenuation using a computer program. It is considered as the most advanced and accurate imaging technique in the study of emphysema. The densitometric quantitation of emphysema is measured in Hounsfield units (HU). The HU scale is a linear transformation of the original linear attenuation coefficient measurement, in which the radiodensity of distilled water at standard pressure and temperature (STP) is defined as zero Hounsfield units (HU), while the radiodensity of air at STP is defined as -1000 HU.

HRCT allows direct visualization of areas of lung destruction, and allows detection of parenchymal changes 0.2-0.3 mm in size. This technique is more sensitive than chest radiography and lung function tests in the detection of early smoking related lung damage. It is also able to identify the presence and to quantify the amount of emphysema present. Chest radiography is a widely used method of detecting pulmonary lesions. It is however, neither sensitive nor specific enough for detecting early COPD and emphysema. There are two methods for assessing and quantifying the amount of emphysema on HRCT. The extent of emphysema can be estimated subjectively by visual inspection of areas of abnormally low attenuation (visual scores), or
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by more objective quantification based on computerised software. The computerized method allows quantification of the total volume of lung showing emphysema on CT scans. It is also able to show the percentage of lung affected by emphysema. Visual inspection to determine the extent of emphysema is generally made independently by 2-3 radiologists. Studies which used visual estimation showed a good intra- and interobserver agreement regarding the extent and grading of emphysema.95-99 Studies have shown a lack of correlation between airway obstruction and parenchymal damage, such as early emphysema on HRCT in smokers with mild airflow limitation.90,100 In established COPD however (i.e. more advanced stages of the disease), a good correlation has been found between the severity of airflow limitation and the extent of emphysema determined.92,101,102 One study reported a sensitivity of 100% and a specificity of 91% with use of HRCT scans in comparison with pathological techniques.103

On the other hand, a significant number of asymptomatic smokers tend to have significant emphysema on HRCT, quantified either by visual scoring or based on computerised attenuation values. HRCT may also detect emphysema in smokers with normal findings in chest radiographies and pulmonary function tests. Studies have demonstrated mild degrees of emphysema in asymptomatic smokers in whom COPD might be developing.97,98 In another study, HRCT detected the presence of emphysema in smokers with anamnestic dyspnoea despite normal chest radiography and normal lung function tests.90 Despite HRCT being the most advanced imaging technique to date for detecting emphysema, one study reported that the computerized method (conventional density mask) was inadequate for detecting mild morphologic emphysema as judged by visual analysis.104

Emphysema and airflow limitation

In theory, airflow limitation in COPD can be due to several reasons, including large airway disease (oedema of the mucus membrane and excessive phlegm production), bronchoconstriction, loss of lung elastic recoil pressure, peribronchial fibrosis of the small airways and emphysema. The contribution of mucus hypersecretion (chronic bronchitis) to the airflow limitation in COPD is uncertain, except for the fact that it contributes little or not at all in the early stages of COPD.105
The inflammation, which results in the destruction of the alveolar attachments on the outer walls of the small airways, is associated with loss of lung elastic recoil pressure, which contributes to an irreversible expiratory airflow limitation. Peribronchial fibrosis of the small airways also contributes to the permanent airway obstruction in COPD. Early in the development of COPD, smokers’ airflow obstruction is due either to intrinsic airway disease or to loss of lung elastic recoil pressure, independent of detectable emphysema.\textsuperscript{106} Emphysema and airflow obstruction appear to occur independently, although both are linked to smoking habits. Clark et al. observed that asymptomatic smokers tended to have either airflow limitation or emphysema, but generally not both.\textsuperscript{100}

**Prevalence of COPD**

Most of the information available on COPD prevalence comes from developed countries. In epidemiological studies the prevalence of COPD is largely dependent on the smoking habits of the examined population, as well as different demographics such as age and gender. The prevalence of COPD tends to be greater in older people, males and people with high smoking habits.\textsuperscript{1,18,107,108}

Another important factor when considering the prevalence of COPD is the actual definition of COPD itself.\textsuperscript{109} There is a heterogeneity of spirometric definitions, and by using different spirometric criteria the prevalence of COPD will invariably alter.\textsuperscript{20,110-112} The variable definitions of COPD used in studies have made it difficult to estimate the true prevalence. It is generally accepted that the fixed FEV\textsubscript{i}/FVC(VC) ratio $<$0.7 is the most important guide when identifying airflow obstruction. Given that FEV\textsubscript{i}/FVC ratios decrease with age, a fixed ratio could result in an increase of falsely positive diagnoses of COPD, resulting in a greater prevalence associated with ageing.

Moreover, to obtain data for the prevalence of COPD, researchers would have relied on either readings based on spirometric criteria\textsuperscript{15}, self-reported respiratory symptoms via a questionnaire\textsuperscript{116}, or a combination of the two.\textsuperscript{18,117} Prevalence based on self-reported respiratory symptoms and physician diagnosis of COPD must be regarded as the most imprecise due to the lack of both sensitivity and specificity. For example, use of self-reported symptoms will include people with chronic bronchitis but without airflow limitation. Furthermore, the disease is usually not diagnosed until COPD patients have
clinically obvious symptoms such as dyspnoea, and smokers with mild to
moderately advanced COPD may not have any symptoms at all.
In the review by Halbert et al 2006, the pooled prevalence of physiologically
defined COPD from 26 studies in adults aged ≥40 years was 9-10%. The
most common spirometric definitions used in that review were those of the
GOLD. The distribution of the disease severity in population based studies among
subjects with COPD showed that the majority of smokers suffer from mild to
moderate stages of the disease. The results of one study from the northern part
of Sweden showed that according to the GOLD criteria 57% had mild, 37%
moderate, 5% severe, and 1% very severe forms of the disease. In the
Spanish epidemiological study by Penna et al, 38.1 had mild, 39.7 moderate
and 22% severe forms of COPD according to ERS criteria.

Smoking cessation

Cigarette smoking is the main cause of COPD, and also the main cause of
preventable deaths in the world. Because smoking has a wide range of
serious effects on health, even a small improvement in cessation rates has been
considered clinically important. Regarding treatment of COPD, smoking
cessation is the most important therapeutic intervention and the only causal
treatment for patients at all stages of COPD. It is the only intervention, which
in several studies has been shown to stop the progression of COPD by
reducing the accelerated decline in pulmonary function, leading to
improvements in respiratory symptoms. In the Lung Health Studies by
Anthonisen et al. and Scanlon et al., 5887 volunteers with mild-to moderate
airway obstruction were randomised to either participate in a 10-week
intensive smoking cessation programme or to receive the standard usual care.
Stopping smoking significantly reduced the age-related decline in FEV₁. The
annual rate of decline in FEV₁ over 4 years was half that observed among those
who continued smoking (31 versus 62 ml/year), and was comparable to rates
for decline in FEV₁ in healthy never-smokers. Near the 15 year follow-up
the effect of the intervention on all-cause mortality and mortality due to
cardiovascular disease, lung cancer and other respiratory disease was
investigated. The all-cause mortality rate was significantly lower in the
special intervention group compared with the usual care group (8.83 vs. 10.38
deaths per 1000 person-years; P=0.03). This corresponds to a relative risk
reduction of 15%.
Dependence on cigarette smoke is a chronic condition and consists of physiological (nicotine) and psychological (behavioural) dependence. Smoking cessation is a dynamic process and often requires repeated interventions. Most smokers go through several stages of preparation before they take the decision to make an attempt at quitting smoking. Treatment of nicotine addiction requires both physiological and psychological/motivational treatment. Major efforts have been focused on identifying mechanisms and developing behavioural methods including developing pharmacological treatments to assist smokers in their process to stop smoking. To prevent relapse into smoking after the initial cessation, guidelines recommend the use of psychological support and/or pharmacological treatment during a period of time after a person has successfully managed to quit smoking. The most important smoking cessation measures in clinical trials are sustained abstinence at 6 and 12 months after discontinuation of the used drug or other intervention, i.e. prolonged abstinence.

The majority of all smokers want to stop smoking, although a significant proportion of them have never actually attempted to do so. Each year approximately 2% of smokers succeed in quitting on their own initiative. Studies have shown that when smokers who perceived that their symptoms were associated to their smoking habits were more likely to intend to stop smoking. According to a review by Morgan et al. 2003, up to 3% of smokers managed to quit smoking without a relapse up to 1 year afterwards, as a direct consequence of being given brief smoking cessation advice (up to 5 minutes duration) from a clinician in routine clinical care as part of an attempt to encourage quitting. According to a Cochrane review from 2004 using pooled data from 17 trials, a single consultation lasting less than 20 minutes and up to one follow-up visit (judged as minimal intervention) increased cessation rate to about 2.5%, (odds ratio 1.74). Brief smoking cessation advice and motivational counselling therapies appears to have their effect by triggering an attempt to quit.

More intense psychological treatment to support smoking cessation could be delivered on an individual basis or in a group setting, or a combination of the two. This intensive counselling consists of several face-to-face meetings or one face-to-face meeting together with further telephone contact delivered by a trained smoking cessation counsellor to the patients. According to the
Cochrane database, Stead 2005, behavioural interventions given as group therapy with the use of a group programme has an effect on quitting smoking at least six months after the start of counselling, with an odds of 2.04. This is in comparison with a self-help programme with an odds of 2.17 with no intervention controls. The odds for successful smoking cessation with individual counselling compared with controls was 1.56.

A great amount of pharmacological, randomised trials have been performed in which different forms of nicotine replacement therapy (NRT) (nicotine gum, nicotine inhaler, nicotine nasal spray, nicotine patch and nicotine tablets) or bupropion were compared to placebo or to no treatment for smoking cessation. NRT has its effects by reducing symptoms of nicotine withdrawal, thereby increasing the likelihood of smoking cessation. Bupropion is a weak dopamine and nor-epinephrine reuptake inhibitor. The exact action of this drug on smoking cessation is not clear, but it has been shown that it can reduce symptoms of nicotine withdrawal.

Due to different designs and variations in characteristics of these trials, the best knowledge about the effect of these medications is found in the Cochrane database. Regarding NRT, a pooled meta-analysis of 103 studies by Silagy and co-workers 2004 showed that the odds ratio for smoking cessation increased 1.5 to 2 times regardless of the setting and forms of NRT used. The pooled odds ratio of abstinence up to 6 months to 1 year for any form of NRT relative to control was 1.77. For the different forms of NRT the OR ranged from 1.66 with nicotine gum to 2.35 with nicotine nasal spray. Wu et al conducted in 2006 a new meta-analysis of randomised controlled trials, only evaluating interventions for smoking cessation at 1 year, through chemical confirmation. They identified 70 trials of NRT versus control, with abstinence from smoking after at least 1 year, and with an odds ratio of 1.71 and 12 trials with bupropion showing significant effect to controls at 1 year, with an odds ratio of 1.56. The latest Cochrane review by Hughes 2007 reported 31 trials of bupropion as the single pharmacotherapy for abstinence from smoking after at least six months follow up, with an odds ratio of 1.94.

Often in clinical trials several components of interventions and several different combinations of interventions are used. The highest cessation rates were achieved when smoking cessation advice was combined with either some kind of NRT or bupropion in addition to multiple other intervention techniques such as spirometry, physician and non-physician advice and
Introductions

psychosocial support on multiple occasions over the longest possible time period.\textsuperscript{139}

Spirometry can be used as a tool to assess lung function and to provide feedback to smokers, and thereby encouraging smoking cessation regardless of symptoms or spirometric status. The role of spirometry as a tool to motivate and improve smoking cessation rates have been unclear, and this was reviewed in 1997 and 2007 by Badgett et al and Wilt et al respectively.\textsuperscript{140,141} Badgett et al. included a total of only seven studies and most studies were from the late 70s and early 80s, and some studies had no control group. Two studies of multi-intervention smoking cessation programs that included spirometry were somewhat efficacious but were only proven effective in symptomatic patients, and there was no effect in a third study that isolated the role of spirometry.

Wilt et al. also included seven randomised controlled studies, four of them were the same as in the review by Badgett et al., and in all of them the spirometry results were incorporated into smoking cessation programs containing other interventions. The duration, format and intensity of the counselling varied widely across the studies. The counselling was up to 50 minutes long and could include 4 visits, and had follow up duration of 6 months or longer. Furthermore, the intervention could vary between the intervention and the control group and some studies had no control group. The range of abstinence rates was 3%–14% for control groups and 7%–39% among intervention groups and the range of absolute abstinence differences between treatment and control groups was 1%–33%.

To summarize, most of the studies were done 20-30 years ago, in both reviews the results was mixed and it was difficult to isolate the role of spirometry in its contribution to smoking cessation as spirometry had been used in combination with multiple other intervention techniques.

Clinical features of COPD

The chronic airflow limitation in COPD is caused by a mixture of small airway disease (obstructive bronchiolitis) and parenchymal damage (emphysema). The relative contributions of each component may vary from person to person and the bronchiolitis component with bronchoconstriction may change over time for the same person. COPD has a variable natural history, and no all individuals follow the same course concerning the loss of lung function and the development of symptoms.
In principal, COPD develops in long-term smokers, and it has a long preclinical and subclinical phase. The pulmonary lesions associated with COPD occur decades before the onset of the airflow limitation and the appearance of symptoms. Since the lesions progress slowly, COPD may take an insidious clinical course and the patients may adapt to the decreased pulmonary capacity. The large telephone survey of more than 3000 patients with COPD by Rennard et al. showed a significant discrepancy between subjects’ perception of disease severity and the degree of severity measured by an objective breathlessness scale. Of those with the most severe breathlessness (too breathless to leave the house), 35.8% described their condition as mild or moderate, as did 60.3% of those with the second most severe degree of breathlessness (breathless after walking a few minutes on level ground). The subjects often attributed their breathlessness to ageing, lack of fitness or as natural for a person who has smoked.

In early stages (mild COPD) the disease is often characterised only by mild airflow limitation and the individuals are usually unaware of the disease. Physical examination findings are often normal in early COPD. As the disease progresses, characteristic symptoms will start to manifest. The characteristic symptoms in patients with more advanced COPD are chronic progressive dyspnoea at ever lower levels of exercise, chronic cough, sputum production and wheezing. Chronic cough and sputum production may precede the development of airflow limitation by many years. Conversely, significant airflow limitation may develop without chronic cough and sputum production.

**Chronic bronchitis**

The inhalation of tobacco smoke and other noxious gases and particles cause an inflammatory response in the bronchi. Histologically, hypertrophy of bronchial submucosal glands and hyperplasia of bronchial surface goblet cells appear. The response of the bronchi to tobacco smoke is hypersecretion of mucus. Increased mucus production together with impaired mucociliary clearance lead to the characteristic productive cough, expressed clinically as bronchitis.

The clinical definition of chronic bronchitis was introduced in the early sixties, and was defined to be present when recurrent or persistent cough and sputum had lasted at least 3 months to a year, for at least two consecutive years, and
could not be attributed to other pulmonary or cardiac causes.\textsuperscript{146} This chronic mucus hypersecretion can occur in the absence of airflow limitation. In smokers, chronic bronchitis can occur as the only smoking related symptom, or it can manifest several years prior to the onset of COPD, and later co-exist with COPD. COPD can also exist without chronic bronchitis, and a smoker may develop COPD without passing through the stage of chronic bronchitis.

Not all smokers with chronic bronchitis will develop COPD. A random general population survey by Vestbo et al which examined a large population with a mean age of 52, found that after 5 and 15 years, 13.2 and 20.5\% respectively, of smokers with chronic bronchitis had developed COPD.\textsuperscript{105} In the Finnish 30-year follow-up survey, Pelkonen et al investigated 1 711 middle-aged, male smokers and their lifetime risk of chronic bronchitis and the effect of chronic bronchitis on pulmonary function and mortality.\textsuperscript{147} The investigators reported that the cumulative incidence of chronic bronchitis was 42\% in continuous smokers and 9.8\% in subjects who had never smoked. Furthermore, they found that almost 50\% of smokers who had chronic bronchitis also developed COPD and that chronic bronchitis was also related to earlier deaths. Subjects with chronic bronchitis had increased all-cause mortality with a hazard ratio of 2.38.

Other studies showing the association between chronic bronchitis and increased total mortality risk include the large Swedish population-based study by Ekberg-Aronsson et al. which showed that among smokers with normal pulmonary function the increased total relative mortality risk was 1.65, and among those with mild and moderate COPD, the relative risk was 1.41 and 2.42, respectively.\textsuperscript{148} In the Copenhagen City Heart study, chronic bronchitis was associated with an increased risk of all cause mortality but statistically significant in men only (relative risk 1.3).\textsuperscript{149} Studies also show that the odds ratio for airflow limitation in smokers with chronic bronchitis/chronic cough is increased.\textsuperscript{150,151}

\textit{Dyspnoea}

COPD has a long, silent preclinical phase with respect to dyspnoea. It is clear that the pathological process leading to deterioration of lung function is evident for decades before the development of dyspnoea. Once dyspnoea develops, it will usually be the primary symptom experienced by patients with COPD, and the symptom that forces most patients to seek medical advice. The
population based survey by Lindberg and colleagues, showed that about 50% of all patients with COPD present with dyspnoea.\textsuperscript{110} The term dyspnoea is generally defined as a \textit{subjective} symptom, experienced by subjects complaining of unpleasant or uncomfortable respiratory sensations.\textsuperscript{152} Patients describe dyspnoea as a sense of increased effort to breathe, heaviness, chest tightness, air hunger, or gasping for breath, and the result of dyspnoea is a reduction in exercise capacity. As lung function deteriorates, dyspnoea becomes increasingly disturbing and is the central symptom of the disease.

There are several factors responsible for the patient’s sense of dyspnoea. The main factors are gas exchange imbalance, static and dynamic hyperinflation of the lungs, abnormal lung and thoracic mechanics, respiratory muscle weakness and increased work of breathing, as well as psychological factors.\textsuperscript{153} It is difficult to be sure which of the factors contribute most strongly to the sensation of dyspnoea, but the consequence of these factors (above all, the gas exchange imbalance) is that the ventilatory system cannot meet the increased oxygen demands during exercise. The result is hypoxemia and hypercapnea, and the clinical outcome is reduced exercise capacity.

Disease severity and progression are traditionally measured as decline in lung function measured by forced expiratory volume in 1 second (FEV\textsubscript{1}). Although there is a correlation between the severity of COPD in terms of the level of FEV\textsubscript{1} and the degree of dyspnoea, this correlation is weak as dyspnoea is affected by several factors other than lung function.\textsuperscript{154,155}

Initially dyspnoea occurs only during physical activities that require strong exertion, but as the disease progresses the threshold of exertion level whereby dyspnoea starts to occur will decrease. When COPD progresses to more severe stages dyspnoea becomes more or less persistent. The dyspnoeaic patient is frequently unable to perform daily life activities, and the quality of life decreases. With further deterioration of the disease, respiratory failure develops due to inadequate gas exchange in the alveoli.

\section*{Screening of COPD}

\subsection*{Early disease detection}

According to the World Health Organization (WHO) COPD is expected to become the third most common cause of death by 2020.\textsuperscript{156} Since the prevalence
of COPD is between 4-10%, COPD is regarded as a national disease.\textsuperscript{157} If not detected early enough to prevent further deterioration of lung function by smoking cessation, COPD will progress and cause high morbidity and mortality.\textsuperscript{15,158} The disease is usually diagnosed late in its course. The mean delay from onset to diagnosis is 20 years (www.nice.org.uk/CG012niceguideline). A clinical diagnosis of COPD is often not made until patients have fairly advanced stages of the disease, and considerable functional impairment. COPD may be detected in its early stages using spirometry, which combined with smoking cessation advice could reduce the burden of COPD by preventing progression to severe, disabling stages of the disease.\textsuperscript{159,160} Early disease detection and smoking cessation are the only available methods to stop the progression of COPD.\textsuperscript{161}

Although standards for performing spirometry are well established, consensus statements recommend a widespread use of office spirometry by primary-care providers for patients >45 years of age and actively smoking, the reality however is different. According to guidelines, smokers should be examined by spirometry regardless of their reason for seeking medical attendance or whether symptoms are present or not.\textsuperscript{162} However, although spirometers are widely available, primary care physicians rarely use spirometry to detect COPD in patients with respiratory symptoms.\textsuperscript{114,163-165}

There is no recommendation for a nationwide screening program for COPD. Mass screening of the smoking population for COPD with spirometry has been controversial and not regarded as feasible.\textsuperscript{164,166,167} However, COPD still remains relatively unknown and ignored by the public, resulting in either underdiagnosis or delayed diagnosis.\textsuperscript{114,168,169}

In most countries, primary care clinicians treat the vast majority of patients with chronic respiratory diseases, as exemplified by the UK and the Netherlands, where approximately 85% of patients with asthma and COPD are managed almost entirely by GPs and primary care nurses.\textsuperscript{150} Access to spirometry is also increasing in primary care. In Sweden, for example, about 90 percent of the Primary Health Care Centres (PHCC) have access to spirometry.\textsuperscript{170} It is, however, reported that primary care physicians seldom use spirometry to detect COPD among smokers or people with respiratory symptoms.\textsuperscript{164}

The definition of screening according to the UK’s national screening committee is: “when members of a defined population, who do not necessarily perceive that they are at risk of, or are already affected by, a disease or its
complications, are asked a question or offered a test to identify those individuals who are more likely to be helped than harmed by further tests or treatment to reduce the risk of disease or its complications. (www.nsc.nhs.uk) Screening for COPD fulfils all WHO criteria for a disease that is suitable for screening. For example: 1) COPD constitutes as an important health problem. 2) The natural course of the disease is well understood. 3) A suitable and accepted screening test is available (spirometry). 4) It is possible to diagnose the condition in a latent or early phase. 5) Treatment (smoking cessation) at an early stage is more beneficial than at a later stage. 6) Screening is a service of reasonable cost compared to other services offered in public health.

Early detection of COPD assumes a systematic method of working on different levels and using different ways to find the disease at a latent or early stage in a population at risk. This systematic approach can be performed at various levels such as national, regional, local, or personal level. The systemic examination can be carried out in mainly two ways. One can distinguish between national population screening, i.e. mass screening of whole population groups thought to be at risk, as in the national programmes for breast or cervical cancer, or targeted, selective screening of certain high-risk groups in the risk population. Regarding COPD, this could include only smokers with a settled age of for example 40-70 years. Another method to identify smokers with a high likelihood of having COPD, for whom spirometric testing is particularly important, is the use of simple patient self-administered questionnaires. This kind of questionnaire could enhance the efficiency and diagnostic accuracy of screening efforts. There are a few large-scale screening surveys for the detection of COPD by spirometry. In order to overcome underdiagnosis of COPD and to increase COPD awareness, Zielinski et al. from Poland showed that mass spirometry in a high-risk population of about 110 000 current or ex-smokers aged ≥40 years with a smoking history of ≥10 pack-years is an effective and easy method for the early detection of COPD.

**Positive/negative effect of screening**

A potentially negative aspect of spirometric screening is the risk of reinforcing the smoking habit in smokers with normal spirometry results. However, it seems that those fears are unfounded. A study by Gorecka et al. showed
that 8.4% of smokers with normal lung function at follow-up stopped smoking after spirometry combined with simple smoking-cessation advice.175

The positive aspects of spirometry use in the diagnosis of COPD has been highlighted in a study by Buffels et al., who analyzed the usefulness of spirometry performed by general practitioners in early diagnosis of COPD. He found that the number of newly diagnosed cases of COPD increased by 42% with spirometry compared to if the diagnosis was based on a questionnaire on signs and symptoms of COPD alone.176 Similar results were found in the study by Geijer et al. in which out of all the subjects who participated it was revealed that 29.9% of them had previously undetected airflow obstruction, with the obstruction being mild (GOLD stage 1) in 86.7% of subjects and moderate (GOLD stage 2) in 13.3%.177

**Underdiagnosis of COPD**

The importance of identifying smokers with COPD at an early stage and supporting smoking cessation is unquestionable.118,121,178,179 According to the ERS, in 1995 only 25% of all smokers with COPD were estimated to have received the diagnosis.1 COPD still remains largely underdiagnosed, and it is not uncommon for smokers to be diagnosed with COPD in moderate or severe stages of the disease (FEV₁<50% of predicted). Population based surveys in different countries which used an objective measurement of airflow limitation for the diagnosis of COPD have shown a large proportion of underdiagnosis.108,180,181

In principle, underdiagnosis of COPD is theoretically caused by either delays by the patient or delays by the doctor. The main causes of patient delay are low awareness of the disease and inherent adaptation to the symptoms of the disease. Figure 1 shows a schematic illustration of the adaptation to the disease and the discrepancy between objective and subjective experience of disease severity (Figure 1). COPD patients gradually adapt to their symptoms, which leads to patient delays in seeking medical care. Data from the Third National Health and Nutrition Examination Survey (NHANES III) by Mannino et al. which was conducted from 1988 to 1994, showed that 63.3% of subjects with documented low lung function had no prior diagnosis of obstructive lung disease.181 In the Spanish study by Pena et al., 78.2% of the subjects with COPD had not been previously diagnosed.108
The concept of patients’ and doctor’s delay was considered in the Swedish population based study by Lindberg et al. Generally in that survey, only 20-30% of the subjects fulfilling the criteria for COPD had been correctly identified prior to the study. Although all COPD diagnosed subjects (+45 years of age) reported respiratory symptoms, only about 50% had consulted the health care system (patient delay) and a minority of those (16%) was diagnosed as having COPD (doctor’s delay). According to GOLD criteria, of those with mild COPD only 5% was priorly diagnosed with COPD. Out of all the subjects suffering from moderate airflow obstruction, FEV$_1$<40% of predicted, (severe according to GOLD) only 50% had been priorly diagnosed. A study by Sundblad et al clearly showed the nature of doctor’s delay. In the study, it was shown that out of 674 diagnosed smokers with COPD only 17.3% had been diagnosed by a physician, despite the fact that all patients had been on a physician-prescribed sick leave for more than two weeks. Bednarek et al conducted a survey in a single primary care setting where all adults, aged 40 years and above were invited to participate. Of all the 2250 eligible subjects, 87% were investigated and COPD was diagnosed in 9.3% of cases, with 81.4% of those cases having been previously undiagnosed.

The severity of undiagnosed COPD in the different studies is consistently mild to moderate. In the study by Bednarek et al, 82% of the previously undiagnosed COPD was mild to moderate. In the study by Lindberg et al 94% of the subjects had mild to moderate COPD.

Data from the Third National Health and Nutrition Examination Survey (NHANES III) showed that a significant proportion of patients with severe COPD (FEV$_1$<50% of predicted) may not report symptoms. The symptoms reported most frequently were wheezing and shortness of breath in 64% and 65% of subjects, respectively. Although the prevalence of COPD is increasing, the public awareness of the condition remains low. The general knowledge that cigarette smoking can cause lung cancer is widespread, but the knowledge that smoking can cause COPD is still low. In a population based study by Van den Boom et al with the aim to detect subjects in the general population with objective signs of COPD or asthma at an early stage, 74% of all subjects with symptoms or signs of COPD or asthma never consulted their general practitioner for their respiratory complaints. On the other hand the study by Lindberg et al., also clearly showed the doctor’s delay. This study revealed that although a majority of the subjects reported respiratory symptoms only about 50% had consulted the health care system (patient delay) and of those consulted only a
minority (16%) was diagnosed as having COPD (doctor’s delay). The reasons for the doctor’s delay could be as many as for the patients’ delay. GP’s are for example pre-occupied a great deal of the time with other national diseases such as hypertension, heart disease, diabetes and frequently also with infectious and orthopaedic diseases. It can be considered that GP’s tend to have a lack of time to investigate this disease thoroughly. Furthermore, in many countries spirometry is not offered in primary care.

Figure 1. Schematic illustration of the discrepancy between objective and subjective experience of disease severity. COPD patients gradually adapt themselves to their symptoms which leads to a patient delay.
Towards early detection and secondary prevention of COPD in primary care

What do we know?

- The prevalence of COPD is high and the disease is expected to be the third most common cause of death by 2020.
- The disease is often diagnosed very late.
- COPD is relatively unknown or ignored due to adaptation by the public.
- Underdiagnosis of COPD is considerable and may be caused by either “patient’s” or “doctor’s” delay.
- There is no nationwide screening program for COPD.

The importance of identifying smokers with COPD at an early stage and supporting smoking cessation is unquestionable and this is recognised in several national and international guidelines. With that in mind, how should we screen smokers for COPD in primary care? Would it be feasible to invite smokers for a free spirometry? And do smokers become motivated to stop smoking if they learn that they have COPD?

For simplicity, the fixed spirometric definition of COPD is more or less set arbitrarily. This implies that there may be individuals with measures close to the decided cut-off limits that are not identified as having COPD and vice versa. Since COPD is caused by a local inflammation in the lungs it seems logical to explore whether this inflammation could be measured systemically. In addition to spirometry, would a simple blood test or measurement of inflammatory markers in exhaled breath condensate be of value to detect smokers at risk of COPD, even before they develop the disease? Furthermore, such tests could be of importance in a screening program to diagnose COPD at an early stage.

These questions highlights the importance of exploring methods for early detection and secondary prevention of COPD.
AIMS OF THE STUDY

General aim

The overall aim of this thesis was to examine the possibilities of early detection and prevention of COPD in primary care.

Specific aim

- To evaluate an invitational method (screening by invitation) for detection of COPD at early stages and assess the prevalence of COPD in a targeted population of smokers (Paper I).
- To investigate if screening spirometry and information on the outcome affects smoking behaviour (Paper II).
- To investigate if a combination of spirometry and brief smoking cessation advice given annually for 3 years could influence smoking habits in smokers with COPD compared with smokers with normal lung function (Paper II).
- To evaluate to what extent emphysema was evident, as identified by High Resolution Computed Tomography (HRCT), in smokers with normal lung function and to relate age, gender, smoking history and Body Mass Index (BMI) to the HRCT results (Paper III).
- To study to what extent emphysema was present in smokers with lower normal values of lung function, near the lower limit of normal values (Paper III).
- To evaluate if concentrations of certain biomarkers in exhaled breath condensate, serum and saliva, or a single breath test for diffusion capacity (DLCO) could identify subjects with COPD or non-COPD smokers and ex-smokers supposed to be at risk of developing COPD (Paper IV).
SUBJECTS AND METHODS

Setting

All studies were performed in southeastern Sweden. The studies in paper I and II were performed in the city of Motala consisting of 45 000 inhabitants and the surrounding suburban areas consisting of 43 000 inhabitants. Six primary health care centres with their respective asthma-COPD nurses participated in the studies. The studies in papers III and IV were performed in the University Hospital in Linköping, department of Radiology and department of clinical Physiology.

Subjects

Papers I, II and III

The targeted population in paper I was smokers, 40-55 years old (Paper I). In the study area a total of 19 750 inhabitants were between 40-55 years old and were served by 9 Primary Health Care Centres. According to Swedish statistics from 2001, when the inclusion of subjects in the study started (Paper I), approximately 27% of the population in the age group 40-55 years were smokers\textsuperscript{184}. The calculated number of smokers in this population would be approximately 5332. In total, 512 subjects were included in the study. In the follow-up study (Paper II) these 512 subjects were examined further. From the original cohort of 512 subjects (Paper I) 60 smokers with normal lung function were invited to participate in the third study (Paper III).

Paper IV

The subjects in the fourth study (Paper IV) were recruited from three sources; 1) 29 smokers with normal lung function were randomly selected from the original cohort of 512 subjects\textsuperscript{185}; 2) 16 smokers and ex-smokers with a clinical diagnosis of COPD were randomly selected from subjects attending a general practitioners office, and 3) 19 age, sex and height matched healthy, non-
smoking controls. With the exception of the healthy non-smoking controls, inclusion criteria included regular tobacco smoking for duration of at least 20 years and exclusion criteria included significant heart or lung disease or any other severe diseases. Four of the controls in group 3 were re-categorised as ex-smokers and one of the four also had COPD, thus leaving 15 healthy controls as reference subjects (Table 1). Twenty-two people who stopped smoking for at least one year prior to the study were classified as ex-smokers according to recommendations by the Society for Research on Nicotine and Tobacco. In total 19 smokers or ex-smokers were categorised as having COPD according to the GOLD definition, of which eight were current smokers and 11 were classified as ex-smokers. 30 smokers had normal or subnormal FEV1 but were not classified as having COPD. Thirty-five subjects had a spirometry recorded approximately five years prior to this study.

Table 1. Demographic data on 49 study subjects and 15 healthy volunteers; classification of subjects as COPD or non-COPD, according to GOLD criteria. Data are present as median (min-max). Statistically significant differences are indicated by • (¶, ¶¶, ¶¶¶) = p<0.05, •• (¶¶, ¶¶¶) = p<0.01, ••• (¶¶¶, ¶¶¶¶) = p<0.001. Mann Whitney U-tests were used in statistical evaluations. ¶ = COPD vs. non-COPD, ¶¶ = COPD vs. healthy volunteers, ¶¶¶ = non-COPD vs. healthy volunteers. * = 3 healthy volunteer with 8-10 smoke years 31-40 years ago. ND = not done.

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<td>FEV1%pred</td>
<td>46 (29-73) ¶ ¶ ¶, ¶ ¶ ¶</td>
<td>93 (75-123) ¶ ¶ ¶</td>
<td>97 (81-121) ¶ ¶ ¶</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>0.56 (0.41-0.68) ¶ ¶ ¶, ¶ ¶ ¶</td>
<td>0.77 (0.70-0.89) ¶ ¶ ¶, ¶ ¶ ¶</td>
<td>0.78 (0.66-0.87) ¶ ¶ ¶, ¶ ¶ ¶</td>
</tr>
<tr>
<td>FEF50%pred</td>
<td>23 (12-40) ¶ ¶ ¶, ¶ ¶ ¶</td>
<td>81 (47-172) ¶ ¶ ¶</td>
<td>92 (51-121) ¶ ¶ ¶</td>
</tr>
<tr>
<td>DLCOc%pred</td>
<td>61 (34-107) (n=8) ¶ ¶ ¶</td>
<td>95 (44-117) (n=27) ¶ ¶ ¶</td>
<td>ND</td>
</tr>
</tbody>
</table>

Pharmacological treatment
ICS           | 5     | 1        | -           |
Bronchodilators | 11    | 1        | -           |
Anti-colinergic | 10    | 1        | -           |
Subjects and methods

Methods

Approval by the Ethical Committee in Linköping for all the papers was obtained, as was the approval by the local committee of radiology for paper III.

Lung function assessment

Paper I

The participants performed at least three dynamic spirometry tests according to standard methodology, including the use of a nose clip at their visit to the asthma/COPD nurses, who all had experience in the field consisting of at least 5 years. The spirometers were calibrated in a standardised manner at the start of each working day. Evaluation of lung function was done by measurement of slow vital capacity (VC) and forced vital capacity (FVC), forced expiratory volume in one second (FEV₁) and forced expiratory flow at 50% of forced vital capacity (MEF₅₀, FEF₅₀). The best of three expirations was documented and expressed as a percentage of predicted normal values. The European Respiratory Society (ERS) criteria were used for diagnosis and classification of COPD.¹ Reversibility tests were done 15 minutes after inhalation of three doses (0.5 mg/dose) of terbutalin (Bricanyl Turbuhaler®, Astrazeneca, Sweden)

The used models of spirometers were Flowscreen, version 3.10gb, Jaeger; Vitalograph-Compact II; Vitalograph Alpha; Vicatest-P2A, Siemens-Elema, all made in the E.C. The spirometry tests were performed and interpreted according to guidelines by the American Thoracic Society.¹⁸⁷ Predicted values were based on reference values according to the European Community for Coal and Steel (ECCS) and according to Hedenström et al.¹⁸⁸,¹⁸⁹,¹⁹⁰

Paper II

The participants were annually invited for further spirometry. The spirometries were performed and interpreted according to the American
Subjects and methods

Thoracic Society. The participants performed at least three dynamic spirometries at their visit with the respective asthma/COPD nurses who participated in paper I. The models of spirometers used were the same as in paper I.

Paper III and IV

Measurements were performed by a trained technician using the Jaeger Master Screen spirometry system (Erich Jaeger GmbH, Hoechberg, Germany). Evaluation of lung function was done by measurement of slow vital capacity (VC) and forced vital capacity (FVC), forced expiratory volume in one second (FEV1) and forced expiratory flow at 50% of forced vital capacity (MEF50, FEF50). The best of three expirations was documented and expressed as a percentage of predicted normal values. Reversibility tests were done 10 minutes after inhalation of four doses (0.4 mg/dose) of salbutamol (Ventoline® Glaxo Smith Kline, UK) and expressed as a percentage change of FEV1. The ERS and GOLD criteria were used for classification of COPD.

Single-breath diffusion capacity of the lung for carbon monoxide (DLCO)(Paper IV) were carried out using a Jaeger PFT MasterScreen Labmaster, MS-PFT analyzer unit (Erich Jaeger GmbH, Würzburg, Germany) according to the clinical routines. The values were adjusted for haemoglobin concentration in blood in accordance with the clinical routine, and documented as a percentage of the predicted normal value (DLCO % predicted). Reference range was 75-125% of the predicted normal value according to the clinical routine.

Research procedures

Paper I

Placards were exposed at each participating health care centre. In addition, twice in the spring and twice in the fall, an advertisement was run in the local newspaper. In the placards and advertisements, smokers between 40 to 55
years of age were invited to have a pulmonary function test (spirometry) performed free of charge in a Primary Care setting. They were also given the information that the aim of the test was to diagnose COPD (or any labeling synonymous with COPD, i.e. emphysema; smokers’ lung) at an early stage. Subjects who had already been diagnosed with COPD were excluded from the study. Information about the relationship between smoking and COPD was also given in the placards and the advertisements. A smoker was defined as someone who smoked at the time of the study, or had stopped smoking less than three months prior to the study.

Venous blood samples were collected by venipuncture for analysis of alfa1-antitrypsin and eosinophils in differential counts. Standard methods were used.

**Spirometry**

An experienced physician re-evaluated all the performed spirometries by the nurses. If the spirometry showing airflow limitation or was judged as not optimal, the subjects were asked to perform a new spirometry 1-2 months later. All the new spirometries were performed by the same physician using a Flowscreen version 3.10gb, Jaeger, Germany, E.C (Figure 2). If the spirometry still showed obstruction, further investigations to rule out asthma were performed, including a beta-2 reversibility-test. Reversibility tests were done 15 minutes after inhalation of three doses (0.5 mg/dose) of terbutalin, (Bricanyl Turbuhaler®, Astrazeneca, Sweden) and a steroid test was also carried out by a 14-day course of oral prednisolon, 30 mg daily.
Subjects and methods

Figure 2. Trial design. 512 responded to the advertisement and were included in the study. In the first screening spirometry for COPD, 160 subjects showed airflow limitation or a questionable spirometric result. At the second confirmative spirometry, 147 smokers still had airflow limitation and of those 141 were judged to have COPD.

Paper II

Out of the original screened cohort of 512 smokers, a total of 445 were included for a follow-up. All smokers with COPD and half of the smokers with normal lung function were annually invited for a new spirometry by postal letter for a duration of 3 years. The other half of the smokers with
normal lung function were examined last year, 3 years after the screening spirometry (Figure 3).
The spirometries were done according to standard methodology by the nurses at the primary health care centres who had done the primary spirometry in the first study.\textsuperscript{185} As a standard part of the visit, discussion was taken up about the subjects’ smoking habits. All participants received brief smoking cessation advice consisting of 3-8 minutes by the study nurse after their spirometry. The discussion was not protocol driven regarding the content of the discussion/advice or how long it should be. The situation should reflect a real life situation. The nurse encouraged the subjects to stop smoking, gave them advice and information about nicotine replacement therapy and bupropion, and if the participants had stopped smoking the nurse encouraged them to remain non-smokers.
The results from all performed spirometries by the nurses and the questionnaires were sent to the prime investigator for evaluation and comparison with the previously performed spirometry. A few weeks after the performed spirometry, all participants received a personal letter from the doctor (less than half of a single sheet of paper, e.g. A4) of any changes in the results of their lung function (FEV\textsubscript{1}), i.e. better, unchanged or worse, in comparison to the previous year’s results. The aim of this was to demonstrate the harmful effects of smoking, to reinforce smoking cessation advice (reinforce trigger attempts), and to encourage continued abstinence. A couple of standardized sentences regarding the health benefits of successful smoking cessation were also included in the letter.

“ Ahead, I hope you find the motivation to make one or more serious quitting attempts. To stop smoking means that the rapid decline of lung function will be stopped. Quitting smoking also provides several other health benefits, since smoking is so strongly linked to many other diseases as well, such as cardiovascular diseases”.

**Smoking cessation measurements**

Smoking cessation measures used were according to recommendations by the Society for Research on Nicotine and Tobacco.\textsuperscript{127} The data on prevalence abstinence rates (smoking free months) were based on the questionnaire answers given by the participants at each annual visit. Participants who did not attend a given visit were assumed to be smokers at that visit.
The primary outcome variables were the annual point-prevalence abstinence rates and the prolonged-prevalence abstinence rates. The number of smokers who were abstinent \( \geq 30 \) days immediately prior to the annual visit was used to calculate the point prevalence abstinence each year. Smokers who were abstinent \( >6 \) and \( >12 \) months, respectively, prior to the annual visit were used to calculate the prevalence of prolonged abstinence. Participants who did not attend a given visit were assumed to be smokers at that visit. The data on point prevalence abstinence- and prolonged abstinence rates were based on the questionnaire answers given by the participants at each annual visit. In addition, the mean number of smoking free months for each group was calculated.
Figure 3. Trial design showing excluded and annual follow-up rates. Twelve of the smokers with normal lung function (NLF) (group B) and 13 of the smokers with COPD didn’t wish to be followed-up at all. A number of smokers dropped out because of moving to another city or died. (*) Smokers not present just at this visit.
Subjects and methods

Paper III

Fifty-nine smokers, with a mean age of 53 years and with normal lung function were examined with High Resolution Computed Tomography (HRCT) (Table 2). Height was measured to the nearest 0.5 cm while the patient stood barefoot. Weight was measured while the patient wore light clothing and no shoes. Body mass index (BMI), defined as weight in kilograms divided by height squared in meters was calculated. High resolution computed tomography was carried out before lung function was assessed. Venous blood samples were collected by venipuncture for analysis of high sensitive C-reactive protein (hs-CRP) and eosinophils in differential counts. Standard methods were used.

Preclinical COPD

For the purpose of this study we defined preclinical COPD as, lower normal values of lung function, when FEV1/FVC ratio in percent of predicted (FEV% of predicted), was 89-93% of predicted value for males and 90-93% of predicted value for females or when FEF50≤ 60% of predicted value.

Assessment of emphysema

High resolution computed tomography was used as the gold standard proof for parenchymal damage due to cigarette smoking. All CT examinations were performed with a General Electric (GE) Light Speed 8 slice machine. Conventional five mm slices at 5 mm increments were reconstructed from a single breath hold, with the subject being supine during the full inspiration. Separate non-single breath 1.25 mm HRCT slices were also collected from the whole thorax with 10 mm increments. The images were displayed on monitors with a 1600 x 1200 matrix at window width 1700 HU and window level –500 HU or at the viewers own discretion to better view emphysema, most likely by altering the window level to about –700 HU. The HRCT images were evaluated visually using structured forms by an experienced radiologist. Densitometric quantitative analysis (quantitative emphysema) of a 3D volume of the lungs with a cut off value of -950 HU as the emphysema limit (according to Park et al.) was carried out after selectively removing the trachea and main stem bronchi.191
The radiologist was blinded to spirometric values, clinical and smoking data. Any sign of centrilobular (CLE) or paraseptal emphysema (PSE) was noted and graded on a 1-5 scale, slightly modified after the criteria of Vehmas et al.99. The following scoring was used:

0 = normal finding
1 = faint abnormality/emphysema usually in a single slice
2 = distinct abnormality/emphysema in one to two slices
3 = abnormalities/emphysema in two to five slices
4 = score between 3 and 5
5 = abnormalities/emphysema in most slices

Table 2. Data showing the two groups of non-COPD individuals regarding gender, mean age, BMI, smoking history, number of pack-years and spirometry variables.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Lower normal values of lung function (Preclinical COPD)</th>
<th>Higher normal values of lung function (n=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female (%)</td>
<td>28</td>
<td>39</td>
</tr>
<tr>
<td>Age (years)</td>
<td>53.2 (4.1)</td>
<td>52.1 (4.3)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.7 (4.0)</td>
<td>25.8 (3.5)</td>
</tr>
<tr>
<td>Smoking (years)</td>
<td>32.0 (7.8)</td>
<td>35.1 (5.7)</td>
</tr>
<tr>
<td>Pack-years</td>
<td>25.0 (11.7)</td>
<td>28.7 (11.9)</td>
</tr>
<tr>
<td>FEV₁ (% predicted)</td>
<td>89% (9%)</td>
<td>110% (13%)</td>
</tr>
<tr>
<td>FEF₂₀ (% predicted)</td>
<td>50% (7%)</td>
<td>91% (24%)</td>
</tr>
<tr>
<td>FEV₁/FVC ratio (% predicted)</td>
<td>93% (4%)</td>
<td>102% (5%)</td>
</tr>
<tr>
<td>FEV₁/FVC ratio (%)</td>
<td>72 (3)</td>
<td>79(4)</td>
</tr>
</tbody>
</table>

Notes: None of the differences were statistically significant. Data are presented as mean and standard deviation (± SD); n=number of subjects; BMI=Body mass index; Pack-years= Number of years of smoking x average number of cigarettes smoked per day, divided by 20.
Subjects and methods

Paper IV

Exhaled breath condensate (EBC)

Exhaled breath condensate (EBC) was collected by means of a commercially available condenser (EcoScreen; Jaeger, Wurzburg, Germany) and according to recommendations from ATS/ERS task force. Subjects were encouraged to breathe normally, i.e. tidal breathing, for a duration of ten minutes through a mouthpiece connected to the condenser whilst wearing a nose clip. Accumulated volume of exhaled air was measured by means of a spirometer (EcoVent, Jaeger, Wurzburg, Germany) connected to the outflow tract of the condenser. Contamination by saliva was prevented by a saliva trap. The total volume of EBC was measured by a spirometer connected to the outflow tract of the condenser. The samples were divided into aliquots and stored at -70°C until they were analysed for chlorine, Lysozyme, Eosinophil Cationic Protein (ECP), Myeloperoxidase (MPO) and α-amylase. Within-subject variability was tested by analysis of repeated measurements of chlorine values in EBC taken from five healthy volunteers, and determined by a Bland Altman analysis.

Serum

Venous blood was collected according to clinical routines and kept at room temperature for 60 ± 15 minutes before being centrifugalized at 3000 rpm for ten minutes. Supernatants were separated and stored at -70°C until analysis of Lysozyme, ECP, MPO or high-sensitivity C-reactive protein (hs-CRP).

Saliva

Subjects rinsed their mouth with de-ionised water before sampling of saliva (Paper IV). Spontaneously secreted saliva was collected in a plastic cup during 2-3 minutes to achieve a volume of approximately 0.5-1.0 ml. Samples of saliva was diluted 1:1 with 1 M acetic acid in order to decrease viscosity. The solution was centrifugalized at 3000 rpm for 10 minutes and supernatants were kept frozen at -70°C until analysed for chlorine, Lysozyme and ECP.
Laboratory analysis

Biomarkers were measured by skilled technicians, according to instructions given by the manufacturer at the department of Medical Sciences, Section of Clinical Chemistry, University Hospital, Uppsala, Sweden. Eosinophil cationic protein (ECP) was measured by means of an immunochemical fluorescence method (Unicap®, Pharmacia Diagnostics, Uppsala, Sweden), LOD was <2 μg/L and inter-assay variation 3%. Reference range in serum of 2.3-16 μg/L. Myeloperoxidase (MPO) was measured by enzyme-linked immunosorbent assay technique (ELISA) (Diagnostics Development, Uppsala, Sweden), LOD was 1.56 μg/L, and the coefficient of inter-assay variability was 7%. Reference range in serum of 8-250 μg/L.

Lysozyme was measured by a radioimmunoassay (RIA), LOD was 3.7 μg/L and inter-assay variability was 7%. Reference range in serum of 615-1383 μg/L. Amylase Activity in Exhaled Breath Condensate was measured according to the Calzyme Laboratories, Inc. (www.calzyme.com) method, modified as follows: 50 μL α-Amylase standards (Sigma-Aldrich Co., Stockholm, Sweden) in duplicates, or 50 μL of freshly-unfrozen EBC samples in triplicates were added on a 96-well plate (Nunc A/S, Roskilde, Danmark). 50 μL of fresh 1% starch solution was added to all wells and incubated for 10 min at 40˚C. 100 μL colour reagent (dinitrosalicylic acid) was added to all wells. The plate was sealed and heated to 95˚C for 15 min, then centrifuged at 2000 rpm for 1 min room temperature (RT), then unsealed and read in a spectrophotometer at 540 nm and concentrations of α-amylase was calculated. For increased sensitivity (<10U/L) the incubation time was increased up to 150 min and the starch was pre-treated in 20 mM NaOH for 30 min at 100˚C and neutralized to 6.9 at RT. LOD was 0.008 U/ml and coefficient of inter-assay variability was <10%.

High sensitive C-reactive protein (hs-CRP) was measured according to clinical routines by a standard method (Architect, Abbott). Reference value in serum is <5 mg/L.

Chlorine in EBC and saliva was measured by means of a modified adsorbed-organic-halogen technique as previously described.193 A sample of 100 μl was combusted with oxygen at 1,000ºC and then measured in a coulometric titration cell (DIN 34809, Euro Glass, Delft, the Netherlands) for measurement of the total chlorine content. Results were validated by measuring standard solutions with known concentrations of chlorine [Titrisol sodium chloride 0.1 mol/L (5.844 g/L), Merck, Darmstadt, Germany] diluted with Milli-Q® water (Millipore, Bedford, Mass., USA) to concentrations of 20, 40, 200, 1000, 2000
and 10000 μmol/L]. Milli-Q® water was used as blank. Limit of detection (LOD) was set to 3 μmol/L and the intra-assay coefficient was 9.7%.

**Questionnaires**

**Paper I**

All participants were asked to fill in a questionnaire regarding age, gender, smoking habits (how many years they had smoked and on average how many cigarettes/day) and profession. They also answered questions regarding symptoms of dyspnoea and symptoms of chronic bronchitis. In addition, they were asked if they had, prior to the study, any knowledge of COPD (or any labelling synonymous with COPD, i.e. emphysema; smokers’ lung) as a disease that could affect smokers. To these questions they could answer either “yes” or “no”.

**Paper II and IV**

In connection to the annual spirometry test, (Paper II) the participants’ smoking status was assessed with a questionnaire. The questions asked if they had stopped smoking or not. Those who had stopped smoking were asked to specify the duration of the non-smoking period and if they had used any nicotine replacement therapies, bupropion or snuff. If they were still smoking they were asked to record the mean number of cigarettes/day they smoked. Prior to the spirometry (Paper IV), each subject rated breathlessness on a ten-grade Borg scale.194
STATISTICAL METHODS

Detailed descriptions of the statistical methods are described in the separate papers. SPSS, version 11.5, Minitab version 13 and CIA version 2 were used in paper I, Minitab 14 in paper II, Stata version 9.1 and SAS version 8.02 in paper III and Statistica 7.0 in paper IV. A two-tailed P-value<0.05 was considered as significant.

Paper I
The variables age, gender, chronic bronchitis, pack-years and dyspnoea were examined for their association with COPD. First, a univariate logistic regression and then both forward and backward stepwise logistic regression were done to estimate the explanatory variables’ influence on the odds of having COPD. A Spearman rank correlation matrix between explanatory variables was done to examine if any relationships existed between the five variables.

Paper II
The Chi-square test was used for analysing the statistical significance, and the z-test was used for statistical significance regarding comparisons of proportions with normal distribution. Mood’s median test was also used.

Paper III
Student’s t-test for the continuous variables and Chi² or Fishers exact test was used for categorical variables. Data that was not normally distributed were log transformed before the t-test. Normally distributed data were expressed as a mean and standard deviation, or as mean with 95% confidence interval.

Paper IV
Normally distributed data were expressed as a mean [95% confidence interval]. All non-normally distributed data were expressed as median values (minimum to maximum). Mann-Whitney U-tests were used to compare groups of data. Bland Altman analyses were used to analyse the agreement between two different assays, and analyses of receiver operating curves (ROC) were performed for diagnostic discrimination. Good discrimination was defined by an area under ROC curves (AUCROC) of at least 0.80, and acceptable discrimination required an AUCROC of 0.75-0.80.
RESULTS

Paper I

Of the total population in the study area, 19 750 subjects were between 40-55 years of age. The calculated number of smokers in this population would be approximately 5332, out of which 512 (9.6%) responded to the advertisement, and were included in the study. The mean age was 48 years.

In the first screening process for COPD conducted by the nurses, 160 subjects showed airflow limitation or a questionable spirometric result. At the second confirmative spirometry test, 147 smokers still had airflow limitation, 13 were judged as normal and 6 were judged as having asthma (Figure 2). In total, 141 (27.5%) were classified as having undiagnosed COPD.

Table 3. Mean values (SD) of pack years and spirometry in relation to classification of COPD according to the ERS definition. Those with normal lung function according to ERS definitions were divided into those with normal spirometry and those with lower normal values of lung function (preclinical COPD), according to our definitions (MEF50 ≤ 60 or FEV% predicted 89–93 for males or 90–93 for females). % predicted = % of predicted value. b There are 11 missing subjects.

<table>
<thead>
<tr>
<th>Normal spirometry according to ERS</th>
<th>COPD</th>
<th>Total Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pack-yearsb</td>
<td>24 (11)</td>
<td>29 (14)</td>
</tr>
<tr>
<td>VCmax (%predicted)</td>
<td>109 (15)</td>
<td>104 (12)</td>
</tr>
<tr>
<td>FEV1 (%predicted)</td>
<td>106 (12)</td>
<td>94 (9)</td>
</tr>
<tr>
<td>MEF50 (%predicted)</td>
<td>91 (20)</td>
<td>60 (8)</td>
</tr>
<tr>
<td>FEV% (%predicted)</td>
<td>78 (4)</td>
<td>71 (3)</td>
</tr>
<tr>
<td>FEV% (%predicted)</td>
<td>99 (5)</td>
<td>93 (2)</td>
</tr>
<tr>
<td>&quot;Preclinical COPD&quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild (n=120)</td>
<td>31 (12)</td>
<td>35 (18)</td>
</tr>
<tr>
<td>Moderate (n=18)</td>
<td>111 (14)</td>
<td>98 (12)</td>
</tr>
<tr>
<td>Severe (n=3)</td>
<td>87 (11)</td>
<td>63 (5)</td>
</tr>
<tr>
<td>Total Mean (SD)</td>
<td>108 (14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>98 (16)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>74 (29)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>73 (9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>98 (6)</td>
<td></td>
</tr>
</tbody>
</table>
Results

Fewer males than females responded to our invitation, 43% and 57% respectively, but the males had smoked significantly more, in terms of pack-years. The mean number of pack-years was 27 (SD 12.4), with the value for males being 29.5 and for females 25.1, with a difference of 4.35 between the two sexes (CI: 1.24-7.46).

Out of all the participants, 41 (8%) received early retirement pension, the majority for musculoskeletal problems, and only 7% had college education. The awareness that COPD (or emphysema or smokers’ lung) is a disease that affected the lungs of smokers was acknowledged by 39% and 42 out of 424 (10%) answered that they would have consulted their physician some time in the future for their symptoms, if the study had not been performed. Among those with COPD, 16 out of 141 (11%) answered that they would have consulted a physician in the future about their symptoms.

Out of the people who responded to the questionnaire regarding symptoms, dyspnoea was experienced by 178 (35%) participants during physical activity (Table 4). Out of the smokers with COPD, dyspnoea was experienced by 48%. Symptoms of chronic bronchitis was evident in 223 (44%) of the 512 participants. Of the participants suffering from chronic bronchitis, 37% of them had COPD. Furthermore, out of the 141 participants with COPD, 57% had chronic bronchitis and among those with normal lung function, 38% had chronic bronchitis.

Table 4. Age distribution in relation to gender, number of pack-years, chronic bronchitis (CB) and lung function.

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Pack-years* Mean (SD)</th>
<th>Dyspnoea n</th>
<th>CB n</th>
<th>Normal lung function n</th>
<th>COPD n</th>
<th>Percentage with COPD by age group n</th>
</tr>
</thead>
<tbody>
<tr>
<td>40-44</td>
<td>Male</td>
<td>49</td>
<td>25 (10)</td>
<td>25</td>
<td>42</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>75</td>
<td>21 (9)</td>
<td>25</td>
<td>28</td>
<td>57</td>
<td>18</td>
</tr>
<tr>
<td>45-49</td>
<td>Male</td>
<td>69</td>
<td>30 (12)</td>
<td>27</td>
<td>32</td>
<td>52</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>92</td>
<td>24 (10)</td>
<td>30</td>
<td>43</td>
<td>65</td>
<td>27</td>
</tr>
<tr>
<td>50-55</td>
<td>Male</td>
<td>103</td>
<td>31 (15)</td>
<td>30</td>
<td>35</td>
<td>71</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>124</td>
<td>28 (13)</td>
<td>41</td>
<td>58</td>
<td>84</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>512</td>
<td>27 (12)</td>
<td>178</td>
<td>223</td>
<td>371</td>
<td>141</td>
</tr>
</tbody>
</table>

*11 missing
The severity of COPD was classified as mild in 120 (85%), moderate in 18 (13%) and severe in 3 (2%) according to the classification of COPD by the European Respiratory Society, 1995 (Table 5). According to the classification by GOLD, 150 (29%) would be classified as having COPD and 95 (63%) was classified as stage I, 52 (35%) as stage II and 3 (2%) as stage III (Table 5).

Table 5. Smokers with previously undiagnosed COPD. Age distribution and gender in relation to FEV₁% predicted according to ERS 1995 and GOLD 2003.

<table>
<thead>
<tr>
<th>Age</th>
<th>ERS</th>
<th>GOLD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild</td>
<td>Moderate</td>
</tr>
<tr>
<td>40-44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>female</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>45-49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>female</td>
<td>26</td>
<td>1</td>
</tr>
<tr>
<td>50-55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>24</td>
<td>7</td>
</tr>
<tr>
<td>female</td>
<td>32</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>18</td>
</tr>
</tbody>
</table>

COPD correlated to a higher number of pack years than if respiratory function was normal: 32.1 and 25, respectively, with a difference of 7.08 (95% CI = 3.73 to 10.43). In participants with 1–20 pack years, 15% had COPD; in those with 21–30 pack years, 27% had COPD; in those with 31–40 pack years, 38% had COPD; and in those with >40 pack years, 48% had COPD. Symptoms of chronic bronchitis was evident in 223 (44%) of the 512 participants and 82 (37%) of them had COPD. Of the 141 participants with COPD, 82 (57%) had chronic bronchitis, and among those with normal pulmonary function, 38% had chronic bronchitis.

Univariate logistic regression, with COPD as the dependant variable, showed that the variables age (OR 1.34), male sex (OR 1.2), pack-years (OR 1.73), chronic bronchitis (OR 2.26) and dyspnoea (OR 1.61) influenced the odds of having COPD. In the multiple regression model, smoking 31-40 pack-years (OR 3.05) and > 40 pack-years (OR 4.58) remained independently associated with COPD.
**Results**

**Paper II**

Out of the initial 512 subjects who were screened by spirometry for COPD in paper I, 22 were excluded due to long distance to the Health Care Centre and 45 due to participation in another study. Of the 445 included subjects, 35 were excluded, of which 25 did not want to participate (Figure 3). In total, 410 smokers were included for a follow-up.

Smokers with COPD had 32 pack-years, which was more pack-years in comparison with those with normal lung function, who had 24 pack-years ($p<0.001$) (Table 6). Smokers with COPD also smoked more, on average 19 cigarettes per day, in comparison with 16 per day among those with normal lung function.

In group A (smokers with normal lung function), at the follow-up at year 3, 144 of 165 (93 %) participated. In group B (smokers with normal lung function), 145 of 161 (90 %) participated in at least one of the yearly follow-ups and 120 (84%) participated in all 3 follow-ups (Figure 3). In group C, the COPD-group, 101 of 119 (85%) were examined more than once and 70 (59%) participated in all 3 follow-ups.

<table>
<thead>
<tr>
<th>Group A (n=165)</th>
<th>Group B (n=161)</th>
<th>Group C (n=119)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>47.6</td>
<td>47.6</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td>Male</td>
<td>74 (45%)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>91 (55%)</td>
</tr>
<tr>
<td><strong>Pack-years</strong></td>
<td>24 (SD 12)</td>
<td>24 (SD 10)</td>
</tr>
<tr>
<td><strong>Cig/day</strong></td>
<td>16 (SD 6)</td>
<td>16 (SD 6)</td>
</tr>
<tr>
<td><strong>FEV1% predicted (mean)</strong></td>
<td>98</td>
<td>95</td>
</tr>
<tr>
<td><strong>Excluded</strong></td>
<td>20 (12%)</td>
<td>16 (10%)</td>
</tr>
<tr>
<td><strong>Participants</strong></td>
<td>164 (99%)</td>
<td>145 (90%)</td>
</tr>
</tbody>
</table>

The point prevalence abstinence, smokers who were abstinent ≥ 30 days immediately prior to the annual visit, and the prolonged prevalence abstinence at 6 and 12 months were higher in group C compared with group B.
Results

At each annual visit (Table 7). At year 3, the point prevalence abstinence was significantly higher (p=0.001) among those with COPD compared to those in group B with normal lung function.

Table 7. Data for subjects who stopped smoking at the annual visit by study group. Group A and group B consist of smokers with normal lung function and group C consists of smokers with COPD. The table shows smoking cessation rates at annual visits throughout the study, point prevalence abstinence (smoking cessation ≥30 days), prolonged abstinence at 6 months and 1 year, and the mean time of being smoke-free in months. Mean number of smoking free months was calculated among those who were smoke free more than ≥30 days at years 1, 2 and 3.

<table>
<thead>
<tr>
<th>Follow-up</th>
<th>Group A (n=165)</th>
<th>Group B (n=161)</th>
<th>Group C (n=119)</th>
<th>Group B versus Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=present at annual visit</td>
<td>3 1 2 3</td>
<td>144 140 138 135</td>
<td>82 90 90</td>
<td>Year 3</td>
</tr>
<tr>
<td>Smoking free ≥30 days</td>
<td>24 (15%)</td>
<td>8 (5%) 14 (9%) 23 (14%)</td>
<td>22 (18%) 28 (24%) 35 (29%)</td>
<td>Chi-square p=0.003 z-test 3.92 p&lt;0.001</td>
</tr>
<tr>
<td>Smoking free ≥6 month</td>
<td>21 (13%)</td>
<td>6 (4%) 10 (6%) 15 (9%)</td>
<td>17 (14%) 25 (21%) 33 (28%)</td>
<td>z-test 3.93 p&lt;0.001</td>
</tr>
<tr>
<td>Smoking free ≥1 year</td>
<td>15 (9%)</td>
<td>4 (2%) 8 (5%) 12 (7%)</td>
<td>12 (10%) 24 (20%) 30 (25%)</td>
<td>Median test NS</td>
</tr>
<tr>
<td>Mean number of smoking free months</td>
<td>19,7</td>
<td>6,4 11,0 15,1</td>
<td>8,4 16,5 26,1</td>
<td>NS</td>
</tr>
</tbody>
</table>

At year 3, the prolonged prevalence abstinence rate (6 or 12 months) was significantly higher in group C than in group B, p<0.001 and p<0.001, respectively. There was no significant difference in prolonged prevalence abstinence between groups A and B at year 3. The P-value at year 3 and abstinent at least 12 months was 0.75. The mean number of smoke-free months for the participants who had stopped smoking at the last annual visit tended to be higher in group C, than in group B (p=0.097). Subjects in group C who stopped smoking used more nicotine replacement therapy and snuff than group B and group A (p<0.001 respectively), but there
Results

was no difference between groups A and B. For the duration of the study, 29% (n=34) of patients in group C used nicotine replacement therapy and 23% (n=27) used snuff.
Results

Paper III

Sixty smokers with normal lung function from the screening study (Paper I), were invited, with 59 consenting to participate in the study. Five smokers that had spirometry values in accordance with the definition of COPD were excluded.

In total, 54 subjects were included, 18 of whom fulfilled the definition of lower normal values of lung function (preclinical COPD). There were more males than females. Subject characteristics and anthropometric measurements are shown in Table 2. Subjects fulfilling the definition of lower normal values of lung function did not differ from the other smokers in terms of age, BMI, number of years of smoking or pack-years (Table 2).

Regarding dyspnoea, all but one subject rated his/her breathlessness between 0-2 on a ten-grade Borg scale. Reversibility after inhalation of four doses (0.4 mg/dose) of salbutamol was significantly higher in the group of smokers defined as lower normal values of lung function than in the group with higher normal values of lung function (p<0.05). Serum values of hs-CRP were within normal limits, with the median at 1.5 (range 0.28-27.5) mg/l, reference value<10mg/l and the median value of eosinophils was 0.13% (range 0.00%-0.78%), reference value 1%-4%. There were no differences between the groups regarding hs-CRP in serum and eosinophils in differential counts. Emphysema, was present by visual inspection in 43% (n=23) of the subjects. There was no difference in the occurrence of emphysema between the group of lower and the group of higher normal values of lung function (p= 0.85) (table 8).
The degree of emphysema was almost exclusively 3-4 (on a 5 grade scale) and did not differ between the groups (p=0.82). The type of emphysema was distributed as CLE-predominant in 43.5%, PSE-predominant in 43.5% and as an equal mixture of the two (CLE = PSE) in 13%, and did not differ between the group of lower normal values of lung function and the rest of the smokers (p=0.11) (Table 8). The quantitative measurements of HRCT attenuation values (quantitative emphysema) did not differ significantly either between the two groups (p=0.11). When the subjects were grouped according to the presence or absence of emphysema, BMI was found to be significantly lower in the group with emphysema (p=0.0011). No differences were found when comparing spirometric parameters, hs-CRP, eosinophils, smoking history or pack-years (Table 9).

Table 8. Findings of emphysema on HRCT in the group of smokers with lower normal values of lung function and the group with higher normal values of lung function. Grade and type of emphysema. None of the differences were statistically significant. >CLE: centrilobular emphysema alone or predominant; >PSE: paraseptal emphysema alone or predominant; CLE = PSE: centrilobular emphysema equal paraseptal emphysema.

<table>
<thead>
<tr>
<th>Lower normal values of lung function (n=18)</th>
<th>Higher normal values of lung function (n=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emphysema</td>
<td></td>
</tr>
<tr>
<td>8 (44%)</td>
<td>15 (42%)</td>
</tr>
<tr>
<td>Grade of emphysema</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Type of emphysema</td>
<td></td>
</tr>
<tr>
<td>&gt;CLE</td>
<td>6</td>
</tr>
<tr>
<td>CLE=PSE</td>
<td>1</td>
</tr>
<tr>
<td>&gt;PSE</td>
<td>1</td>
</tr>
</tbody>
</table>

The degree of emphysema was almost exclusively 3-4 (on a 5 grade scale) and did not differ between the groups (p=0.82). The type of emphysema was distributed as CLE-predominant in 43.5%, PSE-predominant in 43.5% and as an equal mixture of the two (CLE = PSE) in 13%, and did not differ between the group of lower normal values of lung function and the rest of the smokers (p=0.11) (Table 8). The quantitative measurements of HRCT attenuation values (quantitative emphysema) did not differ significantly either between the two groups (p=0.11). When the subjects were grouped according to the presence or absence of emphysema, BMI was found to be significantly lower in the group with emphysema (p=0.0011). No differences were found when comparing spirometric parameters, hs-CRP, eosinophils, smoking history or pack-years (Table 9).
Table 9. Subjects allocated according to findings on HRCT. Data showing mean age, gender, number of pack-years, BMI and lung function values. There were no significant differences except for BMI. Data are presented as mean and standard deviation (SD); % pred=percentage of predicted; Rev-B2=reversibility test with beta-2 agonist. T-tests were used for all variables and hs-CRP and eosinophils were log transformed before the t-test.

<table>
<thead>
<tr>
<th></th>
<th>Emphysema on HRCT</th>
<th>No emphysema on HRCT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=23)</td>
<td>(n=31)</td>
</tr>
<tr>
<td>Female, %</td>
<td>43</td>
<td>39</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52.7 (50.8-54.4)</td>
<td>52.3 (50.7-53.8)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.2 (22.8-25.7)</td>
<td>27.4 (26.2-28.6)</td>
</tr>
<tr>
<td>Smoking (years)</td>
<td>34.2 (31.6-36.8)</td>
<td>34.0 (31.4-36.6)</td>
</tr>
<tr>
<td>Pack-years</td>
<td>29.4 (24.7-34.0)</td>
<td>26.0 (21.4-30.6)</td>
</tr>
<tr>
<td>FEV₁ (% pred.)</td>
<td>103 (96-110)</td>
<td>103 (97-109)</td>
</tr>
<tr>
<td>FEF25 (% pred.)</td>
<td>104 (93-115)</td>
<td>106 (95-117)</td>
</tr>
<tr>
<td>FEF₇₅ (% pred.)</td>
<td>76 (64-88)</td>
<td>78 (68-89)</td>
</tr>
<tr>
<td>FEF₇₅ (% pred.)</td>
<td>52 (43-62)</td>
<td>56 (47-65)</td>
</tr>
<tr>
<td>FVC (% pred.)</td>
<td>110 (104-116)</td>
<td>109 (104-115)</td>
</tr>
<tr>
<td>FEV₁/FVC ratio (%)</td>
<td>77 (74-79)</td>
<td>77 (75-79)</td>
</tr>
<tr>
<td>FEV₁/FVC ratio (% pred.)</td>
<td>98 (96-101)</td>
<td>99 (97-101)</td>
</tr>
<tr>
<td>Reversibility test-β2-agonist (%)</td>
<td>3.5 (1.7-5.4)</td>
<td>4.2 (2.6-5.8)</td>
</tr>
<tr>
<td>HRCT, -950 HU (%)</td>
<td>1.096 (0.34-1.84)</td>
<td>0.665 (0.221-1.108)</td>
</tr>
<tr>
<td>Hs-CRP (mg/l)</td>
<td>2.05 (1.13-2.97)</td>
<td>3.43 (1.50-5.35)</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0.186 (0.117-0.254)</td>
<td>0.145 (0.107-0.182)</td>
</tr>
</tbody>
</table>

Paper IV

Subjects with COPD were distinguished from non-COPD subjects by being older (p<0.001), having exposed themselves to tobacco smoke for a longer period of time (i.e. smoke years, p<0.001), having a lower BMI (p<0.05) and...
lower FEV\textsubscript{1} % of predicted (p<0.001), and having a lower single-breath diffusion capacity of the lung for carbon monoxide (DLCO) (p=0.003) (Table 1). Subjects with COPD had significantly higher values of lysozyme in serum compared to non-COPD subjects (p<0.05), as well as in healthy non-smoking volunteers (p<0.01 (Table 10). There were no differences regarding values of hs-CRP, MPO or ECP between any of the study groups. Furthermore, we found values of serum lysozyme to be significantly higher in subjects with abnormally low DLCO relative to those with values of DLCO within normal limits (1373 (1125-3117) µg/L vs. 1083 (492-1842) µg/L, p=0.011). None of the tested markers in EBC, i.e. ECP, lysozyme and MPO, were detected except chlorine, which was significantly higher in patients with COPD (p<0.05) compared to healthy controls, but no differences were found compared with the non-COPD smokers or ex-smokers. The alpha-amylase used as saliva contamination marker could not be detected in EBC either.

Values of lysozyme in saliva was significantly higher in COPD patients (p<0.05) compared to non-COPD subjects but the highest values were found in the healthy non-smoking volunteers. Values of eosinophil cationic protein (ECP) in saliva was significant lower in COPD (p<0.01) than in non-COPD subjects, but no difference was found compared to healthy controls (Table 10). Generally the spread of data in saliva was considerable.

Inhaled corticosteroid treatment was considered as a possible confounding factor in our evaluations. Apart from being older (p<0.01), treated subjects also had lower FEV\textsubscript{1} % of predicted values (p=0.01), and lower DLCO (p<0.05) than non-treated subjects. Except for higher serum hs-CRP in the corticosteroid treated subjects, (p=0.03) data on biomarkers obtained from these two subgroups (i.e. corticosteriod treated vs non-corticosteriod treated) were not statistically different from each other. Ex-smokers were older (p<0.01) and showed more advanced airway limitation as reflected by lower FEV\textsubscript{1}/FVC (p<0.01) than those who continued to smoke.

Due to difficulty in the interpretation of DLCO data recorded in subjects with unstable airway walls, as suggested by a low value of FEF\textsubscript{50}, DLCO was not measured in all subjects. FEF\textsubscript{50} was significantly lower in those who had no data on DLCO [17 (12-37) % pred.] vs those who had DLCO measured [ 78 (12-172) % pred.] (p<0.001)]. Based on the degree of the fall in FEV\textsubscript{1} during the preceding five-year period, ten subjects were identified as "rapid decliners" according to previously recorded spirometry data. None of the tested
biomarkers or DLCO measured in the subjects classified as “rapid decliners” differed from those measured in re-examined subjects with stable lung function.

Table 10. Results of analyses conducted in EBC, serum and saliva, from 19 subjects with COPD, 30 non-COPD and 15 healthy volunteers. Data are present as median (min-max). Statistically significant differences are indicated by * (¶, Ω, Φ) = p<0.05, ** (¶¶, ΩΩ, ΦΦ) = p<0.01. Mann Whitney U-tests were used in statistical evaluations. ¶= COPD vs. non-COPD, Ω= COPD vs. healthy volunteers, Φ= non-COPD vs. healthy volunteers.

<table>
<thead>
<tr>
<th></th>
<th>COPD</th>
<th>non-COPD</th>
<th>healthy vol</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>19</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td>EBC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorine (μM)</td>
<td>13.3 (6.8-40)</td>
<td>10 (3.6-72.6)</td>
<td>7.8 (3.4-24)</td>
</tr>
<tr>
<td>Amylas, (U/L)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysozyme (μg/L)</td>
<td>1350 (863-3117)</td>
<td>1119 (492-1842)</td>
<td>1020 (603-1410)</td>
</tr>
<tr>
<td>MPO (μg/L)</td>
<td>74 (33-253)</td>
<td>76 (29-659)</td>
<td>105 (41-162)</td>
</tr>
<tr>
<td>ECP (μg/L)</td>
<td>5 (2-29)</td>
<td>5 (2-17)</td>
<td>6 (2-13)</td>
</tr>
<tr>
<td>Hs-CRP (mg/L)</td>
<td>2 (0.4-14)</td>
<td>1.3 (0.3-10)</td>
<td>1.2 (0.6-3.6)</td>
</tr>
<tr>
<td>Saliva</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysozyme (μg/L)</td>
<td>3333 (15-10094)</td>
<td>1206 (15-14816)</td>
<td>6292 (79-15866)</td>
</tr>
<tr>
<td>Chlorine (mM)</td>
<td>13.1 (6.8-56)</td>
<td>16.8 (6.4-24.4)</td>
<td>11.3 (6.8-22.0)</td>
</tr>
<tr>
<td>ECP (μg/L)</td>
<td>44 (2-214)</td>
<td>106 (2-282)</td>
<td>64 (7-270)</td>
</tr>
</tbody>
</table>
DISCUSSION

Prevalence of COPD with invitational targeted screening

Our study focused on a population with a known incipient risk of developing COPD, i.e. smokers 40-55 years old, with the intention to diagnose early, previously unknown COPD (Paper I). Our method for early detection of COPD, by means of an invitational process using advertisements in local media and placards to attract smokers 40-55 years old and offering them voluntary spirometry screening, showed a COPD prevalence of 27% (Paper I). The high prevalence of COPD in our study was most likely the result of the deliberate selection included in this invitational method. Our findings are comparable with other studies from different countries using invitational methods for early diagnosis of COPD.195,161,172, 177 The Polish studies by Zielinski et al. used local media (TV, radio broadcasts, and local and national press) to attract smokers aged ≥40 years with a history of ≥10 pack-years for spirometry test.161,195 The percentage of subjects having airflow limitation in that study was 23%, according to GOLD criteria. The lower percentage of subjects with COPD in that study may be explained by the fact that about 10% of the participants were lifelong non-smokers and about 5% were <40 years of age, an age where COPD is regarded as very rare. On the other hand, the interim report from this study showed that the prevalence of airway obstruction was 29.3% in smokers aged ≥40 years according to ERS criteria.195 The studies by Van Schayk et al and Vandevooorde et al which also used an invitational, targeted method during two consecutive months, offered spirometry to current smokers between 35-70 and 40-70 years of age respectively.150,172 Vandevooorde also had the inclusion of having a smoking history of at least 15 pack-years. The prevalence of COPD was 18% (defined as FEV1< 80% of predicted) and 29.5% respectively. Geijer et al. who performed a targeted screening on 702 smokers with a mean age of 50 years found airflow obstruction in 29.9% of the subjects.177 Table 11 shows the prevalence of COPD in different surveys using invitational methods (Table 11).
Table 11. COPD prevalence in surveys using different invitational, targeted screening.

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Used method</th>
<th>Age limits (years)</th>
<th>Screened population</th>
<th>Mean age</th>
<th>Definition of COPD</th>
<th>Prevalence of COPD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zielinski et al. Poland</td>
<td>11 000</td>
<td>Local media (radio and TV)</td>
<td>40-89</td>
<td>Smokers and ex-smokers</td>
<td>52</td>
<td>GOLD FEV%&lt;70%</td>
<td>30,6</td>
</tr>
<tr>
<td>Van Schayck et al. Netherlands</td>
<td>169</td>
<td>Invitation at the visit on PCC</td>
<td>35-70</td>
<td>Smokers</td>
<td>47</td>
<td>BTS (NICE) FEV%&lt;70% and FEV&lt;80% pred.</td>
<td>18</td>
</tr>
<tr>
<td>Geijer et al. Netherlands</td>
<td>702</td>
<td>Invitation by letter</td>
<td>40-65</td>
<td>Smokers (male)</td>
<td>50</td>
<td>GOLD FEV%&lt;70%</td>
<td>29,9</td>
</tr>
<tr>
<td>Vandervoorde et al. Belgium</td>
<td>146</td>
<td>invitation at the visit on PCC</td>
<td>40-70</td>
<td>Smokers and pack-years &gt;15</td>
<td>52</td>
<td>GOLD FEV%&lt;70%</td>
<td>29,5</td>
</tr>
<tr>
<td>Stratelis et al. Sweden</td>
<td>512</td>
<td>Local media (newspaper and placards at PCC)</td>
<td>40-55</td>
<td>Smokers</td>
<td>48</td>
<td>ERS FEV%pred&lt;88 (male) FEV%pred&lt;89 (female)</td>
<td>27</td>
</tr>
</tbody>
</table>

Notes: Different criteria used for definition of COPD. GOLD=Global Initiative for Chronic Obstructive Lung Disease; BTS=British Thoracic Society; NICE=National Institute for Health and Clinical Excellence; ERS=European Respiratory Society

Epidemiological studies, using the same ERS criteria showed lower prevalence of COPD. In the study by Pena et al. the prevalence of COPD was 15% in smokers between 40-69 years. Data from the Spanish epidemiological study (IBERPOC) by Pena et al. which consisted of a population survey of subjects aged 40–69 years, showed that spirometrically confirmed COPD was present in 9.1 % of that targeted population. The prevalence was 14.3% in men and 3.9% in women, which reflected the smoking habits. The used definition for COPD in the IBERPOC study was by ERS 1995.

In the random general population based study in the northern part of Sweden, (OLIN-studies) the total prevalence of COPD in subjects aged 23–72 years was
Discussion

7.6% according to the BTS definition, whilst it was 14.0% and 14.1% respectively according to the ERS and GOLD definitions. The prevalence of COPD in the age group <45 years was 4.1%, 11.6% and 9.1% respectively, and in the age group ≥45 years the corresponding figures were 9.7%, 15.4% and 17.1% respectively.

In another population based study in the northern part of Sweden for those aged 46-77 years by Lundbäck et al., the prevalence was 8.1% and 14.3% according to the BTS and GOLD criteria respectively. Amongst smokers, the prevalence of COPD according to BTS was 5% in those aged 46-47, 24% in the age group 61-62 years and 45% among the oldest, with the figures according to the GOLD criteria being 11%, 42% and 50% respectively.

In the past, most studies have shown that COPD prevalence was higher among men than women. These gender differences are probably due to the higher prevalence of cigarette smoking in men. The study by Lundbäck et al. showed that the prevalence of the disease is almost equal in men and women, which in all probability reflects changing patterns of tobacco smoking during the last decades. These studies, which all use a spirometric definition of COPD, showed a high prevalence of COPD. The prevalence of COPD in smokers increased almost linearly from the age of 47 years.

The severity of COPD in our study was classified as mild in 85%, moderate in 13 % and severe in 2% according to the ERS classification (Paper I). This is in accordance with other studies. The severity of COPD in the study by Geijer et al. was classified as stage I (mild) in 87% and stage II (moderate) in 13% according to the GOLD definition. The study by Vandevenoorde displayed 42% of smokers with COPD as stage I, 49% as stage II and 9% as stage III according to GOLD definition. When applying the classification by GOLD in our study, 29% of the smokers would be classified as having COPD, 63% as stage I, 35% as stage II and 2% as stage III.

According to a Swedish population based epidemiological survey of smokers fulfilling the criteria for COPD and having mild airflow limitation, (FEV₁≥80% of predicted) 21% were classified as having chronic bronchitis. In our study (Paper I), where the majority of COPD smokers (85%) had mild COPD, the prevalence of chronic bronchitis was 44%, about twice as high as in the epidemiological survey, although that study consisted of middle-aged and elderly subjects. The higher prevalence of chronic bronchitis in our study is probably due to the inherent selection of the used invitational process i.e. selection of smokers with a high prevalence of symptoms. The high rates of
Discussion

dyspnoea shown by 178 (35%) participants during physical activity, is probably explained by the same phenomenon.

In smokers, there are at least six factors associated with the probability of having COPD. The factors are old age, male sex, high pack-years, chronic cough, objective wheezing and dyspnoea. In our study, factors associated with COPD were old age (OR 1.34), male sex (OR 1.2), higher pack-years (OR 1.73), having chronic bronchitis (OR 2.26) and suffering from dyspnoea (OR 1.61). This was in accordance with other studies, both epidemiological and cross-sectional.179,108,114,150,172

To some extent smoking habits and COPD are associated with socio-economic factors, since people with low-levels of education and low income have been shown to be more likely to smoke.21,28,196 We observed that only 7% of the participants (paper I) had college education and 8% received early retirement pension, the majority of cases due to problems with the musculoskeletal system.

Aspects on targeted screening of COPD

Screening requires a systematic approach at different levels and in different ways depending on what you want to screen for. The importance of identifying smokers with COPD at an early stage and supporting smoking cessation is unquestionable, and this is recognised in several national and international guidelines.15,16,197 However, COPD remains relatively unknown to the public.

Out of the responders in paper I (n=512), knowledge of the disease COPD or any labeling synonymous with COPD, (i.e. emphysema; smokers’ lung), was acknowledged by 39%. In a primary care study in Spain only 8.6% of the population surveyed had any spontaneous knowledge about COPD.169 Furthermore, in our study only 10% of all smokers and 11% of those with diagnosed COPD, had planned to consult primary care some time in the future outside the study, according to the questionnaire.

Smokers often consider symptoms of the disease such as “smokers cough” or breathlessness as normal for a smoker, and are therefore reluctant to seek medical advice, which may cause patient delay.198 This low level awareness of the disease combined with the inherent adaptation to the subtle symptoms of the disease in early stages results in a considerable degree of patient delay.
This systematic approach can be done at various levels including national, regional, local, or personal levels. With this in mind, the method used in paper I and the different methods used by others border on screening, i.e. targeted screening of a high-risk population.\textsuperscript{195,150,172} A problem with spirometric measurements performed in primary care settings could be that the practice assistants in primary care can not attain the same low misclassification rates as experienced pulmonary function technologists can at physiology laboratories, or trained nurses at the hospital.\textsuperscript{199} The consequences would be a risk for misclassification of smokers, declaring smokers with COPD as healthy and vice versa, or simply obtaining unacceptable test results. In Sweden however, in the majority of primary care centres, there is a dedicated, specially trained nurse, called an asthma/COPD nurse who performs the spirometry tests. Even if the objective of paper I was not to measure the quality of spirometries done by the asthma/COPD nurses in primary care, of the initial 160 obstructive spirometries performed by nurses in our study, 13 (8\%) were misclassified. In the Dutch study by Geijer et al the percentage of unacceptable spirometry curves was only 12.8\%.\textsuperscript{177} In another Dutch study by Schermer and co-workers designed to investigate the validity of spirometric tests performed in general practice, the results of the study indicated that, on average, the validity and quality of spirometric tests in general practices is satisfactory in comparison with the “gold standard” procedure, a spirometric test performed in a pulmonary function laboratory.\textsuperscript{200} The proportion of non-reproducible tests was 16\% for laboratory tests and 18\% for general practice tests.

**Smoking cessation**

The purpose of identifying smokers with COPD at an early stage (Paper I) is to motivate them to stop smoking, as smoking cessation is the only intervention that has been proven to prevent further decline in lung function.\textsuperscript{4,118,201} It is impossible to isolate the role and effect of spirometry alone (the technical procedure by itself) on smoking cessation because spirometry is much more than just a test procedure. Concerning the smoking cessation rates in paper II, the intervention that theoretically could influence smoking habits a year after the screening process, was the screening spirometry by the nurse and the confirmatory spirometry by the physician of those with pathological spirometry, and brief smoking
cessation advice to all participants (Paper I). This was in line with other studies. Consequently, spirometry in the context of this thesis (paper I, II) includes these components. The studies by Gorecka et al. and Bednarek et al. also included volunteers responding to the offer of free spirometric testing combined with brief smoking cessation advice. One study searched computerised patient records from five general practices, and sent a letter of invitation to relevant people to participate in the study.

In paper II, the intervention at the other annual visits was spirometry performed by a nurse and brief smoking cessation advice as well as a personal letter by the doctor informing any changes in the results of the lung function (FEV1), i.e. better, unchanged or worse, in comparison to the previous year’s results. Each letter also included a couple of standardized sentences regarding the health benefits of successfully quitting smoking.

At the first visit one year after the screening process, 10% of the smokers with COPD were continuously abstinent from smoking for a period of 12 months compared to 2% of smokers with normal lung function (Paper II). Other studies of the benefits of combining spirometry with smoking cessation advice have reported different cessation rates.

In the study by Segnan et al., spirometry combined with smoking cessation advice resulted in a smoking cessation rate of 6.5% which was not significantly different from the 4.5% sustained (1 year) cessation rate in smokers who received only smoking cessation counseling, or only minimal smoking cessation intervention. In the study by Górecka et al., the 12 months cessation rates in smokers with COPD (after the screening process) was generally 10.1% versus 8.4% in smokers with normal lung function, but 16.5% in smokers with moderate and severe COPD. In the study by Bednarek et al. which used the same design as our study and the study by Górecka et al., (screening spirometry by invitation and brief smoking cessation advice) the sustained (12 months) smoking cessation rate in those with COPD was 16.3% compared with 12% in those with normal lung function.

Parkes and colleagues assessed the effect of telling patients over 35 years of age their estimated spirometric “lung age” in combination with brief smoking cessation advice as an incentive to quit smoking. In contrast, the results were given to the control group as raw spirometric values FEV1 (litres per second and not as predicted values). The 12 months carbon monoxide quit rates in the intervention group was 13.6% compared with 6.4% in the control group.
The point-prevalence abstinence rate (cross-sectional quit rates) is usually high in those kinds of studies. For the first annual visit in our study, the point-prevalence abstinence rate was 18% in smokers with COPD and 5% in smokers with normal lung function. In the large Lung Health Study, where spirometry was combined with an intensive behavioural-intervention program, which consisted of a 12-session smoking cessation group meeting during a 10-week period guided by a health educator and nicotine replacement therapy, the point prevalence abstinence rate in those smokers with COPD was 35%.\(^{118}\)

In different studies relapse prevention with behavioural support, nicotine gum or other pharmacotherapy up to six months after stopping smoking is a common procedure. To my knowledge, the Lung Health Study is the only study with a long-term design consisting of several follow-up visits aimed at preventing relapses. The study had 4-monthly interval check-ups that included teaching coping skills for problems such as stress, and encouraging compliance. In addition, the study had annual follow up visits for a duration of 5 years with spirometry and smoking status check-ups. This intervention resulted in a smoking cessation rate of 22% during a 5 year follow up.

In our study, where the relapse prevention consisted of spirometry and brief smoking cessation advice by the nurse and a personal letter by the doctor once a year, the smoking cessation rate (smoking free ≥1 year) at year 2 and 3 was 20% and 25%, respectively, in smokers with COPD compared with 5% and 7% in smokers with normal lung function. At year 3 the median smoking free period was 26.1 months.

**Emphysema on HRCT**

It is obvious that COPD often has its roots decades before the onset of symptoms.\(^{206}\) Given the knowledge of the natural course of COPD, it seems logical that the inflammatory process in smokers predisposed to developing clinical COPD has been going on for many years, resulting in some degree of micro-emphysematous tissue destruction and gradual deterioration of lung function before the obstruction reaches the spirometric definition of COPD.\(^{142}\) Early detection of smoking-induced lung damage would be useful for secondary prevention of COPD since awareness of early harmful effects may promote smoking cessation before a more advanced disease develops.
Preclinical COPD (lower normal values of lung function) and emphysema on HRCT

With the support of relevant literature we defined a state of preclinical COPD as lower normal values of lung function, when spirometry results showed a forced expiratory flow at 50% of Vital Capacity (FEF₅₀ ≤ 60% of predicted) or when FEV₁/FVC ratio (FEV%) was 89-93% of predicted for males and 90-93% of predicted for females. ¹⁴²,²⁰⁷ Eleven percent had preclinical COPD according to our definition (paper I). The smokers with preclinical COPD were presumed to be a group of at high risk of developing COPD.

Emphysema seen on High Resolution Computed Tomography (HRCT) was used as the gold standard for proof of parenchymal damage due to cigarette smoke (paper III). By visual analysis, HRCT showed signs of emphysema in 23 (43%) of the 54 smokers with a mean age of 53, who according to international guidelines all had normal lung function (paper III). When applying the definition of preclinical COPD, the proportion of emphysema was similar in that group of smokers. On a five-grade scale, the degree of emphysema was almost exclusively 3-4 and did not differ between the groups. In comparison, a study found a similar occurrence of emphysema (44%) in healthy symptom free smokers but with a higher mean age of 60 years. ⁹⁸ Additionally, a lower prevalence of emphysema (20%) was found in smokers with a mean age of 33. ⁹⁷ In a smaller study consisting of 26 smokers and ex-smokers with a mean age of 52, emphysema was found in 34,6% of the subjects. ²⁰⁸

Neither of the quantitative computerized measurements of HRCT attenuation values differed significantly between the preclinical COPD group and the group of smokers. Other authors studying emphysema have also found discrepancies between the subjective (visually) and objective (computer attenuated measurements) occurrence of mild emphysema. ²⁰⁹,²¹⁰ Vikgren et al. who examined 55 healthy male smokers, 61-62 years old, found that 49% had emphysema visually, but neither the densitometric nor the reconstruction algorithm had the adequate ability to detect mild emphysema.

Body Mass Index

It was found that the BMI was significantly lower in relatively young and healthy smokers who suffered from emphysema (p=0.0011), than in those who
did not suffer from emphysema (paper III). This is an interesting observation since their spirometry values were within the normal predicted range. The reason for the low BMI in our studied subjects is unclear. Besides the typical pulmonary pathology in COPD due to cigarette smoking, unexplained weight loss is a known and clinically relevant problem in patients with spirometrically confirmed severe to very severe COPD.\textsuperscript{65,66} An accepted explanation of weight loss in established COPD is excess energy expenditure due to the increased energy cost of breathing,\textsuperscript{75,77} and atrophy of skeletal muscle.\textsuperscript{82,83} In the study by Sandek et al. patients with moderate to severe COPD, with a mean FEV\textsubscript{1} of 38.2\% (±15.5) of the predicted value had a reduced BMI correlated with emphysema.\textsuperscript{211} Systemic inflammation could be a potential pathogenic factor that could explain the loss of weight in our subjects, even though our study failed to find a difference in hs-CRP values between those with low and those with high BMI.\textsuperscript{47} Compartmentalization might be a possible explanation since a local inflammation in the pulmonary compartment may not necessarily be detected by measurements of C-reactive protein in the serum. In smokers with very severe emphysema however, prominent neutrophilic inflammation in the alveolar walls and air spaces have been shown.\textsuperscript{40} In the systemic review by Gun et al, all five selected studies regarding CRP showed that patients with COPD had higher levels of CRP than healthy control subjects.\textsuperscript{55}

**Markers of inflammation**

It is acknowledged that the airway inflammation in smokers with COPD is dominated by neutrophils, monocytes and macrophages.\textsuperscript{212} It is now also recognized that COPD is a disease not only restricted to airways and manifested as emphysema and airflow limitation, but also as a systemic disease.\textsuperscript{63,64} Easy diagnosis of early preclinical COPD in smokers at risk of developing COPD would be the optimal diagnostic tool. One hypothesis is that biomarkers in certain body fluids, such as serum, saliva and exhaled breath condensate could be useful in the early detection of tobacco smoke elicited airways disease, and could therefore be used to diagnose the disease.

**Clinical implications of biomarkers in the blood**

The notion of increased neutrophil activation in our subjects with COPD was suggested by significant increases in serum of lysozyme, a granular protein
originating from circulating neutrophils and monocytes. Subjects with COPD had significantly higher values of lysozyme in serum compared to non-COPD subjects (p<0.05) or healthy volunteers (p<0.01) (paper IV). Although the lysozyme in the serum of subjects with COPD was significantly higher, the spread of the values was large, which is why one has to interpret the results with caution. The statistical significances may display a coincidence. Even if our findings de facto is supported by a number of observations on neutrophil inflammation involvement in airways in patients with COPD, there are yet no highly apparent biomarkers in the blood which could be used as indicators for early identification of subjects who will develop symptomatic COPD.49,213,214

Granulocyte peroxidases, such as myeloperoxidase in neutrophils, are supposed to play an important role in the generation of oxidative stress in COPD and may serve as a marker for ongoing COPD inflammation.215 Our study (Paper IV), showed no differences in myeloperoxidase measurements between COPD subjects, non-COPD subjects and healthy non-smoking volunteers. These findings are in contrast to previously published data by Andelid et al. on elevated serum concentrations of MPO in male smokers with mild airflow limitation.216 On the other hand, one large study was in support of our results by showing no significant relation between COPD and MPO.217 We found no significant difference in hs-CRP between smokers with COPD and the other groups (Paper III and IV). In the systematic review by Gun et al. which evaluated the association between COPD and systemic inflammation there was a heterogeneity in the results between the 5 remaining included studies on CRP.55 In this systematic review the authors found that compared with healthy controls, individuals with COPD had significantly raised levels of CRP, although the standardised mean difference in the CRP level between COPD and control subjects was only 0.53 units.

Clinical implications of biomarkers in EBC and saliva

Exhaled breath condensate has received much attention as a new non-invasive technique for measuring markers of airway inflammation. We found the results from analyses of exhaled breath condensate disappointing, since only chlorine was detected in exhaled breath condensates. (Paper IV). There was however no difference in chlorine levels recorded in exhaled breath condensate obtained from COPD and non-COPD subjects, but levels were significantly higher in those with COPD relative to healthy volunteers (Paper IV) (Table 10). Despite a low inter-assay variability of biomarkers, we found
large within-subject variability of chlorine concentrations obtained from healthy volunteers. The range of concentrations of markers was large and may display statistical significances due to coincidence.

Large variability in concentrations of various compounds in exhaled breath condensate has also been observed in other studies, while others showed rather high reproducibility. The reasons for failing to detect other biomarkers in exhaled breath condensate include the fact that the biomarkers are in low concentrations, close to the limit of detection. Water vapor represents at least 99% of the exhaled breath condensate contents.

The clinical implication of chlorine in exhaled breath condensate concerning COPD is uncertain since the anatomical origin of exhaled breath condensate from airway mucosa has not been determined and since the chloride ions, the source of chlorine, are more associated with mucosal glands in large, rather than peripheral airways where the inflammation of COPD takes place. We also investigated the presence of biomarkers in saliva, and although there were significant differences regarding lysozyme and eosinophil cationic protein we found that even here the spread of data was very large and the results contradicting with exhaled breath condensate, which makes clinical interpretations impossible (Paper IV).

The reason for not being able to identify any discriminating inflammatory biomarkers in exhaled breath condensate or saliva in our subjects could be because the study population was too small, with low statistical power as a consequence or, and most likely, the ELISA and RIA techniques were not sensitive enough for detection of the compounds.

**Rapid decliners**

The normal age-dependent decline of FEV\textsubscript{1} in non-smokers is approximately 25 ml/year whereas the decline of FEV\textsubscript{1} in smokers with COPD has been reported to be 50-100 ml/year. Smokers with a rapid decline in FEV\textsubscript{1} are supposed to be at risk of developing tobacco smoke related lung disease. Based on the degree of the fall in FEV\textsubscript{1} during the preceding five-year period, ten out of thirty five subjects were identified as “rapid decliners” (decline in FEV\textsubscript{1} of at least 350 mL). Neither the tested biomarkers in blood or values of DLCO differed rapid decliners from those measured in re-examined subjects with stable lung function (Paper IV). However, other research groups have shown that MPO and falls in DLCO correlate with a decline in FEV\textsubscript{1} and this...
supports the hypothesis that neutrophils may have a key role in progressive inflammation and pathogenesis of COPD.213,222

Despite the low number of participants in our study, we analysed ROC curves with the aim to further search for tests or markers capable of discriminating between COPD and non-COPD subjects. DLCO was the strongest discriminator (AUC ROC 0.85), and serum value of lysozyme was the second strongest discriminator (AUC ROC 0.76) of COPD.

Strengths and limitations

The strengths of the study were that the five participating PHCCs with their respective asthma/COPD nurses worked as a functional unit to perform the screening spirometry and spirometries at follow-up visits. The follow-up of the included smokers was good since at year 3: in group A, 93 % participated; in group B, 90 % participated in at least one of the yearly follow-ups and 84% participated at all 3 follow-ups; in group C, the COPD-group, 85% were examined more than once and 59% participated at all 3 follow-ups.

The study in paper III could be criticized for not having a control group consisting of healthy never-smoking subjects. However, several studies have shown that emphysema is rarely found in lifelong non-smokers, independent of the age of the examined population.97,98,100,104 A limitation could also be the small samples in paper III and IV, but the intention of those works was explorative.

The accuracy of COPD diagnosis

Theoretically, some of the 141 with diagnosed COPD in paper I may have had asthma. This is however not very likely since 90% did a reversibility test with β2 agonists, and out of the 6 smokers with diagnosed asthma in the study all could be diagnosed after the β2-reversibility test.

Recall bias

The information about the subjects’ smoking habits and pack-years in paper I and paper II contain both recall and response bias. Though a recall bias on previous smoking history is known (mainly under-estimating previous smoking), it is considered reliable to use self-reported smoking statuses in
Calculation of pack-years is often used as a method of quantifying the burden of smoking on an individual level, and there is no alternative method than to ask about that. “How many years have you smoked in total, and how many cigarettes/day do you smoke/have you smoked?” We adjusted the pack-years for the time periods of their lives when they did not smoke.

**Selection bias**

In epidemiological studies response rates and high participation is of great importance in order to avoid selection bias. The aim of our study (Paper I) was not to include all eligible smokers from the studied population area. Our study was designed to strive for a selection. The higher the selection, i.e. high prevalence of COPD of the screened smokers, the better the method would be to detect COPD early.

**Smoking cessation**

The subjects in our study were all volunteers and therefore may not be representative of all smokers (Paper I). They also showed an interest in their own health by presenting themselves to the program (Paper II), and thus were probably more motivated to quit than other smokers. The results in our study may be limited to patients with mild COPD, since 85% of those with COPD had mild COPD. There is however, evidence that there is a negative correlation between lung function and likeliness to quit smoking. In one study smokers with worse lung function were more likely to quit smoking.\(^{175}\) All smokers in these 3 groups however, had the same motivation when they entered the study (Paper I). Two of the groups were given the same intervention, i.e. annual visits with a spirometry performed by a nurse, brief smoking cessation advice and a personal letter by the doctor (Paper II).

**Response bias**

The cessation rates may be somewhat over-estimated, since data on smoking was self-reported and not conferred by a biochemical method. However, a study has indicated a deception rate of only 3% in both the control and the intervention group.\(^{205}\) Furthermore, the rate of follow-up was lowest in the COPD group, which indicates that it was smokers who did not manage to stop
smoking who did not attend the annual visit. In calculating our results, we chose to use the intention to treat model. This may under-estimate our results, but was judged to have the closest similarity to daily practice. We did not include a group of subjects who had never smoked to see if emphysema was present (Paper III). It has previously been shown that non-smokers generally do not develop emphysema. In the studies by Tylen et al. and Vikgren et al, which included considerably older subjects, emphysema was diagnosed in only one out of 32 (3%) and none out of 26 subjects who had never smoked. It could be criticised that only one experienced thoracic radiologist evaluated the HRCT, however, previous studies have shown that inter-observer variation for assessment and grading of emphysema is low.
Conclusions

- The used invitational, targeted screening method is feasible in primary care, and attracts smokers with a high prevalence of previously undetected COPD, of which the vast majority were found to have the early, mild form of the disease (Paper I).
- Spirometry in combination with brief smoking cessation advice as part of screening for COPD showed significant prolonged smoking cessation rates for smokers with COPD compared to smokers with normal lung function. A higher number of follow-ups with annual spirometry and brief smoking cessation advice increased the smoking cessation rate (Paper II).
- A high number of middle-aged smokers with normal lung function had emphysema (Paper III).
- High prevalence of emphysema was associated with low body mass index (Paper III).
- The EBC method as a tool to measure exhaled inflammatory biomarkers associated with COPD is uncertain, either because of too low concentrations of biomarkers in EBC or low sensitivity of the assays (Paper IV).
- Although the significantly higher lysozyme concentrations in the serum of subjects with COPD suggests involvement of neutrophil cell activity, the spread of the values was large, which is why the results are not conclusive (Paper IV).
- Single breath test for diffusion capacity (DLCO) appears to be the strongest discriminator between COPD and non-COPD subjects (Paper IV).

Clinical aspects and implications

It seems important to increase the public knowledge and awareness of COPD since the disease is more or less still unknown to the general public according to our questionnaire (Paper I). COPD is currently underdiagnosed due to either patient’s or doctor’s delay. By inviting smokers and offering office spirometry, the method could improve early detection of COPD (Paper I). However, in addition it should be important to offer all smokers visiting
primary care spirometry even if they do not consult for respiratory tract symptoms.

Early detection of smoking-induced lung damage would be useful for secondary prevention of COPD. The diagnosis of COPD could increase the efficacy of smoking cessation advice in affected subjects by a positive incentive to stop smoking, and thereby prevent further decline in lung function (Paper I,II). Repeated communication of the harmful effects of smoking and the health benefits of stopping smoking may be a useful strategy in generating quitting attempts, resulting in improved smoking cessation rates in smokers with COPD (Paper II). A structured COPD-surgery with a dedicated nurse and increased availability to a combination of spirometry and brief smoking cessation advice to smokers with COPD should be a priority in getting more COPD patients to stop smoking (Paper II).

From a general point of view, high resolution computed tomography might detect emphysema in smokers with normal spirometry and before clinical symptoms have developed (Paper III). This illustrates that a state of “preclinical COPD” in smokers may exist, since emphysema was a common finding in middle age smokers. Preclinical COPD seems to be more common than we hypothesized, as emphysema was equally common in subjects with lower normal values of lung function and in the group of subjects with higher normal values of lung function. The identification of those at risk of developing clinical COPD could be a potential use of HRCT, but the future clinical relevance is unclear without long-term follow-up. Furthermore, one must take into consideration that HRCT still is a relatively expensive diagnostic tool, and not easily accessible.

It would be valuable to identify an inflammatory marker, or a lung function test that could predict very early on which smokers are at higher risk of developing COPD (Paper III-IV). The studied exhaled breath condensate as an easy non-invasive tool to measure exhaled inflammatory biomarkers in COPD did not yield any diagnostic markers. Further studies are required to understand the manner in which these biomarkers are generated and how they should be analysed (Paper IV). There is an association between emphysema and low BMI (despite normal lung function), which may be due to systemic inflammation. Further studies are required to confirm and understand this association.
SUMMARY IN SWEDISH


Lindrig KOL har sällan symptom förutom att rökaren kan besväras av kronisk bronkit, dvs. hosta och slem. Ibland kan det förekomma viss andnöd vid hårdare ansträngning. Vid fortsatt försämring av sjukdomen blir andnöd det vanligaste symtomet. I sin mera avancerad form (svår KOL) blir sjukdomen invalidiserade med andnöd redan vid lättare uppförslut och vid gång på plan mark, och ett ökat hjälp- och sjukvårdsbehov adderas. Det är av stor betydelse att upptäcka KOL tidigt och hjälpa rökaren till rökstopp eftersom endast rökstopp stoppar den snabba förlusten av lungfunktionen. KOL diagnostiseras enkelt med spirometri (lungfunktionstest) men någon nationell screeningprogram av rökare för tidig upptäckt av KOL saknas.

Det övergripande syftet med avhandlingen var att undersöka metoder för tidig upptäckt och sekundär prevention av KOL i primärvården. Vi ville utvärdera om en invitativ metod (screening by invitation) för tidig upptäckt av KOL var genomförbar och värdera prevalensen av KOL med denna metod. Dessutom ville vi undersöka om utförd spirometri och kännedom om den nedsatta lungfunktion (KOL) som en funktion av screeningen kunde medföra att rökare slutade röka och om årlig spirometri samt kort rådgivning om rökstopp kunde påverka rökvänorna ytterligare. De övriga målen med avhandlingen var att undersöka förekomsten av emfysem hos normal lungfunktion och hos rökare med normal lungfunktion men som ligger inom det lägre spirometriska normalintervallet och således nära gränsen för KOL och undersöka om vissa biomarkörer i utandningskondensat, blod, saliv eller diffusionsskapacitet skulle kunna identifiera rökare med KOL eller rökare som ligger i risk för KOL.
Delarbete I (Early detection of COPD in primary care: Screening by invitation of smokers aged 40 to 55 years. Br J Gen Pract 2004; 54: 201-206.).

Studien ville undersöka en “invitativ” riktad screeningmetod för tidig upptäckt av KOL hos rökare i åldersgruppen 40-55 år. Via annonsering i lokal massmedia och affischering på vårdcentraler erbjuds rökare i denna åldersgrupp att komma till sin vårdcentral och gratis testa sin lungfunktion. Studien genomfördes på 5 vårdcentraler i västra Östergötland och en vårdcentral i Jönköpings län. Totalt anmälde sig 512 rökare. Screeningspirometrierna utfördes hos astma/KOL sköterskorna i primärvården. Alla patologiska spirometri (nedsett lungfunktion) skickades till huvudansvarig som gjorde om spirometrierna 1 månad senare för bekräftelse. Om spirometrin var patologisk även vid detta tillfälle utfördes reversibilitetstest med luftrörsvidgande läkemedel (β2-agonist) och per oral kortisontest under två veckor för att utesluta astma och annan orsak till den nedsatta lungfunktionen.

Av de 512 som undersöktes hade 141 (27,5%) KOL och 6 st (1%) hade astma. Vid klassificering av svårighetsgraden befanns 85% ha lindrig KOL, 13% medelsvår och 2% svår KOL. Av frågeformuläret framgick att endast 39% av rökarna hade kännedom om sjukdomen KOL eller andra synonyma termer såsom emfysem och att endast cirka 10% hade någon gång i framtiden för avsikt att söka sjukvården för kontroll av lungfunktionen. Konklusionen av studien var att den invitativa riktade screeningmetoden fångar upp hög andel av rökare med KOL i tidig fas av sjukdomen.

Delarbete II (The impact of repeated spirometry and smoking cessation advice on smokers with mild COPD. Scand J Prim Health Care 2006; 24: 133-39.).

Fyrahundratio av de 512 rökare som ingick i delarbete I inkluderades i studie II. Rökarna med normal lungfunktion delades in i 2 grupper, grupp A och grupp B. Rökarna med KOL utgjorde grupp C. Grupp B och C kallades årligen under 3 år för spirometri och kort rådgivning om rökstopp. Grupp A kallades in för ny kontroll endast sista 3:e året. Vid första kontrollen 1 år efter screeningspirometriin var 10% av rökarna med KOL rökfria, grupp C (rökfria >1år) medan de med normal lungfunktion hade slutat att röka i 2%. Vid sista kontrollen var 25% av rökarna med KOL rökfria (i genomsnitt 26 månader) medan de med normal lungfunktion hade slutat att röka i 7% (i genomsnitt 15 månader). Grupp A hade slutat att röka i 9%. Konklusionen av studien var att rökstopp var signifikant högre hos rökare som efter en screeningspirometri och kort rådgivning om rökstopp fick veta att de har KOL i jämförelse med rökare som hade normal lungfunktion och att årlig
spirometri plus kort samtal om rökvanor ökar andelen rökare med långvarigt rökstopp.

**Delarbete III** (*High prevalence of emphysema and its association with BMI: A study of smokers with normal spirometry. Scand J Prim Health Care 2008; 26:241-7*). Femtiofyra rökare med per definition normal lungfunktion (varav 18 av de 54 hade lungfunktion inom det lägre spirometriska normalintervallet, definierad i studien som preklinisk KOL) från studie I ingick i denna studie. Rökarna undersökt med högupplösande datortomografi (HRCT) och spirometri. Resultaten av studien visade att emfysem i lungorna fanns totalt hos 43% av rökarna som enligt alla guidelines bedömdes vara spirometriskt friska i sina lungor. Förekomsten av emfysem skilde sig inte mellan rökare med normal lungfunktion och dem som hade subnormala värden (lungfunktion inom det lägre spirometriska normalintervallet). Efter omgruppering av rökarna, emfysem och ej emfysem på HRCT fann vi att body mass index (BMI) var signifikant lägre hos rökare med emfysem. Konklusionen av studien var att en stor andel av medelålders rökare med normal lungfunktion har emfysem i lungorna som tecken på rökrelaterade lungskador och att emfysem var associerad till lågt BMI.

**Delarbete IV** (*Can we predict development of COPD? Submitted in October 2008*). Nitton patienter med KOL, 30 rökare och ex-rökare med normal lungfunktion från en primärvårdsställning och 15 friska, icke-rökande frivilliga ingick i denna studie. Inflammatoriska markörer mättes i blodet, utandningskondensat och saliv. Dessutom utfördes spirometri och diffusions kapacitet av lungorna. Resultaten antyder att serum lysosym, en biomarkör för ökad neutrofil och monocytt aktivitet var signifikant högre hos rökare med KOL jämfört med rökare utan KOL. Utandningskondensat som ett verktyg för att fånga upp biomarkörer vid KOL var en besvikelser då inga av våra biomarkörer förutom klor kunde mätas i kondensten. Diffusionskapacitet tenderade att vara bästa undersökningen att diskriminera mellan KOL och ej-KOL. Konklusionen av studien är att utandningskondensat som ett verktyg för att fånga upp biomarkörer vid KOL är osäker och behöver utvecklas vidare och att trots att det fanns vissa signifikanta skillnader i biomarkörerna i serum och diffusionskapaciteten så är resultaten osäkra pga. att spridningen av resultaten är stor samt att studiematerialet är litet.
ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to all those who have generously contributed to this thesis. In particular I would like to express my deepest gratitude to:

Professor Sigvard Mölstad my tutor for his excellent scientific guidance, supervision and patience.

PhD Per Jakobsson and professor Olle Zetterström my co-tutors for excellent remarks during the process of the studies and on the manuscripts.

My co-researcher and co-author associated professor Birgitta Schmekel for excellent work with the examinations at the department of physiology and excellent remarks on the manuscripts in paper III and IV.

My co-researcher and co-author associated professor Sven-Göran Fransson for excellent work with the radiologic evaluation of emphysema by high resolution computed tomography and excellent remarks on the manuscript in paper III.

My co-researcher and co-author Anette Davidsson in paper IV.

The asthma/COPD nurses: Gunilla Jansson, Birgitta Carlborg, Thurid Thoor Kerstin Jonsson, Annakarin Eriksson, Marian Areland, Karin Eriksson and Birgitta Wennblad who performed the pulmonary function tests in primary care (Paper I and II).

To all patients who willingly participated in the studies and made this thesis possible.

To Elisabeth Lundborg, Mats Fredriksson and Leif Jörgensen for helping me with statistics.

Anders Niklasson and Bo Björklund former and present head of Brinkens health care centre and the other colleagues and staff for valuable support and interest in my work.
Summary in Swedish

To my wife, Therese, for her patience and support when finalizing this thesis. To my children, for being just children, giving me inspiration, energy and strength.
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