Emphysema and airway wall thickness assessed by quantitative computed tomography

- relation to respiratory symptoms and lung function

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Acknowledgements

It's a long way to Tipperary (1). The thesis you are now reading is the result of several years of hard scientific work, frequently interrupted by shorter and longer periods of not so hard work, strictly scientifically speaking. But not so scientifically speaking, clinical duty and the production of three boys, and the associated paternity leaves are also considered hard work by some. In the 6.5 years that has passed since I first started this project, I have experienced both the ups and downs of scientific work. There have been days of pure scientific joy, after getting a paper accepted or after having solved a particularly difficult problem. And I have very much enjoyed the opportunity to travel the world on scientific meetings together with colleagues that are also considered friends. But there have also been days of quite the opposite, after receiving yet another rejection, or experiencing a particularly long-drawn fit of writers block. Luckily, I'm already starting to forget the downs, and I'm very pleased to have experienced the ups.

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Project summary

Aims

1 – To quantify emphysema (%LAA, percentage Low Attenuation Areas) and airway wall thickness (AWT-Pi10, Airway Wall Thickness in a standardized airway with an internal perimeter of 10 mm) in ever-smoking COPD and non-COPD subjects using quantitative CT analysis, and to determine how these anatomic measures varied with gender, age and smoking habits.

2 – To describe the relationship between respiratory symptoms of COPD and quantitative CT measures of emphysema and airway wall thickness, and to assess how these relationships interacted with COPD-status, gender, age and smoking history.

3 – To examine the relationship between the diffusing capacity of the lung and quantitative CT measures of emphysema and airway wall thickness, and to assess how these relationships varied by COPD status, gender, age and smoking history.

Methods

The subjects included in the current study were all participants in the GenKOLS study, and constitute the approximate half of the GenKOLS population that received an optional CT scan. A total of 463 COPD subjects (65 % men) and 488 non-COPD subjects (53 % men) were included in this study. In paper I we excluded 57 non-COPD subjects (volunteers) from the analyses, and in paper III we excluded 175 COPD subjects and 63 non-COPD subjects from the analyses due to missing or invalid D_LCO measurements. All included subjects were current or ex-smokers older than 40 years. They underwent spirometry (Vitalograph 2160), diffusing capacity tests (SensorMedics Vmax22D) and CT examination (GE LightSpeed Ultra), and completed multiple questionnaires, including an ATS questionnaire on respiratory symptoms. The CT images were quantitatively assessed (Emphylx-J software), giving indices on lung density and airway dimensions.

Results

1 – Median (25, 75-percentile) %LAA was 8.9 (2.8, 19.1) and 4.7 (1.5, 15.5) in male and female COPD subjects, and 0.71 (0.32, 1.58) and 0.32 (0.14, 0.84) in male and female non-COPD-subjects. %LAA was higher in ex-smokers and increased with increasing age and with increasing number of pack years. Mean (SD) AWT-Pi10 (mm) was 5.04 (0.30) and 4.74 (0.31) in male and female COPD subjects, and 4.88 (0.28) and 4.63 (0.25) in male and female non-COPD subjects. AWT-Pi10 decreased with increasing age in cases, and increased with the degree of current smoking in all subjects.

2 – Both %LAA and AWT-Pi10 were independently and significantly related to the level of dyspnea among COPD subjects, even after adjustments for FEV_1 in % predicted. AWT-Pi10 was significantly related to cough and wheezing in COPD subjects, and to wheezing in non-COPD subjects. Odds ratios (95% confidence limits) for increased dyspnea in COPD subjects and non-COPD subjects was 1.9 (1.5, 2.3) and 1.9 (0.6, 6.6) per 10 % increase in %LAA, and 1.07 (1.01, 1.14) and 1.11 (0.99, 1.24) per 0.1 mm increase in AWT-Pi10, respectively.

3 – Multiple linear regression analyses showed significant associations between D_LCO and both %LAA and AWT-Pi10 in the COPD group. The adjusted regression coefficients (SE) for D_LCO were -1.15 (0.11) mmol $\cdot$ min^{-1} $\cdot$ kPa^{-1} per 10% increase in %LAA and 0.08 (0.03) mmol $\cdot$ min^{-1} $\cdot$ kPa^{-1} per 0.1 mm increase in AWT-Pi10, and the adjusted R^2 of the models were 0.65 and 0.49, respectively.

Conclusions

1 – There were significant differences between COPD and non-COPD subjects in quantitative CT measures of emphysema and airway wall thickness. Gender, age and smoking history also had strong effects on these quantitative CT measures and must be considered when comparing quantitative CT studies.

2 – Quantitative CT measures of emphysema and airway wall thickness were significantly and independently associated with respiratory symptoms, and may be used to explain the presence of respiratory symptoms beyond the information offered by spirometry.

3 – Quantitative CT measured emphysema was highly related to both diffusing capacity and diffusing coefficient in COPD subjects, and this relationship was even stronger in men. There was also a positive, but not equally strong, relation between CT measured airway wall thickness and diffusing capacity, and this was contrary to our hypothesis that there would be no relationship between airways and diffusing capacity. Both CT measures provide valuable information about the lungs not readily available from spirometry and diffusing capacity alone, but the modest explained variation attributable to the airway measurements warrants further studies.

Terms and abbreviations

%LAA	Percentage Low Attenuation Areas. A measure of the degree of emphysema. Indicates the relative amount of lung voxels that has a density less than the given cut-off. The most frequently used cut-off is -950 HU (%LAA-950), but other cut-offs are also in use (e.g. -910 HU, -856 HU, etc). If nothing else is specified, %LAA should be interpreted as %LAA-950.
AWT-Pi10	A standardized measure of Airway Wall Thickness at an Internal Perimeter of 10 mm. This measure is calculated by plotting the square root of the measured wall area against the internal perimeter of each individually measured airway, and then using the resulting regression line to calculate the resulting square root of the wall area of a «theoretical airway» with an internal perimeter of 10 mm.
COPD	Chronic Obstructive Pulmonary Disease
CT	Computed Tomography
DICOM	Digital Imaging and Communications in Medicine. A standard for distributing and viewing any kind of medical image.
D_LCO	Diffusing capacity of the Lung for carbon monoxide
D_LCO / V_A	Diffusing capacity of the Lung for carbon monoxide divided by the estimated alveolar volume. The equivalent of KCO.
FEV_1	Forced Expiratory Volume in one second
FVC	Forced Vital Capacity
GenKOLS	Genetic COPD study
GOLD	Global initiative for Obstructive Lung Disease

HCRHS	Hordaland County Respiratory Health Survey
HU	Hounsfield Units. A density scale, ranging from -1000 HU (the equivalent of air) to +1000 HU (the equivalent of dense bone). Water has a density of 0 HU.
HUH	Haukeland University Hospital
Inflation Level	CT measured lung volume divided by the predicted TLC
IVC	Inspired Vital Capacity
kVp	Kilo-Volt peak. Beam energy.
mAs	Milliampere seconds. The product of the tube current and time.
MRCDS	Medical Research Council Dyspnea Scale
OR	Odds Ratio
Pack-years	Number of cigarettes smoked per day divided by 20, and multiplied with the number of years smoked.
Pi	Internal perimeter
PI type	Protease Inhibitor type. Used to classify different forms of A1AT-defiency
RV	Residual volume
SOHAS	Second Oslo and Hordaland Asthma Survey
TLC	Total Lung Capacity
V_A	Estimated Alveolar Volume

Voxel	A three dimensional pixel, or a box. The size of a voxel is decided by the resolution of the image and the slice thickness. A voxel is the smallest part of the lung where individual density measurements can be made using quantitative CT.
WA%	Wall Area Percent. The percentage of the measured total airway area that is made out of wall, and not lumen.

List of publications

Paper I

Quantitative computed tomography: emphysema and airway wall thickness by sex, age and smoking

Grydeland TB, Dirksen A, Coxson HO, Pillai SG, Sharma S, Eide GE, Gulsvik A, Bakke PS.

Eur Respir J. 2009 Oct;34(4):858-65. Epub 2009 Mar 26.
PMID: 19324952

Paper II

Quantitative computed tomography measures of emphysema and airway wall thickness are related to respiratory symptoms

Grydeland TB, Dirksen A, Coxson HO, Eagan TM, Thorsen E, Pillai SG, Sharma S, Eide GE, Gulsvik A, Bakke PS.

Am J Respir Crit Care Med. 2010 Feb 15;181(4):353-9. Epub 2009 Nov 19.
PMID: 19926869

Paper III

Quantitative CT measures of emphysema and airway wall thickness are related to D_LCO

Grydeland TB, Thorsen E, Dirksen A, Jensen R, Coxson HO, Pillai SG, Sharma S, Eide GE, Gulsvik A, Bakke PS.

Respir Med. 2011 Mar;105(3):343-51. Epub 2010 Nov 11.
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Introduction

About COPD

Chronic obstructive pulmonary disease (COPD) is a major cause of chronic morbidity and mortality throughout the world. It was ranked as the sixth most common cause of death worldwide in 1990, and is projected to become the third leading cause of death by 2020 (2).

The main characteristic of COPD is chronic airflow obstruction, and the predominant symptoms are chronic and progressive dyspnea, cough, and sputum production. COPD is usually caused by cigarette smoking, which is the best-studied risk factor, but several other risk factors have been identified (2). These factors include environmental tobacco smoke (passive smoking), indoor and outdoor pollution, occupational dusts and chemicals, and several genetic risk factors, including α_1-antitrypsin deficiency.

Previous COPD definitions have distinguished between different types of COPD, and as illustrated in Figure 1, there is a considerable overlap between the different types of COPD (3, 4).

Asthma is a chronic inflammatory disorder of the airways and cannot be classified as COPD as long as the airflow obstruction is completely reversible (5). Chronic bronchitis is defined by a chronic productive cough for three months in each of two successive years in a patient in whom other causes of chronic cough have been excluded (4). Emphysema is defined by abnormal and permanent enlargement of the airspaces that are distal to the terminal bronchioles, accompanied by destruction of the airspace walls, and without obvious fibrosis (4). As illustrated by the Venn diagram, all three conditions may exist independently or concurrently, and they may or may not result in chronic airway obstruction.

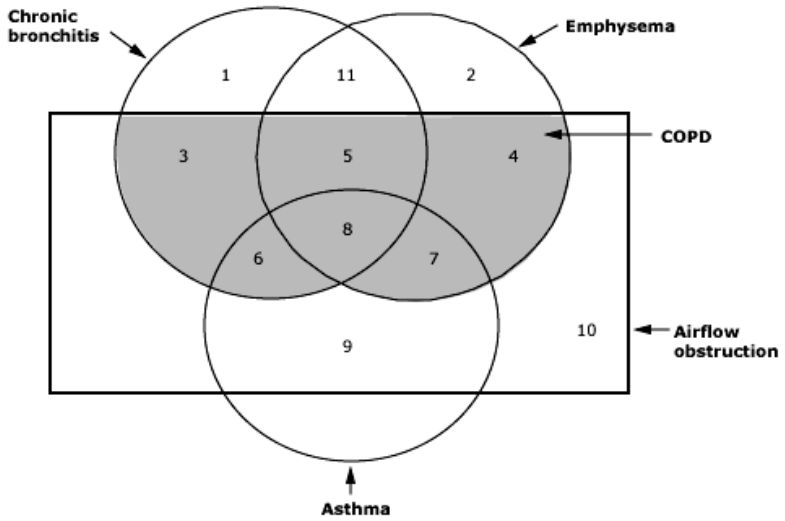

Figure 1 *Non-proportional Venn diagram showing subsets of patients with chronic bronchitis, emphysema, and asthma (black circles). The subsets defined as COPD are shaded gray. The numbers simply indicate the different subsets, and will not be referred to in this thesis. (Taken from ATS. Standards for the diagnosis and care of patients with COPD, 1995 (3))*

It has become clear that COPD is a complex and heterogeneous disease, and that the described Venn diagram should not be taken too literally (6). It has been a useful way of illustrating the different components of COPD, but it is by no means complete. For instance, it does not include small airway disease, while most of the airflow obstruction is due to reduced airflow in the small conducting airways (7). The discussion about how COPD should be divided into various subgroups or phenotypes is ongoing in the scientific community (8).

Today, the most widely used and accepted COPD guidelines is The Global Initiative for Chronic Obstructive Lung Disease (GOLD) (2). GOLD defines COPD as follows: *"COPD is a preventable and treatable disease with some significant extrapulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal*

inflammatory response of the lungs to noxious particles or gases." The previous distinction between asthma, emphysema and chronic bronchitis is not included in the current GOLD definition. Instead it focuses on *a not fully reversible airflow obstruction*, and specifies that the chronic airflow limitation characteristic of COPD is caused by a mixture of small airway disease (obstructive bronchiolitis) and parenchymal destruction (emphysema). The relative contributions of these subtypes vary from person to person.

The chronic airflow limitation in COPD is best measured by spirometry, because this is the most widely available, reproducible test of lung function. The GOLD guidelines recommend that COPD is classified into four stages of severity based on spirometry, ranging from mild to very severe (Table 1).

Table 1 *Classification of COPD severity based on post-bronchodilator FEV_1 and FVC*

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

This classification provides a simple and useful clinical tool for both diagnosis and treatment of COPD, but the cut points are not clinically validated. Especially the use of the fixed FEV_1/FVC-ratio of 0.70 has been debated, and it has been shown that the use of this fixed ratio can lead to an over-diagnosis of COPD in the elderly (9).

The FEV_1 % predicted, as reflected in the four GOLD stages, defines the severity of COPD, but to the individual patient the respiratory symptoms are more important. The characteristic symptoms of COPD are chronic dyspnea, cough and sputum production. The level of dyspnea also corresponds reasonably well to the reduction in FEV_1, but chronic cough and sputum production may precede the development of airflow limitation by several years. And conversely, the patients may develop significant airflow obstruction without any cough or sputum.

Hence, spirometry is not the only instrument needed to assess and monitor COPD. A thorough physical examination and a detailed medical history, including risk factors, respiratory symptoms and co-morbidities are important. A reversibility test may be useful, but cannot reliably differentiate COPD from asthma (10). Arterial blood gas measurements may be important in the more severe stages of COPD that has started to develop respiratory failure. Genetic testing may explain COPD in never-smokers with α_1-antitrypsin deficiency. Lung diffusing capacity may help differentiate between COPD and other interstitial lung diseases, and may also say something about the predominant subtype of COPD (emphysema or small airway disease). Chest X-ray and CT thorax are also widely used, and will be discussed in the next section.

Radiological approaches to COPD

X-rays was discovered by W.C. Roentgen in 1895. Initially its medical use was limited to identifying bone structures, but since then it has been increasingly used as a medical diagnostic method. Conventional plain X-ray of the chest has for many years been used to assess respiratory diseases, including COPD. An abnormal chest X-ray is seldom diagnostic in COPD unless obvious bullous disease is present, but it can be valuable in excluding alternative diagnoses and establishing the presence of significant comorbidities, such as cardiac failure and lung tumours.

Conventional X-ray technology is limited in that the image it produces is a shadow of all the structures in the body that the X-rays pass through. All these structures become superimposed on each other as they are projected on the film or X-ray sensor, and as a

result each single plane blurs the others. To avoid this, Mayer proposed a method known as tomography in the early 1900s, and this was further refined by Vallebona, Bocage and Grossmann (11). The idea was based on the principles of projective geometry. By moving the X-ray tube and the film (inter-attached by a rod) synchronously and in opposite directions, one could produce an image which was sharper in the focal plane (corresponding to the rod's pivot point), while the images from the other superimposed planes would be more blurred. Although tomography was limited by the fact that the absence of blurring occurred in one plane only, it continued to be an important part of radiologic diagnostics for many years, until the development of computed tomography.

The mathematical theory behind computed tomography was also developed in the first half of the 20th century (H.A. Lorentz 1905, J.H. Radon 1917). It was shown that the distribution of material properties in an object layer can be calculated if the integral values along any number of lines passing through the same layer are known. But it was first in the 1970s the development of modern computer technology and the transverse axial scanning method made possible a new approach. And in 1979, G.N. Hounsfield and A.M. Cormack were awarded the Nobel Prize in Medicine in recognition of their important role in the development of computed tomography.

Since the arrival of the first clinical CTs in the 1970s, there has been a tremendous development. The first single-slice units could only do head-scans, had low resolution (80x80 pixels) and were very slow in terms of both scanning and data processing. Today's CTs can scan the whole body, have a much higher resolution and are much faster using multi-slice (128) and dual-source technology. As a result of this ongoing development, the newest CTs can scan the whole thorax well within one breath-hold, and using much lower radiation doses than before.

With regard to COPD, a CT of the chest is not routinely recommended. However, when there is doubt about the diagnosis of COPD, a high-resolution CT scan might help in the differential diagnosis. It is also an important tool to determine the amount and distribution of emphysema and the surgical suitability for lung volume reduction.

Quantitative CT

COPD is characterized by abnormal lung function and structure. Pulmonary function testing, and spirometry in particular, has evolved to become a reliable and safe method to measure the severity and progression of the impaired lung function associated with COPD. A similar assessment of the anatomic and structural changes associated with COPD has until recently not been possible.

Conventional CT uses the density measurements from the scan to produce images that are available for interpretation by a radiologist, yielding semi-quantitative data. The advent of quantitative CT of the chest has made it possible to use the same density measurements to produce quantifiable data on airways and parenchyma. Instead of categorizing emphysema as mild, moderate or severe based on a radiologist's assessment, quantitative CT yields a more accurate and precise measure of lung density and emphysema on a continuous scale. Similarly, instead of a radiologist's description of the presence or absence of bronchiectasis or slightly thickened airway walls, quantitative CT accurately measures the dimensions of both lumen and wall in a large number of airways throughout the lungs.

Over the last few years there has been a considerable development of the technical and methodological aspects of quantitative CT and a growing number of clinical studies have used quantitative CT in their assessment of COPD. But unlike spirometry, quantitative CT has not been fully standardized. There is no general consensus on which of the many possible quantitative CT algorithms or measures that are best at quantifying the airway and parenchymal lesions that are characteristic of COPD. With regard to emphysema, there are several different density cut-offs that are used to calculate the percentage of low attenuation areas, and the most frequently used is -950 HU. In addition, many centres measure the density at a given percentile instead, and the most frequently used is the 15^{th} percentile. With regard to the airways, there are also many different approaches, focusing on both wall area and wall thickness, and there is also a discussion about which airways and which generation of airways to measure.

Relevant literature prior to the current study

In 1988 Gould *et al*. (fifth percentile) and Müller *et al*. ("density mask" at -910 HU) described each their quantitative CT technique, and found good correlation with morphometric measurements of emphysematous pathologic lung sections (12, 13). They also demonstrated that the quantitative lung density measurements were correlated with airflow limitation (FEV_1) and diffusing capacity (13-15). In 1996 Gevenois *et al*. showed that a threshold cut-off value of -950 HU had the best correlation with extent of emphysema (16). Other groups pursued the percentile method, and found that 15^{th} percentile provided the best estimate of the extent of emphysema, especially in subjects with α_1-antitrypsin deficiency and in longitudinal studies (17, 18). These two methods of assessing the extent of emphysema are basically not very different, as they both present the same data in slightly different ways, and there are no big controversies between these two approaches.

The quantitative assessment of airways, on the other hand, has generated more controversy (19). In 1997 McNitt-Gray *et al*. found that airways could be accurately measured using a density cut-off of -500 HU (20), and this was followed up King *et al*. in 2000, using a threshold of -577 HU (21). However, while this method was very good at assessing the airway lumen, it was not very good at assessing the airway wall. The development of the "full width at half maximum" technique solved some of these problems, but has a tendency to overestimate the airway wall area and underestimate the lumen area (22, 23). To overcome these obstacles, several other approaches have been developed, including the "maximum-likelihood method" and the "score-guided erosion algorithm", but no single method has emerged as superior to the others (19). A frequently used way to present the airways measurements is the Wall Area Percent (WA%), which is the percentage of the total airway area that is wall. In 2000 Nakano *et al*. showed that WA% in the right apical segmental bronchus correlated with FEV_1, FVC and RV/TLC (23), and later Nakano *et al*. showed that WA% measured by CT correlated with the wall area measured histologically in the same subjects (24). These findings indicated that the airflow limitation in the small airways (< 2 mm), that cannot be measured by CT, can be assessed using the larger airways (>2 mm) that are

available to quantitative CT measurements. There is however a potential problem with WA%. The smaller the airway, the larger the relative wall area (WA%). To avoid this potential bias, Nakano also plotted the square root of the airway wall area against the internal perimeter of that airway for both CT and histologic measurements, and found good correlation between the two (24).

Thus, at the start of the current study, there were several studies showing that different quantitative CT measures correlated well with both anatomic and functional measures of the lung. But the majority of these studies had small sample sizes and was focused on the methodological aspects. There was limited knowledge on how these quantitative CT measures varied with gender, age and smoking history, and how they related to pulmonary function and respiratory symptoms, but some data had been published:

Gevenois *et* al. looked at the effects of age, sex, lung size and hyperinflation on CT lung densitometry in 1996 (25). They found only a small age-effect, and no gender-effect, but there were only 42 healthy subjects included in the study. In a longitudinal follow-up study of 83 subjects in 2000, Soejima *et al*. also found that quantitative CT measured emphysema increased with age, and more so in smokers, but the study was too small to assess the gender effect (26). Nakano *et al.* found that quantitative CT measurements of both airways and emphysema correlated with pulmonary function (spirometry and diffusing capacity) in 114 smokers, but did not take age, gender or smoking into account (23). In 1999 Park *et al*. reported that emphysema assessed using the -950 HU threshold correlated well with FEV_1 and D_LCO, and better than the -910 and -900 HU thresholds (27). This study comprised 60 subjects, and was also too small to assess gender, age and smoking effects. In 2001 Dowson *et al*. reported that quantitatively assessed emphysema (-910 HU) correlated with health status assessed by St. George's respiratory questionnaire in 125 subjects with α_1-antitrypsin deficiency (28). No studies had looked directly on the relationship between quantitative CT and respiratory symptoms to give quantitative CT measures a clinical correlate.

The above mentioned references are not complete, but illustrate the limited available knowledge concerning quantitative CT assessment of emphysema and airways when this study was initiated. While there was reasonable agreement that quantitative CT measures were correlated with pulmonary function, the technique was not standardized, and there were many unsolved questions (29):

- Disagreement on optimal approaches/techniques, especially with regard to airways
- No established normal reference values
- Limited validation against phenotypic expressions of COPD other than spirometry
- Lack of large studies to assess the effect of gender, age and smoking history in both COPD patients and healthy subjects

The current study was at the time of initiation the largest available study sample with quantitative CT data on lung density and airway dimensions. We hypothesized that gender, age and smoking history would have significant effects on the quantitative CT measures of emphysema (%LAA) and airway wall thickness (AWT-Pi10). We also hypothesized that %LAA would be strongly related to dyspnea score and D_LCO, and that AWT-Pi10 would be strongly related to respiratory symptoms like cough and phlegm.

Aims of the study

The objectives of this thesis were:

1) To quantify emphysema and airway wall thickness in ever-smoking COPD and non-COPD subjects using quantitative CT analysis, and to determine how these anatomic measures varied with gender, age and smoking history.

2) To describe the relationships between respiratory symptoms of COPD and quantitative CT measures of emphysema and airway wall thickness, and to assess how COPD-status, gender, age and smoking history interacted with these relationships.

3) To examine the relationship between the diffusing capacity of the lung and quantitative CT measures of emphysema and airway wall thickness, and to assess how these relationships varied by COPD status, gender, age and smoking history.

Materials and methods

Study design and study population

The subjects included in the current study were all participants in the GenKOLS-study, and constitute the approximate half of the GenKOLS population (951 of 1909) that received an optional CT scan.

The GenKOLS-study was performed at Haukeland University Hospital (HUH) between 2003 and 2005, and was a case/control study designed to look for genetic associations with COPD. GenKOLS recruited potential cases and controls from four different sources:

- Hordaland County Respiratory Health Survey (HCRHS)
- Second Oslo and Hordaland Asthma Survey (SOHAS)
- Haukeland University Hospital COPD registry (HUH COPD registry)
- Volunteers

HCRHS is a large epidemiological cohort study that was performed in Hordaland County, initiated in 1985, and followed up in 1996/97 (30, 31). All subjects from HCRHS that were still living in Bergen and surrounding communities in 2002 were invited to participate in another follow-up of the HCRHS. The HCRHS subjects who were eligible for inclusion in GenKOLS were also invited to participate in GenKOLS.

SOHAS is a cross-sectional population study that was performed in the city of Oslo and Hordaland county in 1998 (32). The HUH COPD registry comprised all patients registered with a diagnosis of COPD or emphysema in the hospital records between 1997 and 2005. Volunteers were people who contacted the study staff and expressed an interest to participate in the study.

Table 2 GenKOLS *study sources and response rates (percent of above)*

	HCRHS		SOHAS		HUH COPD reg		Volunteers		Total	
Invited to participate in GenKOLS	1238		812		2456		291		4797	
Attending Haukeland Hospital	881 (71%)		484 (60%)		1328 (54%)		291 (100%)		2984 (62%)	
	Case	*Ctr*	*Case*	*Ctr*	*Case*	*Ctr*	*Case*	*Ctr*	*Case*	*Ctr*
Included as case/control in GenKOLS	115 (13%)	492 (56%)	71 (15%)	242 (50%)	697 (52%)	73 (5%)	71 (24%)	148 (51%)	954 (32%)	955 (32%)
Included cases/controls with CT	82 (71%)	366 (74%)	30 (42%)	65 (27%)	325 (47%)	32 (44%)	26 (37%)	25 (17%)	463 (49%)	488 (51%)

The table above (Table 2) shows how many subjects from each source that were invited, how many that attended a clinical examination at HUH (GenKOLS screening) and finally how many that were included in GenKOLS and subsequently in the quantitative CT study.

Inclusion and exclusion criteria

Inclusion criteria

- Able and willing to sign an informed consent form.
- Age ≥ 40 years.
- Current or ex-cigarette smoker, minimum 2.5 pack years.
- No evidence of severe α_1-antitrypsin deficiency (ZZ, Z Null, Null-Null, or SZ) assessed by PI type.
- Caucasian − self reported.
- Cases: Diagnosed COPD, GOLD II or worse.
  $FEV_1/FVC < 0.7$, $FEV_1 < 80\%$ (post bronchodilator)
- Controls: Not diagnosed with COPD.
  $FEV_1/FVC > 0.7$, $FEV_1 > 80\%$ (post bronchodilator)

Exclusion criteria

- Unable to give informed consent.
- Severe anaemia as defined by haemoglobin of ≤ 9.0 g/dl.
- Known HIV, hepatitis B or C infection.
- Blood transfusion received within last 4 weeks.
- Chronic pulmonary disorder other than COPD (e.g., lung cancer, sarcoidosis, active tuberculosis, and lung fibrosis). Inactive tuberculosis and previous diagnosis of asthma were not an exclusion criterion.
- Status post-lung or other organ transplantation.
- Status post-lung volume reduction surgery.
- Taken antibiotics for respiratory disease within 1 month or have had a respiratory infection within 6 weeks of the visit.

To be included in the study the subjects had to fulfill all inclusion criteria and no exclusion criteria. However, subjects fulfilling transient exclusion criteria such as a recent respiratory infection or low hemoglobin were eligible for a reconsideration of inclusion at a later date.

Study sequence and data collection

First, the potential study participants from each source were identified. Then, the volunteers and the subjects originating from SOHAS and the HUH COPD registry were screened by telephone interview (Appendix A), to ensure that they were aged 40 years or older, that they were current or ex-smokers with at least 2.5 pack years and that they were willing to participate in the study. During the telephone interview they were also informed about the GenKOLS study, and if they were willing to participate they were given an appointment for a screening visit and clinical examination at HUH. Those willing to participate were subsequently sent a letter (Appendix B, C) containing a confirmation of the appointment, a screening consent form, a screening questionnaire and a description of the GenKOLS study. Eligible subjects (age>40 years, minimum 2.5 pack-years) originating from HCRHS were not screened by telephone, but sent a letter (Appendix B, C) directly containing a description of the GenKOLS study and why they were invited, a screening consent form, a screening questionnaire and an appointment for a screening visit and clinical examination at HUH.

At the screening visit, an extensive description of the study was given to all attending participants, and informed written screening consent was signed. The study staff ensured that the screening questionnaire was correctly completed, and recorded weight, height, waist hip measurements, vital signs and breath sounds on all participants. Then pre- and post-bronchodilator spirometry was performed on all participants.

Based on the post-bronchodilator spirometry and the screening questionnaire, the subjects were then identified as cases or controls in the GenKOLS study, according to

the inclusion and exclusion criteria described. The participants originating from HCRHS that did not meet these criteria were still offered to be included in the longitudinal follow-up of HCRHS using the already obtained screening data.

The subjects that were eligible for inclusion as cases or controls then signed a written case-control study consent form (Appendix D), and completed a larger case-control questionnaire (Appendix E). Then measurements of diffusing capacity and whole body impedance were performed, and blood samples were drawn for biochemical and genetic analyses. The cases and controls were also offered an optional high resolution CT scan for quantitative analyses, to be taken the same day or on a separate later visit. This optional scan was offered until a total of approximately 1000 CTs were acquired, equally distributed between cases and controls.

Questionnaires

All invited subjects were sent the short screening questionnaire (Appendix C). Only included cases and controls were asked to complete the more extensive case-control questionnaire. This questionnaire consisted of 35 pages and more than 200 questions concerning demographics, medical history, family history, smoking history, occupational history and more. The case-control questionnaire is too large to be printed in this thesis. Instead, only the questions that were actually used in papers I-III are given in this thesis. The selected questions from the case-control questionnaire concerned demographics, smoking history, respiratory symptoms and co-morbidities. The exact wording of these questions (not demographics) is given in Appendix E, both in English and Norwegian translation.

Lung function measurements

Spirometry

Spirometric measurements were recorded at the screening visit, using a Vitalograph 2160 Gold Standard Plus (Appendix F), and according to the American Thoracic Society standards (3). All testing equipment was calibrated daily using a Vitalograph 1

liter precision syringe, and a log of the calibration results was maintained. Local reference values for FEV_1 and FVC were used (33).

Subjects were assessed at least 6 weeks after any respiratory infection. Subjects were not asked to withhold regular medication (including bronchodilators), but recent use of bronchodilators was recorded. The pulmonary function tests were supervised by trained technicians, and the subjects were sitting upright using a nose clip to avoid leakage.

Three acceptable forced expiratory maneuvers were recorded, and the highest pre bronchodilator FVC and FEV_1 values were selected. After the baseline spirometry, the subjects were given 400 µg of Ventoline (salbutamol) via a metered dose inhaler and an Aerochamber spacer. 30 minutes after the administration of salbutamol, another three acceptable forced expiratory maneuvers were recorded, and the highest post bronchodilator FVC and FEV_1 values were selected. These post bronchodilation measurements were used for inclusion/exclusion and GOLD classification of the participants, and also as an adjustment factor in the multiple regression analyses.

Diffusing capacity

The single breath diffusing capacity of the lung for CO (D_LCO) was also recorded at the screening visit. We used a SensorMedics V_{max} Spectra 22D (Appendix G) for these measurements, and the system was calibrated daily. We followed the recommended ATS guidelines (34) for D_LCO measurements with one exception. This concerned the criterion that IVC should not be less than 90% of the largest previously measured FVC, and this topic will be discussed later under Methodological Aspects.

We used a gas mixture of 0.3% carbon monoxide, 0.3% acetylene, 0.3% methane, 21% oxygen and nitrogen. Estimated alveolar volume (V_A) was measured from the single breath dilution of methane, and the diffusion coefficient (D_LCO/V_A) was calculated by dividing D_LCO by V_A. Local reference values for D_LCO, V_A and D_LCO/V_A (35) were used.

The subjects were sitting upright and using a nose clip during the measurements, and the tests were supervised by trained technicians. After 4-5 registered tidal volumes, the end-expiratory baseline was determined, and the subjects were then asked to exhale fully. When full exhalation was reached, the subjects made a rapid maximal inhalation, and continued to hold their breath for 10 seconds, before exhaling rapidly. Up to 4 maneuvers were performed in order to obtain 2 error free tests, and there was a minimum interval of 4 minutes between each maneuver.

Computed tomography

CT image acquisition

The high resolution CT images were acquired at Haukeland University Hospital using a GE LightSpeed Ultra CT scanner (Appendix H). The images were taken at suspended full inspiration, and without spirometric gating. A high resolution axial scan was performed, using 120 kVp and 200 mAs, and a slice thickness of 1.25 mm taken at 20 mm intervals from the apex to the base of the lungs. The images were reconstructed using a low spatial frequency algorithm (standard) for the lung density measurements, and using a high spatial frequency algorithm (bone) for the airway measurements. The smallest Field Of View that included both lungs was used. The CT images were then stored on Magnetic Optical Disks (DICOM 3.0 format), and shipped to Vancouver, Canada, for quantitative assessment.

A local radiologist (at HUH) reviewed all CT images for signs of clinically significant abnormalities. A total of 118 subjects were referred for a repeated CT scan (contiguous) due to such abnormalities, and then through the national health care service for further follow-up. The majority of these abnormalities proved to be harmless, but 3 subjects were diagnosed with lung cancer as a result of this process.

Quantitative assessment

The quantitative analysis of the CT images was performed by the iCapture Centre in Vancouver, BC, Canada, under the supervision of Dr. Harvey Coxson (UBC James Hogg Research Centre and Vancouver General Hospital).

All the CT images were first assessed by two independent radiologists, yielding semi-quantitative data on the extent, distribution and type of emphysema, as well as the possible presence of bronchiectasis. The radiologists also reported any clinically significant abnormalities, and if these were not already discovered by the local radiologist at Haukeland University Hospital, the subjects in question were referred through the national health care service for further follow-up. These semi-quantitative data has not been the focus of this thesis.

The DICOM 3.0 format CT image data was imported into Emphylx-J (36), a graphics-based lung analysis program for quantitative analysis of thoracic CT scans developed by the iCapture Centre.

The software automatically segmented the lungs from the chest wall, the trachea and central airways and the mediastinum using a modified border tracing algorithm with prior position knowledge (37). This segmentation was shown visually to the operator, who could check the segmentation slice by slice. Any segmentation errors could then be corrected by the operator by adding, subtracting or reclassifying the segmented areas. After the correct lung segmentation was established, the software used the X-ray attenuation values from the CT images to calculate the lung density, lung volume, lung mass, regional expansion and surface area to volume ratios. Based on operator input, the software also calculated the percentage of low attenuation areas with a density lower than a chosen density cut-off (density mask). All of the above values were calculated for each slice, for each lung and for the total lung.

A custom version of Emphylx-J was also used for the quantitative airway analyses. The operator had to review each slice and manually identify airways that were cut in cross-section (short to long axis greater than 2:3). A seed point was placed in the lumen of each identified airway, and the software automatically computed the inner and outer limits of the airway walls using the Full Width At Half Maximum algorithm (24). This algorithm draws 64 radial lines from the seed point and measures the X-ray attenuation along these lines. Then it chooses the two points where the X-ray attenuation is half of the maximum attenuation value measured along that line as the

inner and outer limit of the actual airway. After the airway wall limits had been established, the software calculated the inner and outer perimeter of each identified airway wall, as well as the area of the lumen and the area of the wall itself. To reduce the technical errors associated with very small airways, only airways with an internal perimeter > 6 mm were included. The number of identified airways was also recorded, but due to the axial scanning method that was used (and not helical) we had no way of tracing the airways and classifying them into generations.

Quantitative CT measures used in this study

The extent of emphysema was assessed using the density mask method described above, to get the percentage of lung voxels with a density lower than a certain threshold. We used the term %LAA (percentage low attenuation areas) to describe these low density regions of the lung. We used -950 Hounsfield Units (HU) as the primary cut-off (%LAA-950), and when nothing else is specified, %LAA should be interpreted as %LAA-950. The -950 HU cut-off has been shown to be appropriate for this CT acquisition technique (16, 38), but several other cut-offs are also commonly in use. For comparison, we also performed the density mask analyses using -910 HU and -856 HU as cut-offs, yielding %LAA-910 and %LAA-856. Another frequently used measure of the extent of emphysema is the 15^{th} percentile density, which is the density (given in HU) at which 15% of the lung voxels have a lower density. This measure was also calculated from our material.

The airway wall thickness (AWT) is often expressed as the Wall Area Percent (WA%), which is the percentage of the whole airway area that is wall (and not lumen). We calculated WA% from this material, but there is a potential problem with this measure: With increasing airway generations, the airways get smaller, and the smaller the airway, the larger the relative wall area. To avoid the potential bias issues surrounding different distribution of airway sizes between subjects, a standardized measure for AWT was derived for each subject by plotting the square root of the airway wall area against the internal perimeter of each measured airway (Figure 2). The resulting regression line was used to calculate the square root of the wall area for a ‘theoretical airway’ with an internal perimeter of 10 mm (AWT-Pi10). Although CT

measured AWT tends to be overestimated compared to histologic AWT, especially in small airways, there is a good correlation between histologic and CT measurements when the square root of the wall area is plotted against the internal perimeter of the airway (24).

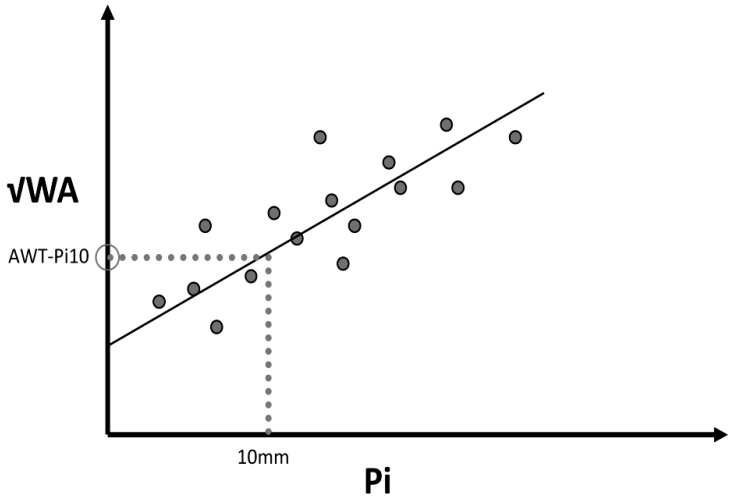

Figure 2 *The calculation of AWT-Pi10. The square root of the wall area of each measured airway (√WA) was plotted against the internal perimeter (Pi) of that airway. This was performed separately for each subject. From the resulting regression line, a standardized measure of airway wall thickness for an airway with an internal perimeter of 10 mm was predicted (AWT–Pi10).*

Data management

All data from the questionnaires and clinical tests were initially recorded on paper, and the paper records were kept in a locked archive facility. The data from the paper records were later punched into an online database by the local study staff, and continuous simple error-checks were applied to reveal and correct punching errors. Examples of this were out of range values and missing values. The online database (GenNet, operated by GSK) was password-protected, and access was only given to

selected members of the local study staff and selected members of the GSK staff. The online database was anonymized and contained no identifiable data except for a unique study ID number. Only the local project manager (Amund Gulsvik) had access to the data key that linked this ID with the subjects' name and date of birth.

After the data sampling phase was completed, an external monitoring agency checked the quality of the sampled data. The paper records from 5 % of the subjects (50 cases and 50 controls) were randomly chosen and re-punched, and the results were checked against the information stored in the online database. The agency concluded that there was good agreement between the paper records and the information stored in GenNet, and noted only a few discrepancies that were mostly related to smoking history. There were 25 observed smoking related discrepancies among these 100 subjects. But each subject answered a total of 36 smoking related questions, yielding a low discrepancy rate (25/3600). The observed discrepancies were later corrected in GenNet.

Then, extensive inconsistency analyses were run manually using Stata 8.0. Several inconsistencies were detected, and these were mainly related to smoking history. For instance, one subject could have answered "no" to ever having smoked, and then later stated that he is currently smoking 20 cigarettes per day. All detected inconsistencies were investigated and sought solved by re-checking the paper records, and also using the recorded data from the postal questionnaire. Some subjects were also contacted by phone for clarification. Solved inconsistencies were updated both in GenNet and in the paper records. Unsolvable inconsistencies were marked as missing values when the variable in question was non-essential. Unsolvable inconsistencies involving essential variables (variables concerning the inclusion/exclusion criteria of a subject) would lead to an exclusion from the study. A total of 23 subjects were excluded in this process.

Statistics

All statistical analyses were performed using the Stata/IC statistical package, releases 8-10 (StataCorp LP, College Station, TX, USA). In all three papers proportions were

tested using Pearson's χ^2-test (39). Normally distributed variables were tested using one way analysis of variance (ANOVA) and reported with means and standard deviations (SD). Not normally distributed variables were tested using the Kruskal-Wallis test (40) and reported with medians and 25 and 75 percentiles. A significance level of 0.05 was applied for all tests.

In papers I and III a multiple linear regression model was used to examine the relationship between the dependent and independent variables. In paper I, the %LAA-values were log-transformed using the natural logarithm, because both the %LAA-values and the resulting regression residuals were right-skewed. In paper I, the results from the whole multiple linear regression model was reported as the regression coefficient for each explanatory variable, along with the standard errors (SE), p-values, intercept and the adjusted R^2. In paper III, only the coefficients and SE for the main explanatory variables (%LAA and AWT-Pi10) were reported, along with the adjusted R^2 and a list of the adjustments or covariates used in the model. The regression coefficient was regarded as statistically significant if the 95 % confidence interval (95 % CI) did not include 0.

In paper II, the dichotomous respiratory symptom outcomes were examined using a multiple logistic regression model, while the ordinal dyspnea measure (MRCDS) was examined using a multiple ordinal logistic regression model (41). The results of the multiple logistic regression models were reported as odds ratios (OR) with a 95 % CIs for the main explanatory variables (%LAA and AWT-Pi10), along with the adjusted R^2 and a list of adjustments or covariates used in the model. The ORs were regarded as statistically significant if the 95% CI did not include 1.

Multiple regression analysis, both linear and logistic, allows for adjustment for confounding factors using covariates. In all regression models adjustments were made for sex, age and smoking history (both current and number of pack-years). Although every CT scan was supposed to be taken at suspended full inspiration, the level of inflation might have varied between subjects. Consequently, all regression models were also adjusted for inflation level (CT measured total lung volume divided by the

predicted total lung capacity (42)). Other adjustments were also used when specified, including FEV_1 in percent predicted, D_LCO, body mass index (BMI), HbCO and co-morbid heart disease. We also tested for some potential interactions between sex, age, smoking and the main explanatory variables (%LAA and AWT-Pi10) as specified in each paper. The analyses were mainly run separately for COPD and non-COPD subjects, but some additional analyses were run on the whole sample to check for interactions with case/control-status.

Synopsis of papers

Paper I

Quantitative computed tomography: emphysema and airway wall thickness by sex, age and smoking

COPD is a complex disorder characterized by chronic airflow limitation. The underlying cause of this airflow limitation is a mixture of small airway disease (obstructive bronchiolitis) and parenchymal destruction (emphysema). COPD is usually assessed by spirometry, which is an integral part of both the definition and the classification of COPD. But spirometry alone cannot differentiate between the underlying causes of this airflow limitation. Quantitative CT can be used to quantify these underlying causes, and to assess the relative contribution of airway disease and emphysema. There is limited data available on how these quantitative CT measures vary with age, gender and smoking history.

The aim of this study was to quantify the CT measured emphysema (%LAA) and airway wall thickness (AWT-Pi10) in a large COPD patient register and community based sample of ever-smokers, and to determine how these anatomic variables interacted with gender, age and smoking history.

The median (25, 75-percentile) %LAA was higher in COPD subjects (7.0 (2.2, 17.8)) than in non-COPD subjects (0.5 (0.2, 1.3)), and higher in men than in women, regardless of COPD status. The mean (SD) AWT-Pi10 (cm) was higher in COPD subjects (0.51 (0.03)) than in non-COPD subjects (0.47 (0.03)), and higher in men than in women, regardless of COPD status. The %LAA increased with increasing age in both COPD and non-COPD subjects, while AWT-Pi10 decreased slightly with increasing age in COPD subjects only. %LAA was higher in ex-smokers than in current smokers, and increased slightly with number of pack years. This pattern was seen regardless of COPD status. The AWT-Pi10 increased with higher daily cigarette consumption and increasing number of pack years in both COPD and non-COPD

subjects. The multiple linear regression analyses including adjustments for gender, age and smoking confirmed the above mentioned crude relationships. Interaction analysis revealed that %LAA increased more rapidly with increasing age in female than in male non-COPD subjects. Interaction analyses also revealed that among COPD subjects %LAA increased more rapidly with increasing age in current smokers, and that AWT-Pi10 increased more rapidly with increasing number of pack years in non-COPD subjects than in COPD subjects.

In conclusion, we found significant differences between COPD and non-COPD subjects in quantitative CT measures of emphysema and airway wall thickness. We also found that gender, age and smoking history have strong effects on these quantitative CT measures and must be considered when comparing quantitative CT studies.

Paper II

Quantitative computed tomography measures of emphysema and airway wall thickness are related to respiratory symptoms

The diagnosis of COPD is usually made by spirometry, but respiratory symptoms are a very important part of the clinical picture. Quantitative CT can be used to assess the pathological changes in lung structure associated with COPD, and to separate different subtypes of COPD according to the contribution of airways disease and emphysema. Quantitative CT measurements have been shown to be significantly correlated with spirometric values, but spirometry alone cannot explain the whole variation of the quantitative CT measurements. Previous studies have shown that there is an association between respiratory symptoms and quantitative CT measures of emphysema and airway wall thickness, but these studies were either very small, or did not fully adjust for pulmonary function or other possible confounders. Hence, there is limited knowledge on the relationship between respiratory symptoms and quantitative CT measurements.

The aim of this study was to describe the relationship between respiratory symptoms of COPD and quantitative CT measures of emphysema and airway wall thickness, and to assess how these relationships interacted with COPD-status, gender, age and smoking history.

About 15% of the non-COPD subjects reported at least dyspnea grade 2 in the MRCDS, while the corresponding figure among the COPD subjects was 70%. The cough, phlegm and wheezing symptoms were 2-5 times more frequent in those with as in those without COPD. In both groups dyspnea grade 2 or above and wheezing was reported more often by women than men, while phlegm was more frequently reported by men. Crude analyses showed that the MRCDS increased with increasing level of %LAA in COPD subjects only, while the airway wall thickness did not significantly affect the degree of dyspnea. We found more emphysema and thicker airway walls in the subjects who answered yes to the cough and wheezing attack questions, but these differences were not always significant. In the multivariate analyses adjusted for gender, age, smoking and level of inflation, the above mentioned crude relationships mainly persisted, but some relationships gained and some lost significance. The odds for increased dyspnea was increased by an estimated factor of 1.87 per 10% increase in %LAA among COPD subjects, while none of the other respiratory symptoms varied significantly with level of emphysema in the multivariate model. Increasing AWT-Pi10 was significantly associated with increased dyspnea level and presence of morning and chronic cough symptoms among the COPD patients, and significantly related to wheezing among both COPD and non-COPD subjects in the multiple logistic regression analyses. Adding FEV_1 to the multivariate models did not alter the observed relationships. Combined analysis of %LAA and AWT-Pi10 in the same model showed that both quantitative CT measures were independently associated with MRCDS.

In conclusion, we have shown that quantitative CT measures of emphysema and airway wall thickness are significantly and independently associated with respiratory symptoms, and may be used to explain the presence of respiratory symptoms beyond the information offered by spirometry.

Paper III

Quantitative CT measures of emphysema and airway wall thickness are related to D_LCO

The observed airflow limitation in COPD patients is caused by a mixture of small airway disease (obstructive bronchiolitis) and parenchymal destruction (emphysema), but neither spirometry nor D_LCO can adequately separate between these two conditions. Quantitative CT is an increasingly used method to assess COPD related changes in the lung structure, including the airway wall thickening associated with small airway disease and the reduced parenchymal density associated with emphysema. The association between these quantitative CT measures and spirometric values is well documented in the literature, while there is limited knowledge on the relationship between D_LCO and quantitative CT measurements.

The aim of this study was to examine the relationship between the diffusing capacity of the lung and quantitative CT measures of emphysema and airway wall thickness, and to assess how these relationships varied by COPD status, gender, age and smoking history.

The non-COPD subjects had higher absolute and percent predicted values of D_LCO, V_A and D_LCO/V_A. Men had higher absolute values of D_LCO and V_A in both COPD and non-COPD subjects. In percent of predicted values, the women had higher values for D_LCO/V_A, especially among COPD subjects. Crude analyses showed that increasing level of both %LAA and AWT-Pi10 were significantly associated with decreasing and increasing level of D_LCO, respectively, in both COPD and non-COPD subjects ($p < 0.01$). D_LCO/V_A also decreased significantly with increasing level of %LAA ($p<0.0001$) in both COPD and non-COPD subjects, while there was no association between AWT-Pi10 and D_LCO/V_A. Multiple linear regression analyses adjusted for gender, hemoglobin concentration, age, height, pack years, current smoking, FEV_1 in percent of predicted and inflation level showed that %LAA was consistently and negatively associated with both D_LCO and D_LCO/V_A in COPD subjects. The observed associations of %LAA were less consistent in the non-COPD

group, and not significant in the fully adjusted model. Using the same model and adjustments, we found a significant and positive association between AWT-Pi10 and D_LCO in both COPD and non-COPD subjects, but significance was lost after adjusting for inflation level in the non-COPD group. We did not find a significant association between AWT-Pi10 and D_LCO/V_A in the multiple linear regression analysis. The explained variation (adjusted R^2) attributable to %LAA was 16% in the adjusted D_LCO model and 26% in the adjusted D_LCO/V_A model among COPD subjects,, while it was negligible among non-COPD subjects. The explained variation attributable to AWT-Pi10 was very small (0-2%) in all models. Interaction analysis of the COPD subjects revealed that there was a stronger negative relationship between %LAA and D_LCO in men than in women.

In conclusion, we have shown that quantitative CT measures of emphysema are highly related to both diffusing capacity and diffusing coefficient, and that this relationship is even stronger in men. We have also shown that there is a positive, but not equally strong, relation between CT measured airway wall thickness and diffusing capacity, and this was contrary to our hypothesis that there would be no such relationship.

Methodological aspects

Study design

All study participants were part of the GenKOLS study, which was a large case/control-study primarily designed to look for genetic risk factors of COPD in ever-smokers aged 40 years or older. The examination of quantitative CT measurements and their relation to age, sex, smoking, respiratory symptoms and pulmonary function were secondary study objectives. Furthermore, the quantitative CT studies included in this thesis did not use the classical case-control approach of comparing the cases and controls, but rather an observational approach to the two separate groups of subjects with and without COPD. The study was cross-sectional in design. We were therefore limited to describing associations and relationships between variables, and no valid inferences could be made about cause and effect.

Reliability

The terms reliability and validity are crucial when it comes to determining whether or not to trust your findings (43, 44). A test is considered reliable when there is reasonable agreement between replicate measurements using that test. The measurements of a reliable test will consequently have a small spread, but do not necessarily measure the "correct" or "true" value. A high reliability is a prerequisite for a high validity, but no guarantee.

This thesis presents data on pulmonary function (spirometry and diffusing capacity), smoking habits, respiratory symptoms and quantitative CT measurements.

The pulmonary function tests were performed using standardized equipment (Appendix F, G), and following the current ATS guidelines (3, 34). The reliability of the pulmonary function tests was therefore not perceived as a problem.

Smoking habits and respiratory symptoms were assessed using questionnaires. This kind of self-reported data may be vulnerable to inaccuracies due to the subjects' memory and perception of the question, but also due to poor data handling afterwards. Every precaution was taken to ensure correct data handling, and the wording of the questions used was based on previously validated studies (45, 46). The reliability of the questionnaire data was not perceived as a problem.

The CT images were acquired using standardized equipment, and the scanner was set up using air and water phantoms according to the manufacturer's recommendations. The reliability of the acquired CT images was therefore not perceived as a problem, and due to the associated risk of radiation, internal repeatability tests were not performed.

The quantitative assessment of the CT images was to a certain degree operator dependent. But there were only a few highly trained operators, and the operator input was minimal with regard to the parenchymal density and volume measurements. We performed analyses of a randomized sub-sample of 20 subjects, whose CT images were quantitatively assessed by two different operators (Figure 3). This analysis showed good correlation between the two operators' measurements, and a mean absolute difference between the two operators' %LAA values of 0.005 (range 0.00 – 0.03).

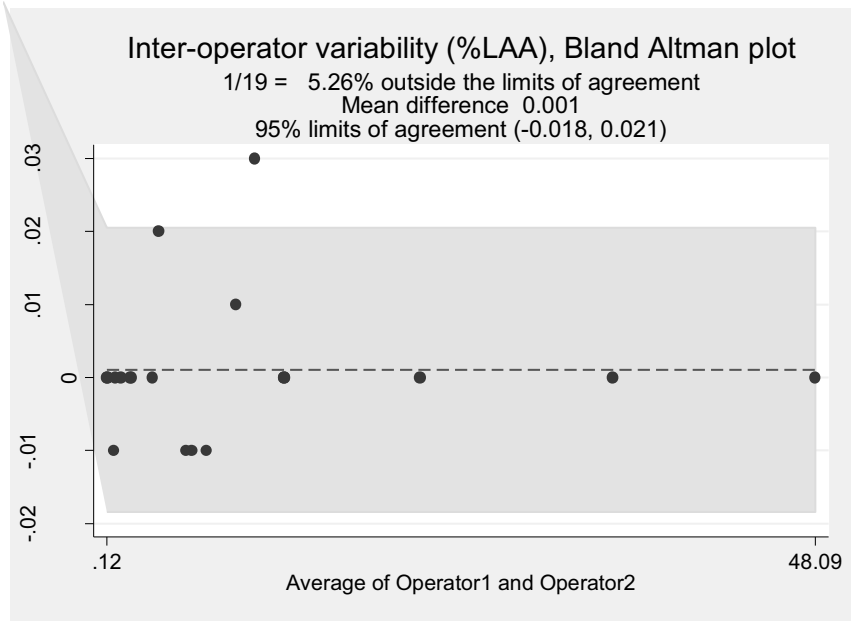

Figure 3 *Inter-operator variability (%LAA), Bland-Altman plot (19 subjects)*

The airway measurements are more operator-dependent, due to the selection of eligible airways, and one would expect a slightly larger inter-operator variability on these. Unfortunately, we do not have enough available data to present a Bland-Altman plot for the airway measurements, but a preliminary analysis of 3 subjects indicated an inter-operator variability of approx. 2 %. As the operators were blinded to other data concerning the subjects, it is reasonable to assume that any misclassification of the airway measurements was non-differential, and consequently the observed associations would have been even stronger without this potential error. The reliability of the quantitative CT assessments was therefore not perceived as a problem.

Validity

The validity of a test concerns to which degree a test is actually measuring what it is intended to measure (43, 44). Validity can be divided into internal and external validity. The internal validity of a study concerns the ability to draw valid conclusions regarding the study population, and may be influenced by several factors, including

random errors, systematic errors and confounding. The external validity of a study concerns the ability to draw valid conclusions about a larger population based on the results of the study. The external validity is compromised if the study population is not representative of the target population.

Given the size of this study, random errors were not perceived as a problem. Systematic errors, or biases, are independent of study size, and cannot be ruled out.

A large fraction of the invited (43 %) and included (57%) subjects in this study were sampled from population based cohorts with high attendance rates (47, 48). However, we also invited/included subjects from the HUH COPD registry (51 % / 38 %) and volunteers (6 % / 5 %). The subjects from the COPD registry are not population based, and although the volunteers did not differ much from the population based subjects, their motives for participating are not known. This study design with recruitment from several sources, made this study vulnerable to selection bias (49).

Furthermore, only one half (951 of 1909) of the GenKOLS population underwent a CT examination, and this selection was not randomized. Due to the potential radiation risk associated with CT, this examination was optional, and offered to included GenKOLS subjects until we had included approximately 500 cases and 500 controls with CT. This may also have introduced a selection bias. However, there were no large differences between the subjects with and without CT with regards to gender, age, smoking and FEV_1 in percent predicted (Table 3).

Table 3 *Comparison of GenKOLS subjects with and without CT*

	GenKOLS (All)		**GenKOLS (no CT)**		**CT sample**	
	Non-COPD	COPD	Non-COPD	COPD	Non-COPD	COPD
n	955	954	467	491	488	463
Male (%)	50	61	47	58	53	65
Age (Mean)	56	65	56	67	56	64
Currently smoking(%)	41	47	40	44	42	49
Pack-years (Median)	16	28	16	29	16	27
FEV_1% pred (Mean)	95	51	95	49	95	53

There was 4797 invited subjects in this study, but only 2984 (62 %) attended the screening visit at HUH. This may have introduced a non-response bias, but a previous population study from Hordaland have shown that increasing the response rate from 65 % to 89 % did not affect the relationship between smoking and lung disease (50).

Confounding may arise when investigating an association between an exposure and an outcome, and both the exposure and outcome are strongly associated with a third (known or unknown) variable (43). In multivariate regression models, known confounders can be adjusted for. We have included sex, age, smoking and several other factors in our regression models, in an attempt to control for potential confounders. However, this study only included smokers with at least 2.5 pack-years. We were therefore unable to fully adjust for smoking, and cannot rule out that there is some residual confounding from smoking.

Diffusing capacity guidelines

According to the current ATS guidelines for accurate measurements of diffusing capacity (34), a test should be rejected if the measured inspired vital capacity is less than 90% of the largest previously measured FVC. This was the case in 148 subjects in the current study. Despite the guidelines, these subjects were not excluded from the study. However, it has previously been shown that it is acceptable to include subjects with an IVC/FVC-ratio < 90% as long as the characteristics of these subjects do not differ much from those with a ratio > 90% (51). As shown in Table 4, there were slightly more women and fewer current smokers among the non-COPD subjects in the low ratio group compared to the high ratio group. Otherwise there were no large differences between the groups, and the 148 subjects were consequently included in the study.

Table 4 *Comparison of subjects with an IVC/FVC ratio over / under 90 %*

	IVC/FVC > 90%		IVC/FVC < 90%	
	Non-COPD	COPD	Non-COPD	COPD
n	332	233	93	55
Male (%)	56	70	47	71
Age (Mean)	56	63	53	64
Currently smoking (%)	51	49	40	56
Pack-years (Median)	16	26	16	26
FEV_1% pred (Mean)	95	59	97	61

Radiation issues

In order to perform quantitative CT analyses, one must first acquire a CT image. And with the acquisition of this CT image, comes the unavoidable radiation risks that accompany any X-ray examination, and especially so with CT. While the standard chest radiography only exposes the subject to a radiation dose equivalent of approx. 3 days of average natural background radiation, the radiation dose from a chest CT is approx. 350 times higher, or the equivalent of more than 1000 days of background radiation (52). There is still incomplete knowledge about the complex link between ionizing radiation exposure and adverse future effects in humans, but even the relatively low radiation doses associated with a chest CT (approx. 7 mSv) carries an increased risk of cancer (53). This risk is probably cumulative, and higher in younger people, women and in certain tissues (e.g. breast and thyroid). And however low this risk may be, there is general consensus that one should keep this exposure to a necessary minimum. And when a CT is warranted, the examination should involve as little radiation as possible, while maintaining the image quality. Fortunately, newer techniques and equipment have made it possible to achieve better image quality using lower radiation doses. With regard to quantitative CT measures of the chest, one can achieve acceptable images from the lung parenchyma using very low radiation doses, while airway analyses may require higher doses (54, 55).

Quantitative CT analyses

There are many different approaches to quantitative CT analysis, and some controversies, especially with regard to airway analysis. But all these approaches are similar in their use of acquired CT images to obtain measurements of lung volume and X-ray attenuation or density measured in Hounsfield units. The measurements of lung volumes are done by using different lung segmentation algorithms. These algorithms have become very precise, and there is general consensus that supine lung volume can be accurately measured using quantitative CT measurements (56).

Parenchymal analysis

The X-ray attenuation values obtained from the CT images gives in indication of the density of the lung, as the HU scale is proportional to the density within the biological range. These density measurements must then be summarized in a way that gives a reasonable estimate of the phenotype in question (emphysema). The two most common ways of doing this are the density mask threshold cut-off and the percentile methods, and both approaches have been shown to correlate with the extent of emphysema in corresponding pathological samples (12, 13). Both methods have been accepted by the scientific community, and the remaining debate concerns which density cut-off, or which percentile to use. In the current study we have chosen the density mask approach, with the most commonly used threshold cut-off value (-950 HU). This was a cross-sectional design, and we could have used either method, but for longitudinal data it is recommended that one uses the percentile method (15^{th} perc), as this method is less sensitive to minor changes in the technical aspects of the CT scan and more sensitive to structural changes (56). These technical aspects (type of scanner, X-ray exposure settings, image noise, reconstruction algorithms) can greatly influence the amount of emphysema measured. It is therefore very important to keep these aspects as constant as possible, especially in longitudinal studies, but also for comparison of different cross-sectional studies. Another important influence on the amount of emphysema measured is the degree of inhalation during the CT scan. This can be controlled using spirometric gating, but that is not very practical. It is therefore recommended, especially in longitudinal studies, that one use a mathematical

adjustment based on the CT measured lung volume (17). In the multiple regression analyses presented in the current study, we used the CT measured lung volume divided by the predicted TLC to adjust for this.

Airway analysis

The characteristic airflow limitation seen in COPD is in large part due to airway remodeling in the small airways (< 2 mm in diameter) (7, 57), and non-invasive measures of these small airways is of great interest. However, CT images, and consequently quantitative CT analyses of the airways, has a limited resolution, both in pixel size and slice thickness. The field of view in each slice limits the pixel size to approx. 0.5 mm, and although some of the newest multi-slice scanners can acquire images with a slice thickness as thin as 0.5 mm, most scanners in a clinical setting are limited to a slice thickness of 1.0-1.25 mm. Due to these resolution limitations, airways smaller than 2 mm in diameter cannot be accurately measured using quantitative CT, and the technology is limited to measuring airways > 2 mm in diameter. However, it has been shown that the remodeling in the small airways can also be seen in larger airways, and that measurements of these larger airways can be used as a surrogate for the processes occurring in the smaller airways (24). This correlation gets stronger the smaller (more distal) the measured airway is, so that a 6^{th} generation airway correlates better than a 3^{rd} generation airway (58, 59). In the current study we used a slice-gap approach, and could therefore not classify the airways into generations. Newer approaches use contiguous scans to produce a three-dimensional airway three, enabling measurements from airways with known generations. This new approach bears promise of a more precise airway assessment, and an even stronger correlation with the small airways actually responsible for the airflow limitation.

An accurate localization of the transitions between airway lumen, airway wall and the surrounding parenchymal tissue is a key element in quantitative CT assessment of the airways. This process gets more difficult when the size of the structures you are measuring (e.g. the airway wall) borderlines the *point spread function* (also known as the “partial volume effect”) of the scanner, resulting in blurring and inaccurate measurements. Several approaches and reconstruction algorithms have been applied to

overcome this problem, one of the first being the “full-width at half-maximum” method (23, 60), which we used in this study. A known problem with this approach is the overestimation of the airway wall, and the underestimation of the airway lumen, and this problem increases with decreasing airway size (22, 60). Several alternative algorithms have therefore been developed, and examples of these are the “maximum-likelihood method” (22), the “score-guided erosion algorithm” (21) and the “elliptical fit algorithm” (61). While each approach has some advantages, there is no solid evidence that one particular algorithm provides more useful information than the others (19).

Discussion of the results

Several topics regarding the results from the current study have already been discussed in the discussion sections of Papers 1-3, and they will not be repeated here. The focus of this section will be on topics that were not discussed thoroughly in the respective papers.

Pre- versus post-bronchodilator CT

In accordance with the current GOLD guidelines (2) , we used post-bronchodilator spirometric values to classify the subjects included in this study. Furthermore, we used post-bronchodilator FEV_1 values as an adjustment in the multiple regression analyses. The CT images that the quantitative analysis was based on were however not acquired after administration of any bronchodilator. There is limited knowledge about the effect of bronchodilation on quantitative CT measures, but this difference could potentially have influenced our results with regard to both airway dimensions and parenchymal density through altered regional ventilation (62). Furthermore, this influence would probably have been even stronger in the non-COPD subjects, as many of the COPD subjects were already using long acting bronchodilators. The exact effect of this difference is not known, but it is reasonable to assume that the observed associations would have been even stronger without this potential influence.

CT versus diffusing capacity: The effect of body position

CT images are usually (and in the current study) acquired in the supine position (although the prone position may also be used), while D_LCO is usually (and in the current study) measured sitting in an upright position. This positional difference may potentially have affected the results of Paper 3.

The density measurements obtained from a CT scan are dependent on the relative amounts of lung tissue, air and blood present in the lung. These components are not homogenously distributed throughout the lung, and the relative proportions

continuously change during respiration (63). The relative distribution of these components is also affected by gravity, and the extent of air trapping follows a gravitational gradient with more air trapping in the dependent lung regions (64). This effect is less pronounced when CT images are acquired at full inhalation, as was done in the current study, but gravity certainly has an effect on the distribution of air and blood.

It has also been shown that both the pulmonary capillary blood volume and D_LCO increases in the supine position as compared to an erect position. These effects were far more pronounced in patients with the chronic bronchitis phenotype as opposed the emphysematous phenotype, and the effect decreased with increasing age (65, 66).

As the current study population was relatively old, the difference in body position has probably not affected the observed relationship between %LAA and D_LCO. We cannot rule out the possibility that the difference in body position may have influenced the observed relationship between AWT-Pi10 and D_LCO, but there are no indications that CT measured airway dimensions are affected by body position.

CT assessed emphysema and airway wall thickness versus respiratory symptoms

Our study showed that level of emphysema and airway wall thickness was related to dyspnea, while airway wall thickness was related to cough, phlegm and wheezing. These findings help to provide a clinical correlate to level of emphysema and airway wall thickness. This is important as the patient does not sense the emphysema or the airway wall dimensions. It also enables the clinician to interpret the CT findings and to put them into a clinical context. It is important to the researcher as our observations imply that the CT assessment may be used to describe potential phenotypes of COPD (8).

The cough and wheezing symptoms were assessed as dichotomous variables. This is a rough way of recording the symptoms and will probably work to underestimate the

strength of the relationship between respiratory symptoms and the quantitative CT measures. Dyspnea was also assessed as a categorical, but ordinal variable. The perception of the symptoms also may influence on their relationships to the CT findings. We have no information as to what extent differences in perception of the various symptoms influence the observed associations between symptoms and quantitative CT measures.

Dyspnea, cough and wheezing may be seen in other conditions than COPD, as for instance coronary heart disease, cardiac heart failure and asthma. All of these conditions may be related to COPD, and we adjusted for some of these conditions. However, the information indicative of these diseases was self-reported. If more valid information about these co-morbidities had been available and added to the equation, one may speculate that more specific relationships between respiratory symptoms and quantitative CT measures would have been observed than was actually the case.

The technology and data handling available at the time of the present study did not allow us to assess the level of bronchial generation studied. Up to date technology in CT allows the researcher to generate a three-dimensional bronchial tree, and estimate the generation of the measured bronchia down to the sixth generation bronchus (67). This allows a more precise characterization of the airways than we have been able to perform in the current study. This new technology will hopefully work to further clarify the relationship between the airway wall dimensions and the respiratory symptoms.

A final comment to the relationship between the CT findings and the respiratory symptoms has to do with the study design. As the current study is a cross sectional survey, it offers only one point of time in this relationship. A longitudinal study would have allowed another perspective of the association between symptoms and CT findings. One may speculate that the observed relationships then would have been stronger than what was observed cross-sectionally. However, from a clinical point of view, the doctor often has only one CT available when making his or her clinical decisions.

CT assessed emphysema and airway wall thickness by gender and smoking

As the first study we observed that smoking is related to airway wall dimensions (Paper I). When evaluating these results it is important to bear in mind that other potential risk factors of COPD have not been taken into account. Examples of such factors include occupational airborne exposure, impaired socio economic status and poor indoor air quality. These risk factors are also related to cigarette smoking and may act as confounders in the relationship between smoking and the CT measured airway wall dimensions. They may theoretically also interact with smoking aggregating its effect on the airway wall measurements.

Several studies have observed a stronger relationship between smoking and the pulmonary outcome variables in women than in men (68, 69). One interpretation has been that women are more susceptible to smoking than men (70). The pulmonary outcomes have mainly been respiratory symptoms and quality of life assessments. Hence, gender differences in perception of these outcomes could explain these dissimilarities between men and women. Level of emphysema and airway wall dimensions are beyond perception. Our findings do not support the hypothesis that women are more susceptible to smoking than men. On the other hand we used self-reported information on both current smoking status and total smoking consumption. This might have worked to obscure any gender difference in the effect of smoking. Recent data suggest that there are biological mechanisms indicating increased susceptibility of COPD in female smokers (71, 72).

The lower airways are not sterile, and culture-independent molecular methods have shown that the microbiota of humans is far greater in extent than previously recognized (73). Symbiotic bacteria like *Bacteriodetes* and *Firmicutes* elicit tonic signals in the gut epithelium that prevent activation of innate and adaptive immune responses (73, 74), and symbiotic bacteria down-regulate immune responses to pathogens in the nasal mucosa (75). It is possible that similar mechanisms may be operational in healthy airways.

It is not known if smoking may influence the distribution and relative content of symbiotic and pathogenic bacteria in the airways, but smoking is related to thicker airway walls. One may speculate that at least part of this effect may be due to the influence of smoking on the airway microbiome.

When interpreting the observations of the current study it is important to bear in mind that it is cross sectional in design. As such, we are not able to take into account any cohort effects or any influence of different time periods. In a cross sectional setting any difference between a 40 years old and an 80 years old subject may be interpreted as being due to the age. To be able to examine the cohort and period effects, a longitudinal survey with repeated data samplings is needed.

Conclusions

This study has shown that COPD subjects and men have significantly more emphysema and thicker airway walls than non-COPD subjects and women. Furthermore, both these quantitative CT measures are substantially affected by age and smoking habits. This has not previously been shown in a large study like this, and it underlines the importance of taking gender, age and smoking habits into account when designing and interpreting these kinds of studies.

This study has also shown that these quantitative CT measures are strongly and independently associated with respiratory symptoms, and that they may be used to explain respiratory symptoms beyond the information that is available from a spirometry.

This study has also confirmed our hypothesis that CT measured emphysema is highly related to diffusing capacity, and that this relationship is even stronger in men. The finding of a significant and positive relationship between diffusing capacity and airway wall thickness was contrary to our hypothesis, and has not been shown previously.

Perspectives

1. The analyses of this thesis are based on cross sectional study design. It would be of interest to examine the relationships of gender, age and smoking to level of emphysema and airway wall thickness in a longitudinal study design. Such a study would give a more valid estimate of these relationships than offered by a one point in time examination.
2. A similar consideration as above can be made for the associations between the quantitative CT variables and the respiratory symptoms.
3. Longitudinal data on the development of emphysema and airway wall thickness in terms of distribution and degree will improve our knowledge of the natural history of COPD and its phenotypes. A further objective would be to examine predictors for these developments.
4. Apart from smoking, limited data is available on how other airborne exposures like occupational airborne exposure and indoor air pollution including passive smoking affect the level of emphysema and airway wall thickness. As CT is increasingly used to characterize patients with COPD both in the clinic and in research, data on the relationship between CT findings and other airborne exposures than smoking is warranted.
5. Limited data is available regarding the prognostic value of these quantitative CT variables. This particularly relates to the airway wall thickness. Such knowledge will work to improve the clinical validity of the CT findings. This goes for both subjects with and without COPD.
6. Improvements in both CT technology and data handling in the years to come will enable the use of quantitative CT to better characterize the anatomical correlates of for instance respiratory symptoms. One may also be able to examine the generations of the bronchial tree more specifically than it is done today.
7. Little is known regarding the use of these quantitative CT variables in combination with other COPD key variables like spirometry, exercise capacity, exacerbation rates or inflammatory markers in the characterization of phenotypes of COPD.

8. We defined COPD based on the GOLD criteria. It would be interesting to examine how the observed relationship in this thesis would be affected if COPD had been defined based on the lower limit of normal of the FEV_1/FVC ratio.

Errata

a) *Methods sections of Papers I, II and III*

In all three papers, it was specified that we used a slice thickness of 1.0 mm at 20 mm intervals when acquiring the CT images. This is incorrect. The actual slice thickness used was 1.25 mm at 20 mm intervals. The error was due to a misunderstanding in an early phase of the project, and was not discovered until June 2011. However, this error has had no consequences with regard to the data presented in the three papers, as the error was in the description only, and the correct slice thickness was used for all the quantitative analyses.

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