Detection of COPD, lung function decline and emphysema progression in heavy smokers

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Detectie van COPD, longfunctiedaling en emfyseemprogressie in zware rokers

(met een samenvatting in het Nederlands)

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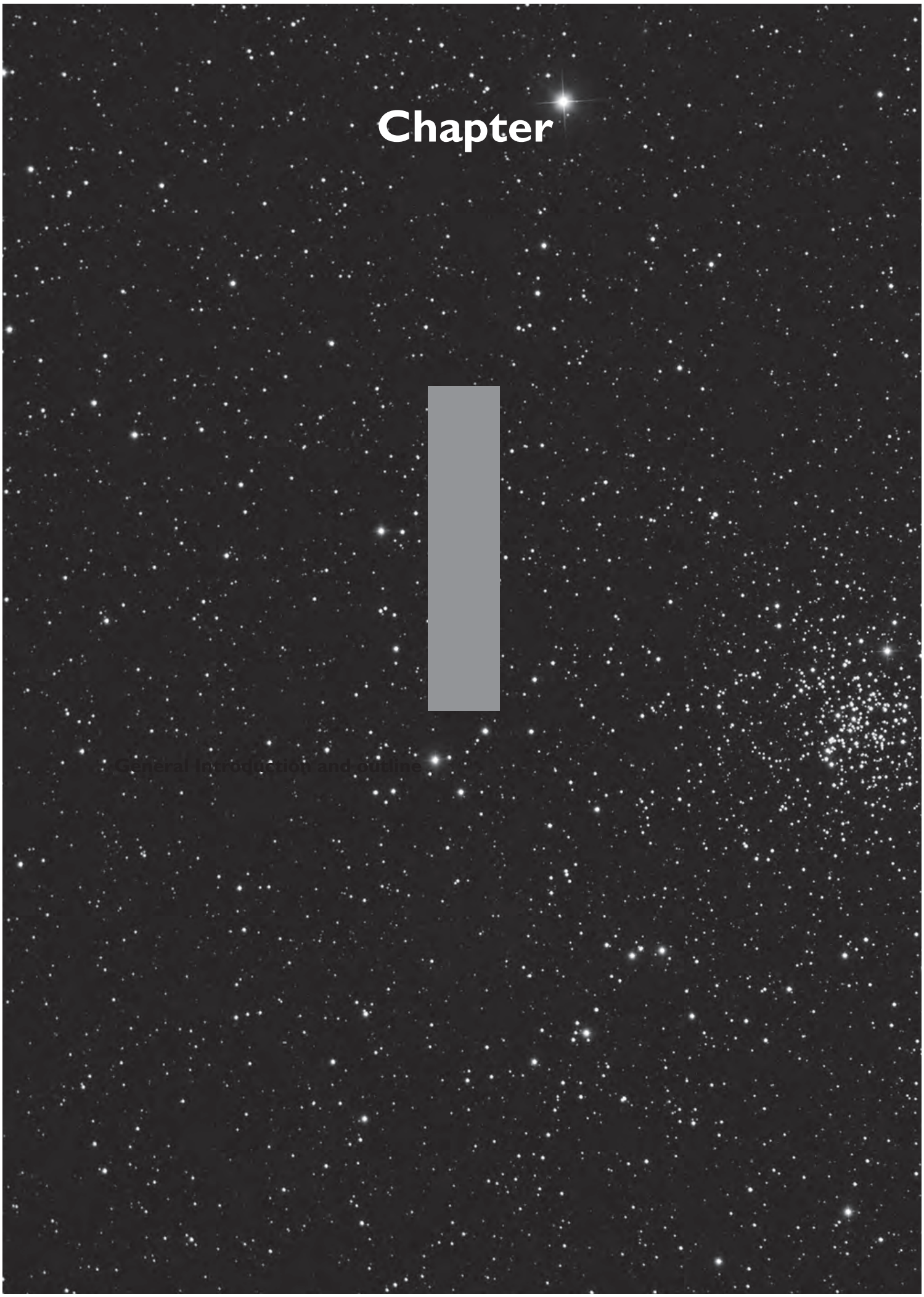
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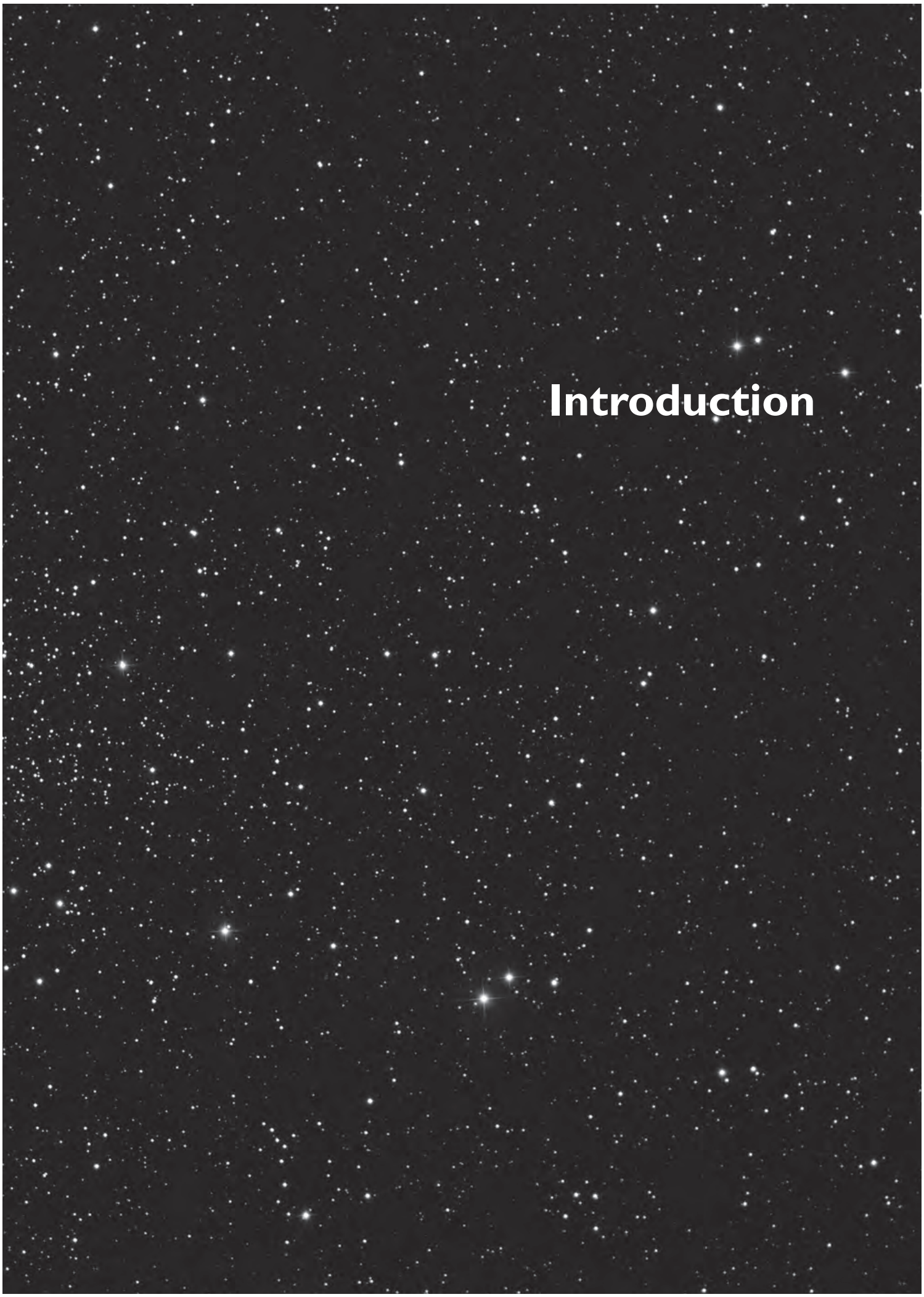
1
General introduction and outline



Introduction

Introduction

Chronic obstructive pulmonary disease (COPD) classically refers to two disorders both leading to disturbance of airflow in the airways: chronic bronchitis and emphysema. The prevalence, as well as the mortality and morbidity rates, of COPD are still rising.[1] [2] Strikingly, COPD is the only chronic disease world-wide with an increasing mortality rate and it will constitute the third cause of death by 2020 [3]. Early recognition and detection of COPD is of utmost importance as this may enable timely interventions such as smoking cessation. Furthermore, because COPD is an irreversible and progressive disease early recognition may prevent future disease progression. Several factors are known to cause COPD, however it is well-established that the main cause is tobacco smoking. Other causes include environmental pollution (bio-gasses), occupational exposure (chemicals and dusts) and inherited deficiencies (α_1-anti-trypsin deficiency). Also genetic factors presumably play a role. COPD is diagnosed when the forced expiratory volume in 1 second to the forced vital capacity (FEV1/FVC) is below a predefined threshold.[4] However, the pathological processes leading to this lowered FEV_1/FVC probably start earlier in time. These pathological processes can not be detected by spirometry only. Lung density measures at computer tomography (CT) of the lungs, assessing CT-detected emphysema, may be used to detect structural changes before spirometry becomes abnormal. Following the same line of reasoning, diffusion tests may also be helpful to detect these structural changes. Subjects with an increased lung function decline, but with FEV_1/FVC values still above or in the normal range, will not be labeled as having COPD. However, these subjects may show their largest decline at an early stage. [5] The objective of this thesis is to investigate the role of clinical, radiological and genetic determinants of lung function decline and emphysema progression in heavy smokers and to examined the validity of the spirometric diagnosis of COPD.

Chronic obstructive pulmonary disease

COPD classically refers to two disorders both leading to disturbance of airflow in the airways: chronic bronchitis and emphysema. It is highly recognized that COPD is multifactorial in its etiology and pathophysiology and heterogeneous in its phenotype. The pulmonary component is mainly characterized by the presence of progressive airflow obstruction associated with abnormal inflammatory responses of the lung to noxious particles or gases. The main pathogenic mechanism in COPD is the abnormal

inflammatory response of the lungs on inhaled noxious particles, like present in tobacco smoke. [6]

Several factors are known to cause COPD, however it is well-established that the main cause is tobacco smoking. Other causes include respiratory infections, environmental pollution (bio-gasses), occupational exposure (chemicals and dusts) and inherited deficiencies (α_1-anti-trypsin deficiency). Besides α_1-anti-trypsin deficiency, which in daily practice rarely causes COPD, also other genetics factors are presumed to play a role in the development of COPD [7] [8]. Fletcher and Peto found that only 10-20% of smokers develop the disease, which makes the presence of genetic causes more convincing [9]. Furthermore, familial clustering of lung function and COPD susceptibility have been reported . Extensive research is being conducted to unravel the role of these genetic factors in COPD, nevertheless it is acknowledged that complicated genotype-environment and genotype-genotype interactions may be present making interpretation more complicated [10] [11].

The abnormal inflammatory response is determined by both the innate and the adaptive immune response, and numerous inflammatory cell-types appear to be involved. Like pointed out before, COPD refers to two disorders both leading to disturbance of airflow in the airways: chronic bronchitis and emphysema. Although both entities can be more or less present in the same individual and histopathological emphysema is always accompanied by a certain degree of small airway disease, they have different pathogenic features and therefore will be shortly discussed separately.

Chronic bronchitis is caused by an innate immune response leading to inflammation in the airways and glands producing mucus. Hypersecretion of mucus is a key feature of chronic bronchitis and is due to an increased number of goblet cells and enlarged submucosal glands. The hypertrophy and hyperplasia of the mucus glands results, together with infiltration of the airway wall by neutrophils, CD8+-cells and macrophages, in an increased airway wall thickness. Furthermore, smoking negatively affects the ciliary clearance of mucus, resulting in more mucus stasis.

Emphysema is caused by parenchymal destruction due to alveolar detachments and decrease of the elastic recoil of the parenchymal structures resulting in an abnormal permanent enlargement of air spaces distal to terminal bronchioles [12]. Destruction of

lung tissue is amongst otherinduced by oxidants from tobacco smoke and by oxidants and proteases from inflammatory cells. Morphologically two subtypes can be distinguished; panacinar and centriacinar emphysema. Panacinar emphysema is associated with α_1-anti-trypsin deficiency, while centriacinar emphysema is associated with tobacco smoking. [13] Emphysema eventually leads to gas exchange abnormalities. The correlation between histological emphysema and lung function is not strong. Autopsy studies have shown that one third of the lungs can be obliterated by emphysema without lung function parameters being impaired [14]. Especially mild emphysema does not correlate well with lung function parameters.

Lung function decline

In healthy non-smoking individuals lung function declines at a constant rate with increasing age. The normal age related FEV_1 decline depends on the reference equation and the characteristics of the study population Based on the reference equation from the European Community for Coal and Steel (ECCS), which is most used in West-European countries like the Netherlands, this is 29 mL/ year. [15] Smokers have an accelerated lung function decline. However, several other factors are also associated with an accelerated lung function decline like airways reactivity, COPD disease exacerbations and air pollution. [16] [17] [18] According to a pooled analysis of seven randomized controlled trials investigating the effects of inhaled corticosteroids on FEV_1-decline, a decline of 37 ml/ year and 49 ml/year, in male current and ex-smokers, respectively, was present in the placebo groups. [19] There is however a large variation in the decline among current smokers. Some current smokers show a considerably larger decline while others only show a slightly faster decline compared to non-smokers. This difference in lung function decline among smokers may well be an explanation why some smokers develop COPD and others do not. Subjects with a faster lung functiondecline will pass the threshold of airflow obstruction earlier in time than those with smaller declines. Factors associated with faster lung function decline in (heavy) smokers may be useful to select smokers eligible for aggressive stop smoking therapies.

An accelerated lung function decline is considered as a hallmark of COPD and could be regarded as an important phenotype of COPD. [20] It is known that lung function decline can serve as a prognostic factor. Mannino et al found that an accelerated lung function decline was independently associated with an increased risk of hospitalization

and mortality. [21] Similar results were also found by Rodriguez et al. and Ryan et al. [22;23] Fletcher et al. were the first to show that an accelerated FEV_1 decline is an indicator of COPD. In their study subjects with COPD showed higher FEV_1- declines than non-COPD subjects. The lung function decline according to their classical concept increased with decreasing FEV_1 rates. In contrast to the classical findings of Fletcher and Peto, secondary analyses from the TORCH and UPLIFT studies showed that the rate of lung function decline is steepest in those with mild disease. [5;24] Both in the UPLIFT and TORCH studies subjects in GOLD stage II had a decline of approximately 50 ml/year compared to approximately 30 ml/year in GOLD IV subjects. Whether this phenomenon also occurs in less diseased subjects remains to be determined. Since the NELSON trial, a lung cancer screening trial in healthy smokers, included heavy smokers with no airflow obstruction yet or those with only mild COPD we investigated whether the lung function decline would also be steepest in less diseased subjects, i.e. with higher lung function levels. These results are described in **chapter 3.** If our findings would be in line with the findings of the UPLIFT and TORCH investigators they would implicate that functional deterioration in heavy smokers is greatest at a very early stage.

CT quantification of emphysema and lung function decline

Destruction of alveoli in persons with emphysema results in a lower lung density, which can be quantified with CT lung densitometry. The best way to quantify this CT-detected emphysema is still controversial. Lung densitometry assesses the relationship between tissue density and its X-ray attenuation on CT [25]. Emphysema is characterized on CT by voxels with abnormally low X-ray attenuation. The extent of X-ray attenuation is measured by Hounsfield units (HU) ranging from 0 to -1000. Arbitrarily air has the lowest X-ray attenuation (-1000 HU), while water is 0 HU.

Several thresholds of emphysema scores have been proposed to quantify this CT-detected emphysema. The >10% of total voxels with <-910 HU for moderate and >1% of total voxels with <-950 for severe emphysema are two fixed cut-offs for the presence of emphysema. The <-950 HU is most frequently used for the quantification of emphysema. The 15th percentile (Perc15) technique provides the HU below which 15% of all voxels are distributed and is a continuous measure. The lower the Perc15 values are, i.e. closer to -1000 HU, the more emphysema is present. All these emphysema scores have been validated against pathology, the gold standard of emphysema [26 27 28].

The Perc15 measurement showed to be the most robust parameter of emphysema progression compared to the <-950 HU. [29] [30]

It has been shown that spirometry do not correlate well with histological proven emphysema and that it even can be present without resulting in airflow obstruction. [31] Only 15% of smokers develop airflow obstruction, while up to 40% of smokers develop emphysema. [6] With the introduction of the CT-scan, emphysema was found to be increasingly present in subjects with a normal lung function. Especially the presence of mild emphysema was larger than expected. Sanders et al. found that in up to 69% of smokers, with normal lung diffusion test results and without airflow obstruction, visually estimated emphysema was present on CT of the lungs.

Participants of the NELSON study all smoked enough to have emphysema present on the CT, however the predictive value of this emphysema is unclear. Yuan *et al.* found no significant effect of emphysema on annual lung function decline. A study by Remy-Jardin *et al.* found that emphysema was significantly associated with a more rapid lung function decline. However, a drawback of the latter study was that the extent of emphysema was visually estimated, and that there were no corrections made for differences in age, pack years and smoking status (current/ former smokers) between included subjects. The predictive value of CT-quantified emphysema in heavy smokers thus remains unclear. To unravel the role of CT-quantified emphysema a study investigating the predictive value of CT-quantified emphysema on lung function decline was performed. The results of this study are described in **chapter 3**.

An advantage of CT is that besides the extent of emphysema also information about the distribution and localization of emphysema is provided. Next to the total extent of emphysema, also associations between the distribution of emphysema, upper or lower lobe predominant, have been described with lung function in cross-sectional studies. [32] [33] In subjects with alpha1-anti-trypsin defiency (AATD) it has also been shown that the distribution pattern is associated with lung function decline.[29] The questions arise whether in heavy smokers the distribution pattern of emphysema would also be associated with disease progression, i.e. lung function decline. in heavy smokers. The results of such a study are described in **chapter 4**.

Interventions: smoking cessation

In spite of an increasing understanding of the pathophysiology of COPD in recent years, treatments intervening in the fundamental pathophysiological processes causing COPD are still lacking. Intervention options can be divided in non-pharmacological and pharmacological. Of the non-pharmacological options, smoking cessation plays an important role. Smoking cessation is the only known intervention capable of altering the increased lung function decline. Unfortunately, up to now no current pharmacologic treatment is able to modify the increased lung function decline over time [34].

In the past several studies have been conducted on the effect of smoking cessation in subjects with and without COPD [35]. It can be generally concluded that the accelerated FEV_1-decline in subjects in time normalizes or slows down. One of the hallmark studies on smoking cessation, the Lung Health Study (LHS), showed that after 11 years of cessation, lung function decline normalized. The LHS included only subjects with airflow obstruction (FEV_1/FVC<70%). The effect of smoking cessation on emphysema progression has not been thoroughly investigated. Bellomi et al. found that after a two year follow-up that progression of emphysema was smaller in former smokers in comparison to current smokers. [36] A drawback of the study is that pulmonary function parameters were not taken in account and that also the length of cessation was not taken in account. Differences in lung function values and lengths of smoking cessation could have obscured the outcomes. Thus far, no study assessed the effect of length of smoking cessation on lung function decline and emphysema progression in a cohort of heavy smokers. **Chapter 5** describes a study investigating the effect of the length of smoking cessation on lung function decline and emphysema progression in heavy smokers. The outcomes of this study may be of special interest because no studies, to our knowledge, have examined the effects of duration of smoking cessation on lung function decline and emphysema progression in heavy smokers, without severe COPD yet.

Lung diffusion testing

Testing the diffusion capacity of the lung is frequently used to evaluate obstructive as well as restrictive pulmonary diseases. Diffusion testing using carbon monoxide as tracer gas (DLco) is most frequently used and measures the ability of the lung to

transport gas to the red blood cells situated in the pulmonary capillaries. As such they reflect the properties of the alveolar-capillary membrane. Diffusion of CO over the alveolar-capillary membrane is comparable to that of oxygen, however the uptake of CO is limited by the pulmonary flow. In case of decreased pulmonary blood flow the diffusion capacity will be negatively affected. Diffusion capacity testing is indicated for the evaluation of subjects with airway obstruction or lung volume restriction. There are no absolute contra-indications or adverse events of diffusion testing, however when the vital capacity is below 1.5 liters most instruments are unable to measure the diffusion capacity reliably.

In the presence of emphysema the alveolar-capillary membrane is damaged, which will result in a lowered DLco. In subjects without emphysema, but with chronic bronchitis the DLco will be normal. Otherwise in subjects with emphysema and with or without chronic bronchitis the DLco will be lowered. A lowered DLco thus can aid in distinguishing subjects with emphysema from those without emphysema. Emphysema is by definition a pathological diagnosis. Good correlations between histological proven emphysema and CT-quantified emphysema (low attenuation areas) have been described. Holme et al showed that there was evidence for emphysema at CT present in subjects with a lowered transfer coefficient for carbon monoxide (Kco) but wit a still normal FEV_1/FVC. There is however little information present about the prognostic value of diffusion testing in heavy smokers. In **chapter 6** the predictive value of lung diffusion testing on the decline of lung function and progression of emphysema in heavy smokers is further explored.

Genetic risk factors for COPD

It is well known that not every smoker develops COPD. [37] However, it is not well understood why it is some smokers are susceptible and some are not. Genetic factors are deemed to play a role. Two important findings support this idea, firstly, severe $alpha_1$-anti-trypsin deficiency is a proven genetic risk factor for the development of emphysema, and secondly, lung function values are shown to be inheritable. [38] [39] Unfortunately no other single gene, besides the one for $alpha_1$-anti-trypsin, has been discovered yet.

A recent step in finding genetic factors associated with COPD was the introduction of genome-wide association scans (GWAS). This technique enables scanning of large

amounts of single nucleotide polymorphisms (SNPs) and provides a hypothesis-free approach. Current technique makes it possible to study 500,00 to 2 million SNPs at a time. SNPs are base pairs which may differ between subjects. Pillai et al were the first to publish a GWAS on COPD. They included 823 COPD cases and 810 smoking controls and identified two SNPs located on the alpha-nicotinic acetylcholine receptor loci 3 and 5 (*CHRNA* 3/5). [40] Both loci have been associated with lung cancer and nicotine dependence.[41] [42] Lambrechts et al. found that a polymorphism (rs1051730) of the nicotinic acetylcholine receptor on chromosome 15q24/25 locus, which is associated with lung cancer and COPD, is also associated with emphysema and emphysema severity.[43]

Hancock et al. performed a large meta-analysis including GWAS results from 20,890 identified five significant loci for pulmonary function. [44] Interestingly, the loci identified in the meta-analysis were most likely involved in the development of lung growth. These results indicate that the found SNPs are associated rather with lung development than with COPD.

Although, polymorphisms of *CHRNA* 3/5 have been associated with COPD and lung function at a cross-sectional level it is unclear whether these polymorphism also associate with lung function decline. It may be of importance to distinguish subjects with a more rapid lung function decline from those with merely a lower starting lung function level. In **chapter 7** we describe the results of a study investigating the association of two common polymorphisms of *CHRNA*, rs1051730 and rs8034191, with lung function decline.

Spirometric diagnosis of COPD

Since many years there has been discussion about the correct definition of COPD [45]. In 2001 the Global Initiative for Chronic Obstructive Lung Disease (GOLD) committee published a first consensus statement including recommendations on diagnosing COPD in order to standardize diagnosis worldwide. The most recent (GOLD) definition states that COPD is a preventable and treatable disease with both pulmonary and extra-pulmonary effects. The pulmonary component is mainly characterized by the presence of progressive airflow obstruction associated with abnormal inflammatory responses of the lung to noxious particles or gases. The extra-pulmonary components of COPD can vary considerably among patients leading to several of disease phenotypes. Due to this

heterogeneity it remains difficult to have unambiguous diagnostic criteria for COPD.

The GOLD defines COPD spirometrically as an FEV_1/FVC below 0.70 after administration of a bronchodilator. The severity of COPD is classified in four classes based on the post-bronchodilator FEV_1 level. However, the presence and severity of airflow obstruction not necessarily depict the severity of a subject's clinical symptoms like breathlessness and exercise capacity. The GOLD therefore states that COPD is a clinical diagnosis and that the presence of airflow obstruction is used to signal the diagnosis.

The European Respiratory Society (ERS) and the American Thoracic Society (ATS) however, define airflow obstruction as a FEV_1/FVC below the lower limit of normal (LLN) [46]. The LLN is statistically defined by the lower fifth percentile of a reference population and can be calculated by subtracting 1.645 times the standard deviation from the mean FEV_1/FVC in a reference population. [47] This mean FEV_1/FVC is the expected value for an individual given a certain age, height and gender. The LLN is therefore age, height and gender corrected. If in an individual the FEV_1/FVC value is below the expected LLN this indicates that their FEV_1/FVC is lower than 95% of individuals in an age, height and gender matched healthy reference population. Therefore the LLN heavily depends on the reference equation used to calculate the LLN. If the reference equation is not representative for the population it is used for it is questionable if the LLN will point at true abnormal values.

It is heavily debated which threshold is best. The main argument to prefer the LLN is that a fixed criterion may lead to over diagnosis in elderly subjects because the FEV_1/FVC is inversely correlated to age. One of the aims of the GOLD to introduce a fixed criterion of 0.70 is the fact that it is simple to use and that there is no need for reference values. Because it is recognized that COPD is still being underdiagnosed [48] increasing awareness of COPD is important.

In the past decade several studies have tried to elucidate which threshold is to prefer, but there is still no consensus. It has been shown that the sensitivity and specificity of the LLN were higher than that of the fixed value of 70%. However, the outcomes of those studies are predictable because they used the LLN as reference standard. Instead they should use an independent test as reference standard and compare both the 70% and LLN against this reference standard. Because there is no study that objectively

summarizes all studies comparing the <70% with the <LLN we performed an evidence-based literature search. In **chapter 8** an overview and appraisal is given of available literature comparing either definition of airflow obstruction, FEV_1/FVC <70% or <LLN.

According to the classical principles of clinical epidemiology one should evaluate diagnostic tests by comparing them to the gold standard test. In absence of a true gold standard for COPD it is difficult to compare the <70% approach to the <LLN approach. To overcome the current debate on the most appropriate cutoff of spirometric COPD we designed a study in which the FEV_1/FVC<70% and <LLN were compared against a panel diagnosis of COPD. This panel diagnosis of COPD acted as reference standard and enabled us to compare both thresholds against an independent reference standard. By using an independent reference standard, instead of using the LLN as reference standard, the presence of an imperfect-gold standard bias is minimized. The results of this study are presented in **chapter 9**.

Study populations: NELSON and FRESCO studies

The subjects studied in part 1 of this thesis all participated in the Dutch Belgian Lung Cancer Screening Trial, the NELSON, which is a randomized multi-center population-based study aimed on detecting lung cancer in heavy smokers (>20 packyears or more).[49] Due to their high tobacco exposition subjects included in the NELSON trial have a high-risk of developing lung cancer, but also a high risk to develop COPD. The exclusion criteria were aimed to include heavy smokers, who were otherwise relatively healthy. This group is of special interest because all participants have smoked enough to develop COPD and this allowed us to investigate the determinants of development of COPD in this population. Emphysema is frequently found on CT scans of heavy smokers, and therefore also in subjects participating in the NELSON study. For the same reason mentioned above, the NELSON population offers a unique opportunity to study the determinants of emphysema progression. Because the participants underwent CT-scanning and pulmonary function tests at baseline and after follow-up we could investigate factors associated with disease progression in time. Most of the previous published studies examined lung function decline and emphysema progression in subjects already with airflow obstruction present and in cross-sectional study designs.

The subjects described in part 2 of this thesis participated in the FRESCO study (From Respiratory Symptoms to COPD).[50] This study included subjects older than 50 years of age who visited their primary care physician because of persistent cough, but were not known with a respiratory disease. In these patients the diagnostic dilemma of COPD is most urgent and therefore this study population is well suited to compare the < 70 % and the LLN thresholds of FEV_1/FVC.

Aim and outline of this thesis

The major goal of the studies described in this thesis is to elucidate the role of lung function tests and CT scans in the early detection of COPD and to examine the validity of spirometric diagnosis of COPD

Part I of this thesis focuses on the determinants of lung function decline and emphysema progression in current and former heavy smokers, with or without airflow obstruction. Better characterization of subjects with an accelerated lung function decline and emphysema progression may enable a more aggressive risk-modifying approach in order to prevent COPD or to slow down the disease in an early stage of the disease.[51] Factors associated with future disease progression will provide important information about prognosis. It has been shown that smoking cessation therapy is more effective when subjects know that they have airflow obstruction.[52;53] The same could be true for knowledge on the risk of future disease progression or development. The combination of CT-scanning and PFT performed at the same day with follow-up CT-scanning and PFT enabled us to determine the role of emphysema and small airway disease in the development of airflow obstruction and progression of emphysema. **Chapter 2** describes the lung function decline and progression of emphysema according to the classification of airflow obstruction. The goal of this chapter is to investigate whether there are differences between subjects with a FEV_1/FVC (*i*) >70%, (*ii*) <70%, but >LLN and (*iii*) <LLN. **Chapters 3** and **4** focus on the role of CT-quantified emphysema in heavy smokers in predicting lung function decline and development of airflow obstruction. **Chapter 3** describes the association of the total extent of emphysema with lung function decline while **chapter 4** describes the association of the distribution of emphysema with lung function decline. **Chapter 5** includes the results of a study investigating the effect of duration of smoking cessation on lung function decline and emphysema progression. In **chapter 6** it was investigated whether diffusion testing

can aid to differentiate the lung function decline and emphysema progression in heavy smokers. Chapter **8** deals with the question whether variants in the 15q24/25 are associated with lung function decline in heavy smokers.

Part 2 of this thesis focuses on the question which definition of airflow obstruction is the most appropriate for diagnosing COPD. Because the prevalence of COPD is rising, but is still being under diagnosed in primary care, consensus on the spirometric definition of COPD is needed. **Chapter 8** describes the results of and evidence-based literature search aiming on answering whether there is sufficient evidence in the literature to conclude which definition of airflow obstruction is superior. **Chapter 8** describes results of a study comparing the diagnostic values of FEV_1/FVC <70% and LLN in diagnosing COPD in subjects with persistent cough by using a panel diagnosis as reference standard. In addition, **chapter 9** also describes whether there were differences in diagnostic values when using pre- and post-bronchodilator values.

In **chapter 10** the findings of the presented studies are discussed and recommendations are given for future research. **Chapter 11** summarizes the results of this thesis.

Reference List

1. Lopez AD, Shibuya K, Rao C et al. Chronic obstructive pulmonary disease: current burden and future projections. Eur Respir J 2006; 27(2):397-412.
2. Mannino DM, Buist AS. Global burden of COPD: risk factors, prevalence, and future trends. Lancet 2007; 370(9589):765-773.
3. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. Lancet 1997; 349(9064):1498-1504.
4. Qaseem A, Wilt TJ, Weinberger SE et al. Diagnosis and Management of Stable Chronic Obstructive Pulmonary Disease: A Clinical Practice Guideline Update from the American College of Physicians, American College of Chest Physicians, American Thoracic Society, and European Respiratory Society. Ann Intern Med 2011; 155(3):179-191.
5. Celli BR, Thomas NE, Anderson JA et al. Effect of pharmacotherapy on rate of decline of lung function in chronic obstructive pulmonary disease: results from the TORCH study. Am J Respir Crit Care Med 2008; 178(4):332-338.
6. Hogg JC. Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. Lancet 2004; 364(9435):709-721.
7. Kueppers F, Miller RD, Gordon H et al. Familial prevalence of chronic obstructive pulmonary disease in a matched pair study. Am J Med 1977; 63(3):336-342.
8. McCloskey SC, Patel BD, Hinchliffe SJ et al. Siblings of patients with severe chronic obstructive pulmonary disease have a significant risk of airflow obstruction. Am J Respir Crit Care Med 2001; 164(8 Pt 1):1419-1424.
9. Fletcher C, Peto R. The natural history of chronic airflow obstruction. Br Med J 1977; 1(6077):1645-1648.
10. Wood AM, Stockley RA. The genetics of chronic obstructive pulmonary disease. Respir Res 2006; 7:130.
11. Smolonska J, Wijmenga C, Postma DS et al. Meta-analyses on suspected chronic obstructive pulmonary disease genes: a summary of 20 years' research. Am J Respir Crit Care Med 2009; 180(7):618-631.
12. Snider G.L., Kleinerman J, Thurlbeck W.M. et al. The definition of emphysema. Report of a National Heart, Lung, and Blood Institute, Division of Lung Diseases workshop. Am Rev Respir Dis 1985; 132(1):182-185.
13. Snider G.L., Kleinerman J, Thurlbeck W.M. et al. The definition of emphysema. Report of a National Heart, Lung, and Blood Institute, Division of Lung Diseases workshop. Am Rev Respir Dis 1985; 132(1):182-185.
14. Uppaluri R, Mitsa T, Sonka M et al. Quantification of pulmonary emphysema from lung computed tomography images. Am J Respir Crit Care Med 1997; 156(1):248-254.
15. Quanjer PH, Tammeling GJ, Cotes JE et al. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993; 16:5-40.

16. Donaldson GC, Seemungal TA, Patel IS et al. Airway and systemic inflammation and decline in lung function in patients with COPD. Chest 2005; 128(4):1995-2004.
17. Rijcken B, Schouten JP, Xu X et al. Airway hyperresponsiveness to histamine associated with accelerated decline in FEV1. Am J Respir Crit Care Med 1995; 151(5):1377-1382.
18. Forbes LJ, Kapetanakis V, Rudnicka AR et al. Chronic exposure to outdoor air pollution and lung function in adults. Thorax 2009; 64(8):657-663.
19. Soriano JB, Sin DD, Zhang X et al. A pooled analysis of FEV1 decline in COPD patients randomized to inhaled corticosteroids or placebo. Chest 2007; 131(3):682-689.
20. Han MK, Agusti A, Calverley PM et al. COPD Phenotypes: The Future of COPD. Am J Respir Crit Care Med 2010.
21. Mannino DM, Reichert MM, Davis KJ. Lung function decline and outcomes in an adult population. Am J Respir Crit Care Med 2006; 173(9):985-990.
22. Rodriguez BL, Masaki K, Burchfiel C et al. Pulmonary function decline and 17-year total mortality: the Honolulu Heart Program. Am J Epidemiol 1994; 140(5):398-408.
23. Ryan G, Knuiman MW, Divitini ML et al. Decline in lung function and mortality: the Busselton Health Study. J Epidemiol Community Health 1999; 53(4):230-234.
24. Decramer M, Celli B, Kesten S et al. Effect of tiotropium on outcomes in patients with moderate chronic obstructive pulmonary disease (UPLIFT): a prespecified subgroup analysis of a randomised controlled trial. Lancet 2009; 374(9696):1171-1178.
25. Parr DG, Stoel BC, Stolk J et al. Validation of computed tomographic lung densitometry for monitoring emphysema in alpha1-antitrypsin deficiency. Thorax 2006; 61(6):485-490.
26. Coxson HO, Rogers RM, Whittall KP et al. A quantification of the lung surface area in emphysema using computed tomography. Am J Respir Crit Care Med 1999; 159(3):851-856.
27. Gevenois PA, De VP, Sy M et al. Pulmonary emphysema: quantitative CT during expiration. Radiology 1996; 199(3):825-829.
28. Gould GA, MacNee W, McLean A et al. CT measurements of lung density in life can quantitate distal airspace enlargement - an essential defining feature of human emphysema. AM REV RESPIR DIS 1988; 137(2):380-392.
29. Parr DG, Stoel BC, Stolk J et al. Validation of computed tomographic lung densitometry for monitoring emphysema in alpha1-antitrypsin deficiency. Thorax 2006; 61(6):485-490.
30. Coxson HO. Quantitative chest tomography in COPD research: chairman's summary. Proc Am Thorac Soc 2008; 5(9):874-877.
31. Hogg JC, Wright JL, Wiggs BR et al. Lung structure and function in cigarette smokers. Thorax 1994; 49(5):473-478.
32. Gietema HA, Zanen P, Schilham A et al. Distribution of emphysema in heavy smokers: impact on pulmonary function. Respir Med 2010; 104(1):76-82.
33. Mair G, Miller JJ, McAllister D et al. Computed tomographic emphysema distribution: relationship to clinical features in a cohort of smokers. Eur Respir J 2009; 33(3):536-542.

34. Telenga ED, Kerstjens HA, Postma DS et al. Inhaled corticosteroids in chronic obstructive pulmonary disease: a review. Expert Opin Pharmacother 2010; 11(3):405-421.

35. Willemse BW, Postma DS, Timens W et al. The impact of smoking cessation on respiratory symptoms, lung function, airway hyperresponsiveness and inflammation. Eur Respir J 2004; 23(3):464-476.

36. Bellomi M, Rampinelli C, Veronesi G et al. Evolution of emphysema in relation to smoking. Eur Radiol 2010; 20(2):286-292.

37. Fletcher C, Peto R. The natural history of chronic airflow obstruction. Br Med J 1977; 1(6077):1645-1648.

38. Wilk JB, DeStefano AL, Arnett DK et al. A genome-wide scan of pulmonary function measures in the National Heart, Lung, and Blood Institute Family Heart Study. Am J Respir Crit Care Med 2003; 167(11):1528-1533.

39. McCloskey SC, Patel BD, Hinchliffe SJ et al. Siblings of patients with severe chronic obstructive pulmonary disease have a significant risk of airflow obstruction. Am J Respir Crit Care Med 2001; 164(8 Pt 1):1419-1424.

40. Pillai SG, Ge D, Zhu G et al. A genome-wide association study in chronic obstructive pulmonary disease (COPD): identification of two major susceptibility loci. PLoS Genet 2009; 5(3):e1000421.

41. Hung RJ, McKay JD, Gaborieau V et al. A susceptibility locus for lung cancer maps to nicotinic acetylcholine receptor subunit genes on 15q25. Nature 2008; 452(7187):633-637.

42. Spitz MR, Amos CI, Dong Q et al. The CHRNA5-A3 region on chromosome 15q24-25.1 is a risk factor both for nicotine dependence and for lung cancer. J Natl Cancer Inst 2008; 100(21):1552-1556.

43. Lambrechts D, Buysschaert I, Zanen P et al. The 15q24/25 susceptibility variant for lung cancer and chronic obstructive pulmonary disease is associated with emphysema. Am J Respir Crit Care Med 2010; 181(5):486-493.

44. Repapi E, Sayers I, Wain LV et al. Genome-wide association study identifies five loci associated with lung function. Nat Genet 2010; 42(1):36-44.

45. Ciba Guest Symposium. Terminology, Definitions, and Classification of Chronic Pulmonary Emphysema and Related Conditions: A Report of the Conclusions of a Ciba Guest Symposium. 1959: 286-299.

46. Celli BR, MacNee W. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. Eur Respir J 2004; 23(6):932-946.

47. Pellegrino R, Viegi G, Brusasco V et al. Interpretative strategies for lung function tests. Eur Respir J 2005; 26(5):948-968.

48. Hill K, Goldstein RS, Guyatt GH et al. Prevalence and underdiagnosis of chronic obstructive pulmonary disease among patients at risk in primary care. CMAJ 2010; 182(7):673-678.

49. van Iersel CA, de Koning HJ, Draisma G et al. Risk-based selection from the general population in a screening trial: selection criteria, recruitment and power for the Dutch-Belgian randomised lung cancer multi-slice CT screening trial (NELSON). Int J Cancer 2007; 120(4):868-874.

50. Broekhuizen BD, Sachs AP, Hoes AW et al. Undetected chronic obstructive pulmonary disease and asthma in people over 50 years with persistent cough. Br J Gen Pract 2010; 60(576):489-494.

51. Decramer M, Cooper CB. Treatment of COPD: the sooner the better? Thorax 2010; 65(9):837-841.

52. Parkes G, Greenhalgh T, Griffin M et al. Effect on smoking quit rate of telling patients their lung age: the Step2quit randomised controlled trial. BMJ 2008; 336(7644):598-600.

53. Bednarek M, Gorecka D, Wielgomas J et al. Smokers with airway obstruction are more likely to quit smoking. Thorax 2006; 61(10):869-873.


Chapter
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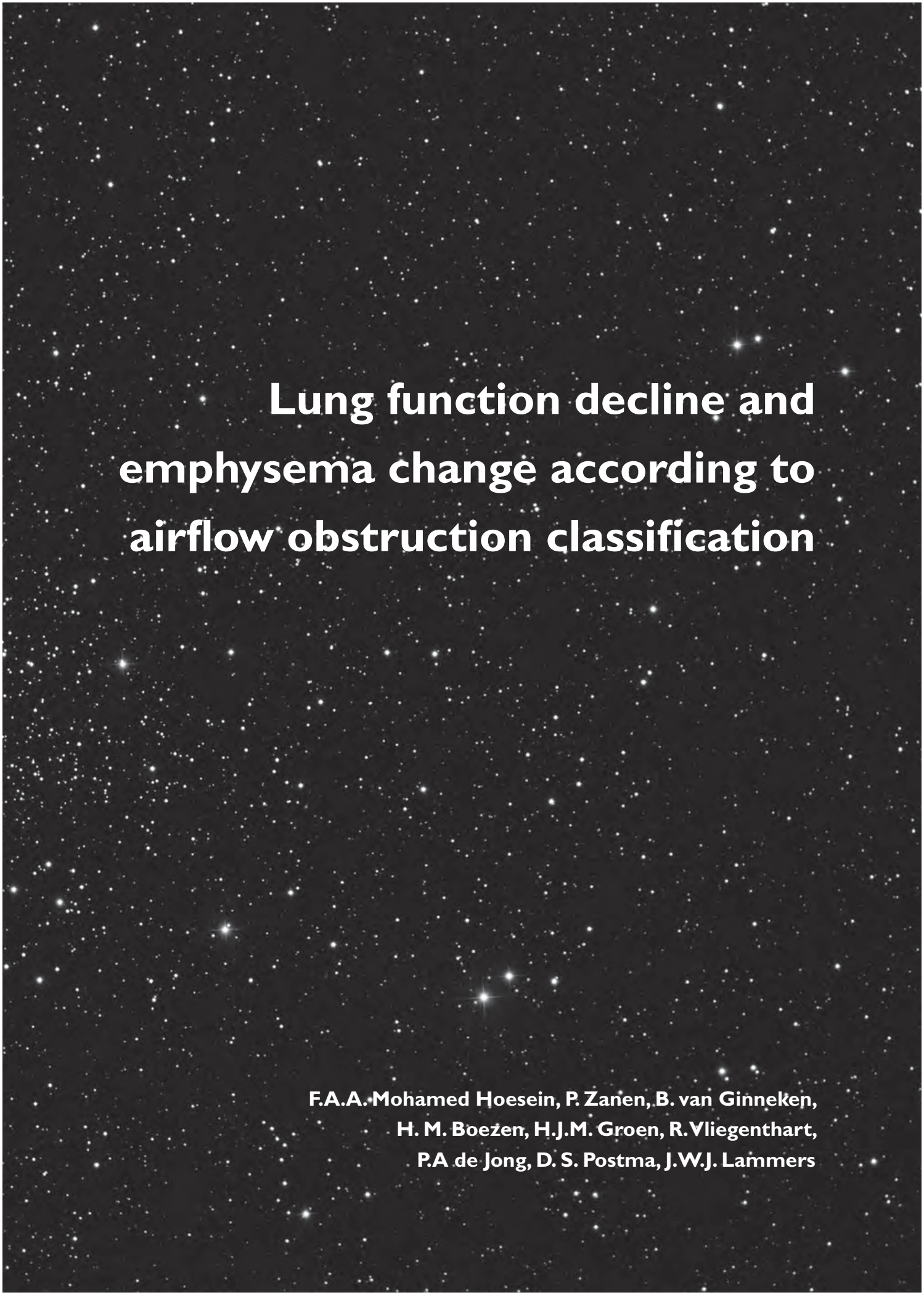

Lung function decline and emphysema change according to airflow obstruction classification

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Abstract

Purpose This study was conducted to assess lung function decline and CT-quantified emphysema change according to the severity of airflow obstruction in heavy smokers.

Methods In total, 2,003 male smokers with a mean (SD) age of 59.8 (5.3) years underwent pulmonary function testing and CT scanning at baseline and after follow-up. Participants were classified by entry FEV_1/FVC as follows: Group 1, >70%; Group 2, <70% but >LLN; and group 3, <LLN. Emphysema was quantified by lung densitometry (Perc15). Differences in lung function decline and Perc15 change among the groups were assessed using multiple regression.

Results Over three years, the mean (SD) FEV_1/FVC and FEV_1 decreases in Group 1 were 3.1% (1) and 0.21 L (0.07), respectively. In Group 3, these decreases were 2.4% (1.1) and 0.15 L (0.08), respectively. The mean (SD) emphysema change was 3.7 (0.4) HU in Group 1 and 9.1 (0.7) in Group 3. All lung function parameters showed the greatest decline in Group 1, but emphysema change was most substantial in Group 3 (all $p < 0.001$).

Conclusion The LLN approach selects the smallest number of subjects with rapid lung function decline but with the largest emphysema change.

Introduction

Chronic obstructive pulmonary disease (COPD) will be the third leading cause of death by 2020 and is the only chronic disease with increasing morbidity and mortality rates. [1] COPD includes emphysema and chronic bronchitis, which can coincide and both contribute to airflow obstruction. According to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria COPD is present when the ratio of forced expiratory volume in one second to the forced vital capacity (FEV_1/FVC) is below 70%, but the European Respiratory Society (ERS) and the American Thoracic Society (ATS) recommend the lower limit of normal (LLN) as the level for diagnosing COPD. [2 3] Thus far, no consensus exists as to which of these thresholds is better. [4 5]

Accelerated lung function decline is an important hallmark of COPD; however the diagnosis of COPD is often based on pulmonary function testing at a single time point. Emphysema can be assessed with chest computed tomography (CT) analysis of lung densitometry and may be present even before airflow obstruction manifests. [6] Furthermore, because COPD has a prolonged course, it likely begins before the FEV_1/FVC ratio falls below a predefined threshold.

Two large randomised clinical trials, the UPLIFT and TORCH studies, recently assessed the effect of pharmacological treatment on lung function in COPD patients. [7 8] The rate of decline was steepest in mild COPD and slower in moderate to severe COPD. However, differences in the dropout rates among the COPD stages offered an alternative explanation for this phenomenon, and no further analyses were performed to investigate this effect in more detail. We hypothesised that this phenomenon might also be present in heavy smokers with only mild or absent airflow obstruction.

The aim of the current study was to assess the decline in lung function and change in CT-quantified emphysema in relation to the severity of airflow obstruction in a cohort of relatively healthy heavy smokers. We compared three groups: Group 1 with FEV_1/FVC >0.70; Group 2 with FEV_1/FVC between 70% and LLN and Group 3 with FEV_1/FVC <LLN.

Methods

Participants

The study was conducted among participants of the Dutch Belgian Lung Cancer Screening Trial (NELSON), conducted by the University Medical Centres in Utrecht

and Groningen, the Netherlands. The NELSON study includes subjects at a relatively high risk of developing lung cancer/COPD and is population-based. [9] [10] At the start of the investigation, it was decided that this study provided the unique opportunity to also assess lung function in all participants and to investigate this factor in relation to CT measures. Therefore, spirometry was performed in all individuals.

The NELSON trial was approved by the Dutch Ministry of Health on December 23, 2003 and by the institutional review board of the University Medical Centre Utrecht, the Netherlands (approval number 03/040) The NELSON trial is registered at www.trialregister.nl with trial number ISRCTN63545820. Informed consent was obtained from all participants.

Subjects with a smoking history of at least 20 pack-years who were fit enough to undergo surgery were invited to participate. Only males with a high risk of developing lung cancer/COPD were included, as fewer women in the Dutch population have accrued long-term tobacco exposure. Patients with a moderate or poor self-reported health status or who were unable to climb two flights of stairs were excluded. Detailed information on smoking habits (duration of smoking, number of cigarettes per day), smoking status (current/former smoker) and self-reported respiratory symptoms (cough, mucus, dyspnea and wheezing) were collected via questionnaires at baseline.

Pulmonary function testing

Pulmonary function tests (PFT) were performed at baseline and after 3 years, according to ERS/ATS guidelines and with standardised equipment. [11] No broncho-dilatation was applied. FT measurements included forced expiratory volume in one second (FEV_1), FEV_1/forced vital capacity (FVC) ratio and maximum expiratory flow at 50% (MEF_{50}). The PFT was performed on the same day as the CT for each patient. Lower limits of normal were calculated using the reference equations of the European Community of Coal and Steel (ECCS). [12]

Baseline classification of airflow obstruction

At baseline, participants were classified according to FEV1/FVC. Group 1 comprised participants without airflow obstruction (FEV1/FVC >70% and hence >LLN), Group 2 included participants with an FEV1/FVC <70% but >LLN, and Group 3 included participants with airflow obstruction according to both criteria (FEV1/FVC <70% and <LLN).

CT Scanning

The CT protocol has been reported before. [13] All participants received low-dose CT without intravenous contrast injection. Sixteen-detector MDCT scanners were used (Mx8000 IDT or Brilliance 16P, Philips Medical Systems, Cleveland, OH; or Sensation-16 Siemens Medical Solutions, Forchheim, Germany). The scan data were obtained in spiral mode, with 16 x 0.75-mm collimation and at full inspiration No spirometric gating was applied since this does not improve reproducibility of lung density measurements. [14;15] Axial images were reconstructed with a 1.0-mm thickness at an increment of 0.7 mm. All scans were reconstructed with a soft reconstruction filter (Philips B, Siemens B30f) using a 512x512 matrix. The exposure settings were 30 mAs at 120 kVp or 140 kVp, depending on the participant's weight. This low-dose CT protocol has previously been used to quantify emphysema in COPD patients and heavy smokers. [16-18]

Emphysema quantification

All CT scans were automatically analysed. [19] Airways were excluded to ensure that only the lung parenchyma was analysed. Air calibration is critical in multicentre lung densitometry studies, and the incorporation of a correction factor is essential for quantitative image analysis [20]. Therefore, CT examinations were recalibrated using air in the trachea to ensure comparability between the two centres.

Emphysema severity was based on the 15th percentile (Perc15) technique. This technique provides the cut-off value in Hounsfield Units (HU) below which 15% of all voxels are distributed. Lower Perc15 values, i.e. those closer to -1000 HU, correspond to the presence of more emphysema. This method of emphysema quantification has been validated against pathology [6] and applied in multiple studies [21]. Because the Perc15 method is the most robust measurement of emphysema and its progression, it is preferred to the <-950 HU measurement (which is defined as the proportion of low-density voxels below -950 HU). [22]

Statistical analysis

Mean and standard deviation (SD) values were calculated for normally distributed data and median values and interquartile range (Q1-Q3) were calculated for non-normally distributed data. The distribution of normality was confirmed visually using probability plots. Previous research has shown that lung function decline can be assumed to have a linear course over a time span of 3 years. [7] Lung function parameters (FEV_1/FVC, FEV_1, MEF_{50}) and Perc15 at the end of the observation period were analysed using multiple

linear regression analysis with adjustment for packyears, smoking status (current/former smoker), centre, height, age and the appropriate baseline values of FEV_1/FVC, FEV_1, MEF_{50} and Perc15. A one-way ANOVA was used to compare declines among the three groups of airflow classifications. P <0.05 was considered significant. All statistical analyses were performed using SPSS 18 for Windows (SPSS, Chicago, Illinois, USA).

Results

Demographics, lung function and Perc15 at baseline

In total, 2,003 participants were included, with a mean (SD) age of 59.8 (5.3) years and 40.2 (17.6) smoked pack-years. Additional baseline demographics for the population are provided in Table 1.

FEV_1, FEV_1/FVC, MEF_{50} and Perc15 values at baseline for Group 1 (FEV_1/FVC>70% and >LLN), Group 2 (FEV_1/FVC<70% but >LLN) and Group 3 (FEV_1/FVC<70% and <LLN) are shown in Table 1.

Table 1. Baseline demographics of included participants for the total population and according to groups. Group 1 FEV_1/FVC >70% and >LLN, Group 2 FEV_1/FVC <70% but >LLN, and Group 3 FEV_1/FVC <70% and <LLN

	Total *n = 2,003*	Group 1 *n = 1,337*	Group 2 *n = 317*	Group 3 *n = 349*	P-value for group differences
Age [years]	59.8 (5.3)	59.5 (5.2)	61.1(5.4)	60.0 (5.5)	<0.001
Current smokers [%]	55.6	54.1	53.7	63.0	0.005
Packyears	40.2 (17.6)	39.3 (17.9)	41.7 (15.6)	42.2 (17.8)	0.004
FEV_1 [L]	3.4 (0.73)	3.65 (0.6)	3.22 (0.6)	2.63 (0.7)	<0.001
FEV_1 [% predicted]	98.5 (18.5)	105.1(14.3)	94.4(13.1)	76.4 (17.9)	<0.001
FEV_1/FVC [%]	72.2 (9.3)	77.3(4.7)	67.4(1.8)	56.8 (7.4)	<0.001
MEF_{50} [L]	3.2 (1.4)	3.80 (1.2)	2.36(0.5)	1.49 (0.6)	<0.001
Perc15 (HU)	-934.9 (19.5)	-931 (19.2)	-938.7 (15.5)	-946.1 (18.9)	<0.001

Lung function and Perc15 after follow-up

After 3 years of follow-up, the mean (SD) FEV_1/FVC was 69.3% (9.9); the FEV_1 was 3.21 L (0.72), or 95.4% (19.0) of the predicted value; and the MEF_{50} was 2.88 L/s (1.33), or 64.2% (29.1) of the predicted value (%pred). The mean (SD) Perc15 was -938.3 (15) HU. The data show a 2.9% (1.2) mean decline in the FEV_1/FVC; a 0.21 L (0.07) decline, or 3.2%pred (2.1), for FEV_1 and a 0.32 L/s (0.29) decline, or 5.5%pred (15.1), in MEF_{50}. The mean Perc15 decline was -3.5 (5.7) HU. The significances of the adjustment factors in the models are listed in Table 2.

Table 2. P-values for the adjustment factors in the regression model of follow-up lung function and Perc15 values. Only significant adjustment factors (in bold) were retained in the final model (see Table 3). * Baseline FEV_1/FVC

	Dependent variables			
Covariate	**FEV_1/FVC**	**FEV_1**	**MEF_{50}**	**Perc15**
Study Center	**<0.001**	**<0.001**	0.566	**<0.001**
Years in study	0.470	0.233	0.858	0.969
Age [years]	**0.020**	**<0.001**	**0.001**	0.208
Height [cm]	0.585	**0.002**	0.959	**0.045**
Smoking status (current/ former)	**0.001**	**<0.001**	**<0.001**	**0.004**
Packyears	0.065	**0.013**	**0.002**	0.689
Baseline lung function	**<0.001**	**<0.001**	**<0.001**	**<0.001***
Baseline Perc15 (HU)	**<0.001**	**0.004**	**<0.001**	**<0.001**

The final model and the change in FEV_1/FVC, FEV_1 and MEF_{50} per covariate unit change are given in Table 3.

Table 3: Results of the multiple linear regression analyses. Changes in A) FEV1/FVC, B) FEV1 [mL], C) MEF50 [mL/s] and D) Perc15 [HU] per covariate unit change and 95% confidence intervals (CI95%) over the 3-year follow-up period. Only the significant factors were retained in this model.

A.

Estimated effects of specified changes in covariates: effects on FEV_1/FVC in % after follow-up			
Covariate	**Increment or Comparison**	**Change in FEV_1/FVC**	**CI95%**
Study centre	Utrecht vs. Groningen	1.71	1.31 - 2.11
Age [years]	plus 10 years	-0.57	-0.92 – -0.35
Smoking status	current vs. former	-1.03	-1.4 – -0.66
FEV_1/FVC at baseline [%]	plus 1 %	0.93	0.91 – 0.95
Perc15 at baseline [HU]	decrease of 10 HU	-0.18	-0.19 – -0.07

B.

Estimated effects of specified changes in covariates: effects on FEV_1 in mL after follow-up			
Covariate	**Increment or Comparison**	**Change in FEV_1**	**CI95%**
Study centre	Utrecht vs. Groningen	23	0.00- 47
Age [years]	plus 10 years	-40.0	-60.0 – -20.0
Smoking status	current vs. former	-48	-68 – -28
Height [cm]	plus 10 cm	25	10 – 40
Packyears	plus 10 years	-10	-15 – -5
FEV_1 at baseline [mL]	plus 1 mL	0.91	0.89 – 0.93
Perc15 at baseline [HU]	decrease of 10 HU	-8.3	-14.6 – -2.1

C.

Estimated effects of specified changes in covariates : effects on MEF_{50} in mL/s after follow-up			
Covariate	**Increment or Comparison**	**Change in MEF_{50}**	**CI95%**
Age [years]	plus 10 years	-100	-150 – -50
Smoking status	current vs. former	-138	-189 – -87
Packyears	plus 10 years	-20	-30 – -10
MEF_{50} at baseline [mL/s]	plus1 mL/s	0.84	0.82 – 0.86
Perc15 at baseline [HU]	decrease of 10 HU	-30	-42 – -12

D.

Estimated effects of specified changes in covariates: effects on Perc15 in HU after follow-up			
Covariate	**Increment or Comparison**	**Change in Perc15**	**CI95%**
Study centre	Utrecht vs. Groningen	-4.86	-6.00 – -3.76
Height [cm]	plus 10 cm	+0.10	0.02 – 0.18
Smoking status	current vs. former	-1.64	-2.69 – -0.58
Perc15 at baseline [HU]	decrease of 1 HU	-0.74	-0.76 – -0.72

Values for adjusted lung function decline according to airflow obstruction classification (Groups 1-3) are provided in Table 4 and depicted in Figures 1-4. The declines in FEV_1/FVC, FEV_1 and MEF_{50} were significantly greater in Group 1 than in Group 3 ($p<0.001$). The FEV_1 and MEF_{50} declines in Group 2 were also significantly (all $p <0.001$) greater than in Group 3, but the decline in FEV_1/FVC was significantly ($p=<0.001$) more substantial in Group 3 than in Group 2, at 2.4% and 2.2%, respectively (see Table 4).

Figure 1. Mean (CI95%) adjusted FEV1/FVC decline over 3 years stratified by baseline classification of airflow obstruction.

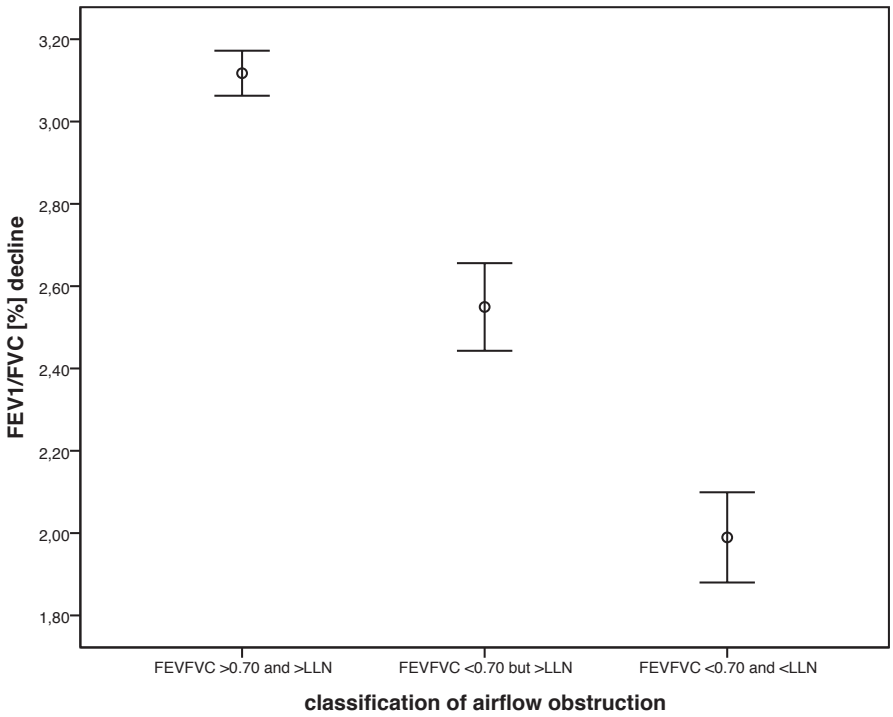


Figure 2. Mean (CI95%) adjusted FEV1 [L] decline over 3 years stratified by classification of airflow obstruction

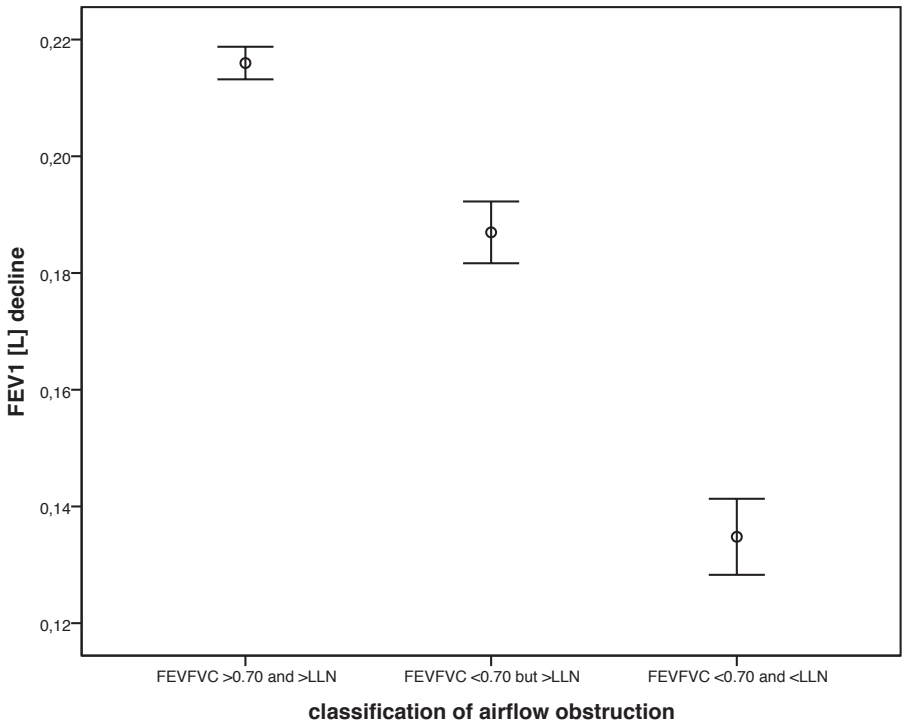


Figure 3. Mean (CI95%) adjusted [mL/s] decline over 3 years stratified by classification of airflow obstruction

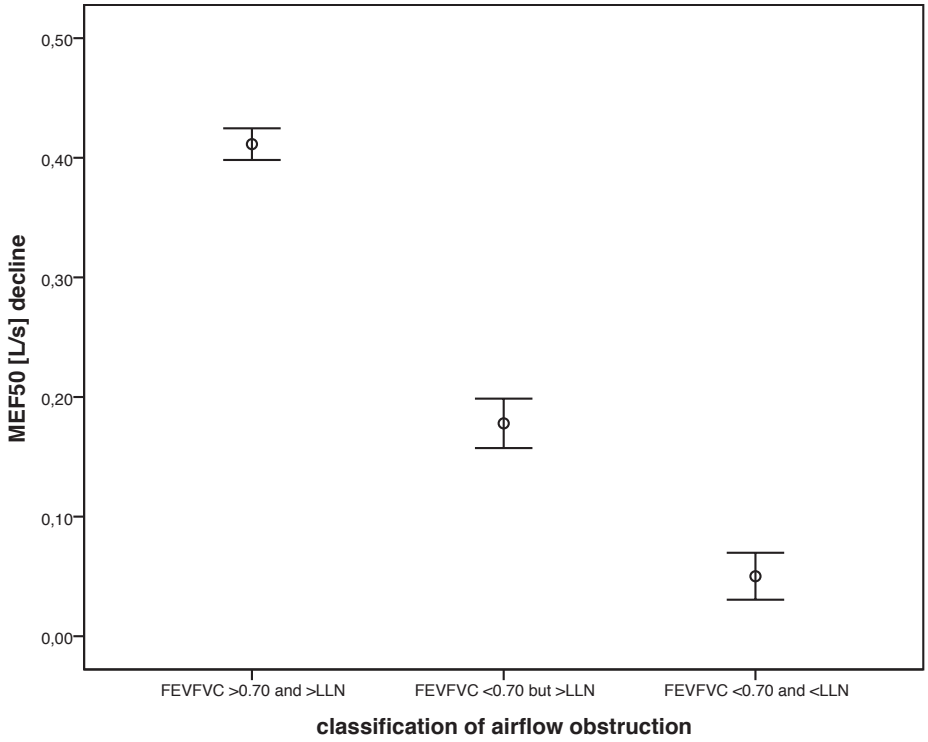
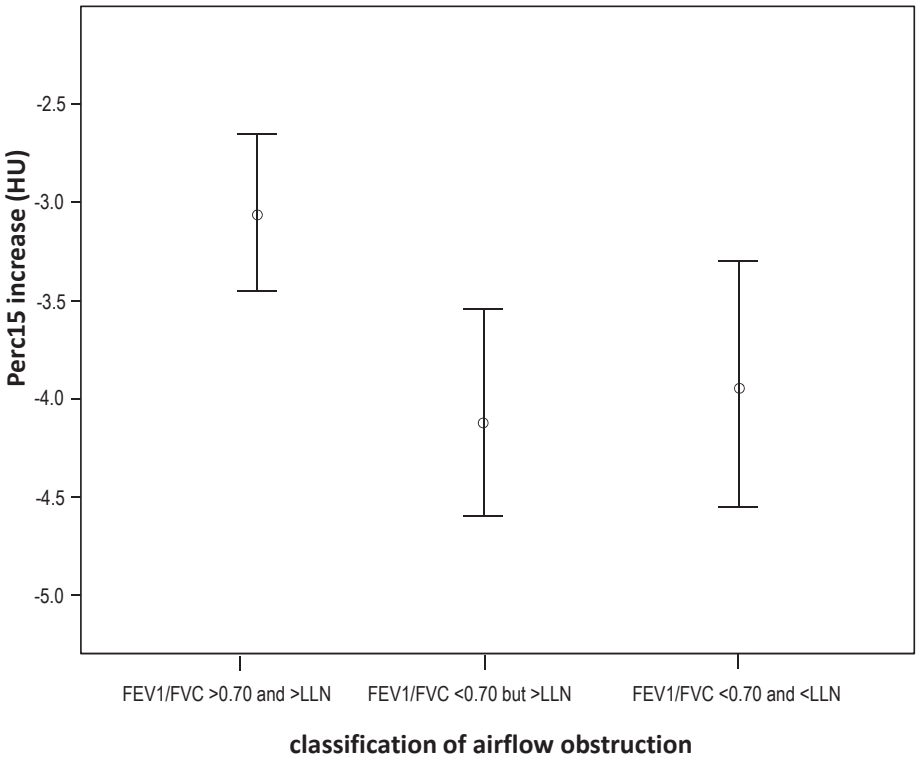


Figure 4. Mean (CI95%) adjusted Perc15[HU] change over 3 years stratified by classification of airflow obstruction



The Perc15 change was significantly reduced in Group 1 compared with Groups 2 and 3 (see Table 3 and Figure 4). There was no significant (p=0.681) difference in Perc15 change between Groups 2 and 3 (-4.1 and -3.9 HU, respectively).

Smoking status had comparable effects on the decline in lung function parameters and Perc15 change in all three groups, and the interaction between smoking status and the groups was not significant (p= 0.55).

Table 4. Mean adjusted declines in lung function parameters and Perc15 over the 3-year follow-up period, according to baseline classification of airflow obstruction. Significance levels indicate differences in comparison with Group 3 (one-way ANOVA). Group 1: FEV_1/FVC >70% and >LLN, Group 2: FEV_1/FVC <70% but >LLN, and Group 3: FEV_1/FVC <70% and <LLN

	FEV_1/FVC in % (SD)	**p-values**
group 1	3.1 (1.0)	<0.001
group 2	2.2 (1.0)	<0.001
group 3	2.4(1.1)	.
	FEV_1 in L (SD)	
group 1	0.21 (0.06)	<0.001
group 2	0.19 (0.05)	<0.001
group 3	0.14 (0.06)	.
	MEF_{50} in L/s (SD)	
group 1	0.40 (0.26)	<0.001
group 2	0.18 (0.20)	<0.001
group 3	0.06 (0.19)	.
	Perc15 in HU (SD)	
group 1	-3.1 (5.9)	0.004
group 2	-4.1 (4.8)	0.013
group 3	-3.9 (5.6)	.

Discussion

The results of this study show that the lung function decline is most pronounced in former and current heavy smokers with FEV_1/FVC >70% and less pronounced in those with more severe airflow obstruction. In addition, the Perc15 change is lowest in subjects with an FEV_1/FVC >70%. Our data suggest that screening for COPD based on the presence of more pronounced airflow obstruction as defined by FEV_1/FVC <LLN means that by the time of diagnosis, heavy smokers have already passed the phase of most rapid lung function decline and entered the phase of the strongest emphysema progression. These findings demonstrate the importance of the early recognition of airflow obstruction.

To our knowledge, no human studies thus far have assessed the effect of airflow obstruction classification on emphysema change. Animal models of smoking-induced emphysema have shown that once a critical threshold of smoking exposure has passed, emphysema progression will continue. [23] In humans, smokers with severe emphysema have increased inflammation in the lungs compared with smokers with no or mild emphysema. [24] In our cohort of heavy smokers, those with lower FEV_1/FVC values (<70% and <LLN) showed a greater Perc15 change than those with normal FEV_1/FVC values (>70%), indicating that prompt intervention, i.e., by smoking cessation, will have the greatest impact at an early stage of the disease.

Our findings are in agreement with the results from the UPLIFT and TORCH studies, but our data extend their observations, as we included subjects with better lung function [7 8] The UPLIFT placebo-treated GOLD Stage I/II subjects showed a greater decline in FEV_1 than Stage III subjects, and Stage III subjects showed a more substantial decline than Stage IV. The varying dropout rates among the UPLIFT GOLD groups prevented a valid analysis. A similar pattern was found in the placebo group of the TORCH study. The main difference between our study and the UPLIFT and TORCH studies is that UPLIFT and TORCH only included individuals with established COPD, whereas the majority of our subjects had either no or mild COPD. In addition, the studies by Donaldson et al. and Higashomoto et al., which investigated determinants of more rapid lung function decline in moderate to severe COPD subjects, found that an initially high baseline FEV_1 value predicted a faster decline. [25 26] We have confirmed these observations, but we also show these findings to be true for subjects with an FEV_1/FVC ≥70%.

The well-known Fletcher-Peto curve, which was originally published in 1977, has been used as the reference for lung function decline in smokers and ex-smokers. [27] According to this curve, the FEV_1 decline in smokers is initially slow and increases when lung function decreases, which is known as the horse-racing effect. [28] The results of our study indicate the opposite: lung function decline is steep at first and then becomes less profound as the lung function decreases (an inverse horse-racing effect). Although the study by Fletcher and Peto has been extensively cited, there is some criticism of its quality control. At the time, the spirometry equipment used in the study was not standardised, and measurements errors are likely to have occurred. Additionally, there was a large loss to follow-up, i.e., 344 subjects of 1,136 initially included, which may have led to under- and/or overestimation of the observed FEV_1 decline. Thus, the actual decline in subjects with normal FEV_1 values could have been underestimated if subjects with normal lung function were more likely to be absent from the follow-up data. Furthermore, it is

recognised that it was not feasible for the researchers to appropriately and adequately study changes over time at that period. [29] Taking all of these factors into account, it is at least debatable whether the findings by Fletcher and Peto are valid or need to be qualified.

The overall FEV_1 decline in Group 1 after a median 3-year follow-up period was 210 mL (70 mL/ year), which is higher than the values found in the general population, i.e., 29 mL/year [14], and hence 87 mL in three years. Even in Group 3, the 3-year FEV_1 decline exceeded the normal value by nearly a factor two. In the Atherosclerosis Risk in Communities (ARIC) cohort, the annual decline in FEV_1 was slightly lower (62 mL/ years) but comparable to ours. [30] The obvious explanation for this relatively high decline is that the NELSON trial included heavy smokers. Interestingly, in the ARIC cohort, subjects without airflow obstruction but with chronic cough and mucus (former GOLD 0) had a higher risk of COPD-related hospitalisation and mortality compared with subjects at higher GOLD stages, which indicates the importance of recognising patients exhibiting rapid decline without airflow obstruction. Participants in the Lung Health Study, all of whom had an FEV_1/FVC <70% but a comparable number of pack-years smoked, showed a decline of 52.3 ml/year. [31]

It is difficult to provide a pathophysiological explanation for our results, and it should be realised that the pathological processes leading to either airflow obstruction or emphysema likely overlap. In addition, emphysema also contributes to airflow obstruction via the loss of elastic recoil of the lung tissue. Previous studies have shown that inflammation is present in smokers with normal lung function, and that the number of inflammatory cells in the lungs is increased in these patients. [32;33] [34] Pierrou et al. found that the expression of genes involved in the oxidant/anti-oxidant response was higher in patients at GOLD stages 0 and 1 compared with GOLD stages 2-4. [35] Other studies have shown that the expression of matrix metalloproteinase 2 (MMP2), which is involved in extra-cellular matrix breakdown, decreased as FEV_1 declined. [36] Together, these studies indicate that part of the immune response and reaction to smoking exposure occurs in the early stages of the disease and also that this response declines in advanced COPD stages. The effects of this early immune response may elicit an FEV_1 decline even when FEV_1 values are normal.

Regression towards the mean could be suggested as a possible explanation for our findings, [37] as it can occur in longitudinal studies when subject dropout is non-random and when repetitive measurements are imperfectly correlated. [38] We have examined the occurrence of both of these factors and concluded that regression towards the mean

is unlikely in our study. The correlation coefficient between the baseline and follow-up lung function measurements was high (Pearson's *r*= 0.89). Furthermore, we analysed the data with an analysis of variance (ANOVA), which is the preferred method for analysing a continuous variable that is measured at baseline and again at the outcome. [39] Results from a more detailed analysis of the probability of regression towards the mean are presented in the supplementary files.

One of the strengths of this study is that it included a large number of smokers with a high number of pack-years smoked. These patients were frequently in a pre-COPD phase, which allowed the observation of early phenomena not seen in patients diagnosed by physicians. Furthermore, all participants underwent both longitudinal pulmonary function testing and CT scanning, allowing us to study the relationship between the two diagnostic modalities. Additionally, lung function was determined by well-qualified lab technicians and according to ATS/ERS standards, and the equipment used in both centres was shown to be interchangeable. [40] Furthermore, emphysema scores were quantified by the same software package, which eliminated the inter/intra observer variability that occurs with visual assessment of emphysema. Each scan was also recalibrated on tracheal air to avoid scanner bias. Failure to inspire at the same level during the follow-up could influence the emphysema quantification; however, in the current study, no significant differences were detected in inspiratory CT volumes at baseline and follow-up.

This study also has some limitations. First, the smoking status and the presence of symptoms were determined via questionnaires at baseline. As a result, we could not take into account that some quitters could have started smoking again, and conversely some smokers could have quit during the follow-up period. Second, no reversibility testing was applied, which could have led to lower FEV_1/FVC values. However, because the follow-up spirometry was similarly performed without reversibility testing, we believe that this limitation did not bias our results. Third, no females were included. Previous studies have shown that emphysema scores in females are lower than in men, and females also show a slower progression of emphysema. [41-43] Lastly, our follow-up time was 3 years; however, this interval is comparable to the follow-up period of the UPLIFT study. In addition, because we included a large number of former and current heavy smokers, we were able to report significantly large declines over the 3-year follow-up period. When relevant changes in factors such as lung function and CT-quantified emphysema are evident, additional observation time becomes unnecessary.

In conclusion, lung function decline in heavy smokers is greatest in those patients with a normal lung function according to GOLD or ERS/ATS criteria. Neither approach classifies

these individuals as having COPD, and these methods therefore miss the phase with the most rapid decline, which is the hallmark of COPD. Importantly, the emphysema change is lowest in subjects with normal lung function and highest in subjects with an FEV_1/FVC <0.70 and <LLN. These findings show that a diagnosis of COPD cannot be excluded in heavy smokers when based on an above-threshold PFT score at a single time point.

Reference List

1. Murray, C. J. and A. D. Lopez. 1997. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. *Lancet* 349:1498-1504.
2. Rabe, K. F., S. Hurd, A. Anzueto, P. J. Barnes, S. A. Buist, P. Calverley, Y. Fukuchi, C. Jenkins, R. Rodriguez-Roisin, W. C. van, and J. Zielinski. 2007. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am.J Respir Crit Care Med* 176:532-555.
3. Celli, B. R. and W. MacNee. 2004. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. *Eur.Respir J* 23:932-946.
4. Mannino, D. M. 2007. Defining chronic obstructive pulmonary disease... and the elephant in the room. *Eur.Respir J* 30:189-190.
5. Miller, M. R., O. F. Pedersen, R. Pellegrino, and V. Brusasco. 2009. Debating the definition of airflow obstruction: time to move on? *Eur.Respir J* 34:527-528.
6. Gould, G. A., W. MacNee, A. McLean, P. M. Warren, A. Redpath, J. J. Best, D. Lamb, and D. C. Flenley. 1988. CT measurements of lung density in life can quantitate distal airspace enlargement--an essential defining feature of human emphysema. *Am.Rev.Respir.Dis.* 137:380-392.
7. Tashkin, D. P., B. Celli, S. Senn, D. Burkhart, S. Kesten, S. Menjoge, and M. Decramer. 2008. A 4-year trial of tiotropium in chronic obstructive pulmonary disease. *N.Engl.J Med* 359:1543-1554.
8. Celli, B. R., N. E. Thomas, J. A. Anderson, G. T. Ferguson, C. R. Jenkins, P. W. Jones, J. Vestbo, K. Knobil, J. C. Yates, and P. M. Calverley. 2008. Effect of pharmacotherapy on rate of decline of lung function in chronic obstructive pulmonary disease: results from the TORCH study. *Am.J Respir Crit Care Med* 178:332-338.
9. van Iersel, C. A., H. J. de Koning, G. Draisma, W. P. Mali, E. T. Scholten, K. Nackaerts, M. Prokop, J. D. Habbema, M. Oudkerk, and R. J. van Klaveren. 2007. Risk-based selection from the general population in a screening trial: selection criteria, recruitment and power for the Dutch-Belgian randomised lung cancer multi-slice CT screening trial (NELSON) 1. *Int.J.Cancer* 120:868-874.
10. van Klaveren, R. J., M. Oudkerk, M. Prokop, E. T. Scholten, K. Nackaerts, R. Vernhout, C. A. van Iersel, K. A. van den Bergh, W. S. van 't, A. C. van der, E. Thunnissen, D. M. Xu, Y. Wang, Y. Zhao, H. A. Gietema, B. J. de Hoop, H. J. Groen, G. H. de Bock, O. P. van, C. Weenink, J. Verschakelen, J. W. Lammers, W. Timens, D. Willebrand, A. Vink, W. Mali, and H. J. de Koning. 2009. Management of lung nodules detected by volume CT scanning. *N.Engl.J.Med.* 361:2221-2229.
11. Miller, M. R., R. Crapo, J. Hankinson, V. Brusasco, F. Burgos, R. Casaburi, A. Coates, P. Enright, C. P. van der Grinten, P. Gustafsson, R. Jensen, D. C. Johnson, N. MacIntyre, R. McKay, D. Navajas, O. F. Pedersen, R. Pellegrino, G. Viegi, and J. Wanger. 2005. General considerations for lung function testing. *Eur.Respir J* 26:153-161.
12. Quanjer, P. H., G. J. Tammeling, J. E. Cotes, O. F. Pedersen, R. Peslin, and J. C. Yernault. 1993. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur.Respir J Suppl* 16:5-40.

13. Mohamed Hoesein, F. A., H. B. de, P. Zanen, H. Gietema, C. L. Kruitwagen, G. B. van, I. Isgum, C. Mol, R. J. van Klaveren, A. E. Dijkstra, H. J. Groen, H. M. Boezen, D. S. Postma, M. Prokop, and J.W. Lammers. 2011. CT-quantified emphysema in male heavy smokers: association with lung function decline. *Thorax*.

14. Gierada, D. S., R. D. Yusen, T. K. Pilgram, L. Crouch, R. M. Slone, K. T. Bae, S. S. Lefrak, and J. D. Cooper. 2001. Repeatability of Quantitative CT Indexes of Emphysema in Patients Evaluated for Lung Volume Reduction Surgery. *Radiology* 220:448-454.

15. Newell, J. D., Jr., J. C. Hogg, and G. L. Snider. 2004. Report of a workshop: quantitative computed tomography scanning in longitudinal studies of emphysema. *Eur Respir J* 23:769-775.

16. Mair, G., J. J. Miller, D. McAllister, J. Maclay, M. Connell, J. T. Murchison, and W. MacNee. 2009. Computed tomographic emphysema distribution: relationship to clinical features in a cohort of smokers. *Eur Respir J* 33:536-542.

17. Shaker, S. B., N. Maltbaek, P. Brand, S. Haeussermann, and A. Dirksen. 2005. Quantitative computed tomography and aerosol morphometry in COPD and alpha1-antitrypsin deficiency. *Eur Respir J* 25:23-30.

18. Sverzellati, N., E. Calabro, G. Randi, V. C. La, A. Marchiano, J. M. Kuhnigk, M. Zompatori, P. Spagnolo, and U. Pastorino. 2009. Sex differences in emphysema phenotype in smokers without airflow obstruction. *Eur Respir J* 33:1320-1328.

19. van Rikxoort, E. M., de Hoop B, M. A. Viergever, M. Prokop, and B. van Ginneken. 2009. Automatic lung segmentation from thoracic computed tomography scans using a hybrid approach with error detection. *Med.Phys.* 36:2934-2947.

20. Parr, D. G., B. C. Stoel, J. Stolk, P. G. Nightingale, and R. A. Stockley. 2004. Influence of calibration on densitometric studies of emphysema progression using computed tomography. *Am J Respir Crit Care Med.* 170:883-890.

21. Parr, D. G., B. C. Stoel, J. Stolk, and R. A. Stockley. 2006. Validation of computed tomographic lung densitometry for monitoring emphysema in {alpha}1-antitrypsin deficiency. *Thorax* 61:485-490.

22. Parr, D. G., M. Sevenoaks, C. Deng, B. C. Stoel, and R. A. Stockley. 2008. Detection of emphysema progression in alpha 1-antitrypsin deficiency using CT densitometry; methodological advances. *Respir.Res.* 9:21.

23. Wright, J. L. and A. Churg. 1990. Cigarette smoke causes physiologic and morphologic changes of emphysema in the guinea pig. *Am.Rev Respir Dis* 142:1422-1428.

24. Retamales, I., W. M. Elliott, B. Meshi, H. O. Coxson, P. D. Pare, F. C. Sciurba, R. M. Rogers, S. Hayashi, and J. C. Hogg. 2001. Amplification of inflammation in emphysema and its association with latent adenoviral infection. *Am.J.Respir.Crit Care Med.* 164:469-473.

25. Donaldson, G. C., T. A. Seemungal, I. S. Patel, A. Bhowmik, T. M. Wilkinson, J. R. Hurst, P. K. Maccallum, and J. A. Wedzicha. 2005. Airway and systemic inflammation and decline in lung function in patients with COPD. *Chest* 128:1995-2004.

26. Higashimoto, Y., T. Iwata, M. Okada, H. Satoh, K. Fukuda, and Y. Tohda. 2009. Serum biomarkers as predictors of lung function decline in chronic obstructive pulmonary disease. *Respir.Med.* 103:1231-1238.

27. Fletcher, C. and R. Peto. 1977. The natural history of chronic airflow obstruction. *Br.Med J* 1:1645-1648.

28. Burrows, B., R. J. Knudson, A. E. Camilli, S. K. Lyle, and M. D. Lebowitz. 1987. The "horse-racing effect" and predicting decline in forced expiratory volume in one second from screening spirometry. *Am.Rev Respir Dis* 135:788-793.

29. Linn, R. L. and J. A. Slinde. 1977. The Determination of the Significance of Change Between Pre- and Posttesting Periods. *Review of Educational Research* 47:121-150.

30. Mannino, D. M., M. M. Reichert, and K. J. Davis. 2006. Lung function decline and outcomes in an adult population. *Am.J.Respir.Crit Care Med.* 173:985-990.

31. Scanlon, PD, Connet , JE, Waller, LA, Altose, MD, Bailey, WC, Buist, SA, and Tashkin, DP. Smoking cessation and lung function in mild-to-moderate chronic obstructive pulmonary disease. Am J Respir Crit Care Med 161, 381-390. 2000. Ref Type: Generic

32. Cosio, M., H. Ghezzo, J. C. Hogg, R. Corbin, M. Loveland, J. Dosman, and P. T. Macklem. 1978. The relations between structural changes in small airways and pulmonary-function tests. *N.Engl.J.Med.* 298:1277-1281.

33. Wright, J. L., L. M. Lawson, P. D. Pare, S. Kennedy, B. Wiggs, and J. C. Hogg. 1984. The detection of small airways disease. *Am.Rev.Respir.Dis*. 129:989-994.

34. Amin, K., A. Ekberg-Jansson, C. G. Lofdahl, and P. Venge. 2003. Relationship between inflammatory cells and structural changes in the lungs of asymptomatic and never smokers: a biopsy study. *Thorax* 58:135-142.

35. Pierrou, S., P. Broberg, R. A. O'Donnell, K. Pawlowski, R. Virtala, E. Lindqvist, A. Richter, S. J. Wilson, G. Angco, S. Moller, H. Bergstrand, W. Koopmann, E. Wieslander, P. E. Stromstedt, S. T. Holgate, D. E. Davies, J. Lund, and R. Djukanovic. 2007. Expression of genes involved in oxidative stress responses in airway epithelial cells of smokers with chronic obstructive pulmonary disease. *Am.J.Respir.Crit Care Med.* 175:577-586.

36. Gosselink, J. V., S. Hayashi, W. M. Elliott, L. Xing, B. Chan, L. Yang, C. Wright, D. Sin, P. D. Pare, J. A. Pierce, R. A. Pierce, A. Patterson, J. Cooper, and J. C. Hogg. 2010. Differential expression of tissue repair genes in the pathogenesis of chronic obstructive pulmonary disease. *Am.J.Respir.Crit Care Med.* 181:1329-1335.

37. Suissa, S. 2008. Lung function decline in COPD trials: bias from regression to the mean. *Eur.Respir.J.* 32:829-831.

38. Davis, C. E. 1976. The effect of regression to the mean in epidemiologic and clinical studies. *Am.J Epidemiol.* 104:493-498.

39. Vickers, A. J. and D. G. Altman. 2001. Statistics notes: Analysing controlled trials with baseline and follow up measurements. *BMJ* 323:1123-1124.

40. Munnik, P., P. Zanen, and J. W. Lammers. 2006. A comparison of lung function equipment with emphasis on interchangeability and methods. *Physiol Meas*. 27:445-455.

41. Bellomi, M., C. Rampinelli, G. Veronesi, S. Harari, F. Lanfranchi, S. Raimondi, and P. Maisonneuve. 2010. Evolution of emphysema in relation to smoking. *Eur.Radiol.* 20:286-292.

42. Grydeland, T. B., A. Dirksen, H. O. Coxson, S. G. Pillai, S. Sharma, G. E. Eide, A. Gulsvik, and P. S. Bakke. 2009. Quantitative computed tomography: emphysema and airway wall thickness by sex, age and smoking. *Eur.Respir J* 34:858-865.

43. Soejima, K., K. Yamaguchi, E. Kohda, K. Takeshita, Y. Ito, H. Mastubara, T. Oguma, T. Inoue, Y. Okubo, K. Amakawa, H. Tateno, and T. Shiomi. 2000. Longitudinal follow-up study of smoking-induced lung density changes by high-resolution computed tomography. *Am. J Respir Crit Care Med* 161:1264-1273.

Lung function decline and emphysema change according to airflow obstruction classification

Results analyses regression towards the mean

In theory, the drop-out of non-declining FEV_1/FVC >70% and of rapid-declining FEV_1/FVC <LLN subjects could explain the outcome and decline becomes over and under estimated, respectively. In other words, the drop out of non-susceptible or highly susceptible subjects, respectively. We feel that this is improbable as it requires sensing of no / rapid decline without having access to PFT equipment or of diseases status. Secondly, regression toward the mean occurs when repetitive measurements are imperfectly correlated. [1] A last reason why regression towards the mean is not probable is that emphysema progression was strongest in group 3. Selective drop out as a sole explanation of the lung function decline / emphysema increase outcomes would require the drop-out of non-declining FEV_1/FVC >70% with strong progression of emphysema subjects and of rapid-declining FEV_1/FVC <LLN with no progression subjects. Therefore regression toward the mean can not explain our results.

Methods

Differences in presence of self-reported respiratory symptoms at baseline (cough, mucus, dyspnea and wheezing), percentage of current smokers and smoked packyears between subjects who underwent PFT and CT-scanning twice and those only once, were assessed by chi-square tests and independent sample t-tests. For subjects who underwent PFT twice Pearson correlation was calculated between first and second PFT.

Results

Drop-out was first of all minimal and baseline respiratory symptoms (cough, mucus, dyspnea and wheezing) of those, who did not return, were not significantly different to those who did return. Non drop-outs had lower number of packyears than drop-outs, respectively 40.2 (17.6) and 42.4 (18.4) ($p = 0.003$), but these small differences offer no good explanation.

2,003 subjects were included in this study from a total of 3,048. All 2,003 included subjects underwent PFT and CT-scanning at least twice and were a random sample from the total study population of 3,048 subjects. There were no significant differences in the presence of any respiratory symptoms (cough, mucus, dyspnea and wheezing) between subjects included in this study and subjects excluded. Included subjects had a significantly (p value = 0.003) lower smoked packyears than excluded subjects, respectively 40.3 (17.1) and 42.4 (18.4).

In our study there was a high correlation ($r=0.89$) between the first and second PFT rendering regression toward the mean unlikely.

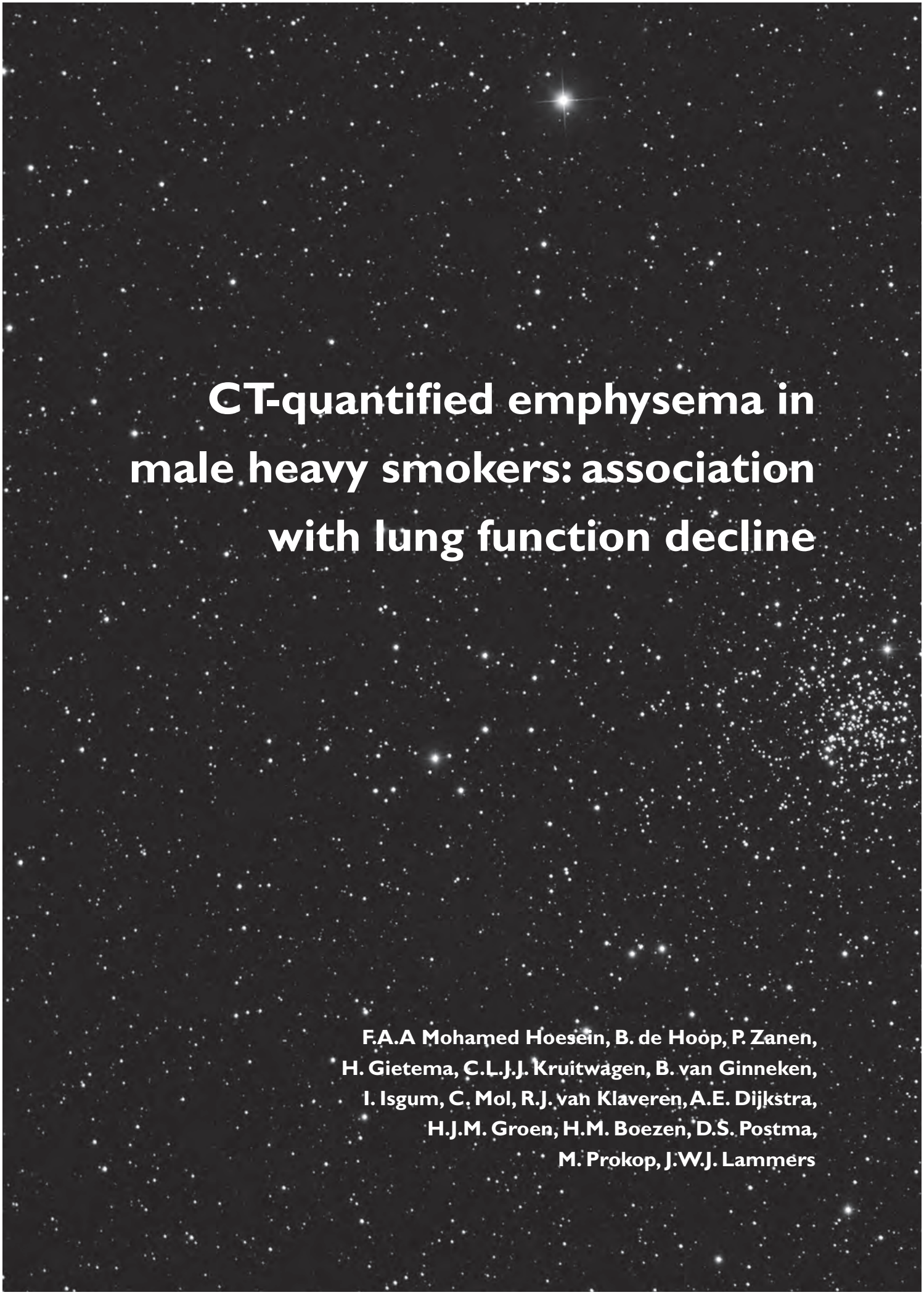
Reference List

1. Davis, C. E. 1976. The effect of regression to the mean in epidemiologic and clinical studies. *Am.J Epidemiol.* 104:493-498.

Part I

Lung function decline and progression of emphysema


Chapter
3



CT-quantified emphysema in male heavy smokers: association with lung function decline

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Abstract

Purpose Emphysema and small airway disease both contribute to COPD, a disease characterized by accelerated lung function decline. We investigated whether the extent of emphysema in male current and former smokers associates with stronger lung function decline.

Methods We included current and former heavy smokers participating in a lung cancer screening trial and all underwent CT. Spirometry was performed at baseline and at 3-year follow-up. The 15th percentile (Perc15) was used to asses the severity of emphysema.

Results We included 2,085 males, mean age 59.8 years. Mean (standard deviation) baseline Perc15 was -934.9HU (19.5). A lower Perc15 correlated with a lower FEV_1 ($r=0.12$) at baseline ($p<0.001$). Linear mixed model analysis showed that a lower Perc15 significantly related to a stronger declined in FEV_1 ($p<0.001$) after follow-up. Participants without baseline airway obstruction, but developing it after follow-up, had significantly lower mean (SD) Perc15 values at baseline than those who remained non-obstructive: -934.2HU (17.1) versus -930.2HU (19.7) ($p<0.001$).

Conclusion Greater baseline severity of CT-emphysema is related to lower baseline lung functions and stronger rates of lung function decline, even in those without airway obstruction. CT-detected emphysema aids to identify non-obstructed male smokers who will develop airflow obstruction.

Introduction

Chronic obstructive pulmonary disease (COPD) is a common lung disease that will likely become the fourth ranking cause of death worldwide by 2020.[1] Unfortunately, it often remains under diagnosed, especially in elderly people.[2] Tobacco smoking is the main risk factor for developing COPD but not every smoker will develop COPD. [3] Currently, it is hard to predict which persons are susceptible to smoking and will develop COPD. Factors predictive of future pulmonary function decline in subjects who do not show airflow obstruction yet may help to identify COPD patients in early stages of their disease, when treatment and stabilizing the disease via smoking cessation are most effective.

COPD is characterized by a mixture of small airway disease (obstructive bronchiolitis) and parenchymal destruction (emphysema) and their relative contributions vary among COPD patients. [4] Signs of emphysema are frequently found on Computed Tomography (CT) scans performed in lung cancer screening trials, studying heavy smokers who are also at great risk to develop COPD, but this CT-detected emphysema often present without airway obstruction. [5] Destruction of alveoli in persons with emphysema results in a lower lung density that can be quantified with lung densitometry, which assesses the relationship between tissue density and its X-ray attenuation on CT.

Whether the presence of low attenuation areas is a risk factor for stronger lung function decline has not been established so far. Yuan et al. reported a significant relation of lung function decline with overinflation on CT-scan, but not with the extent of low attenuation areas (assessed via the percentage lung volume <-950 HU). [6] On the other hand, Remy-Jardin et al. reported that subjects with visually scored emphysema showed a more rapid decline in pulmonary function than in those without emphysema. [7] Although the sample sizes of both studies were rather small: respectively 143 and 111 subjects, respectively, these results suggest that signs of emphysema on CT scans may be predictive of future lung function decline.

Since not all (heavy) smokers will develop COPD, factors predictive for lung function decline such as the extent of low attenuation areas in e.g. individuals without airway obstruction, may help to identify smokers who will develop COPD. This is important since stabilizing the disease via smoking cessation is a major component of COPD management.

The purpose of the study was to assess whether the presence / severity of low attenuation areas at CT as measured at the start of a lung cancer screening trial is

associated with additional lung function decline in heavy (non-obstructive) smokers or the development of COPD.

Material and Methods

Participants

The study was conducted among participants of the Dutch-Belgian Lung Cancer Screening Trial (NELSON), included by the University Medical Utrecht and the University Medical Groningen, the Netherlands. The NELSON trial included subjects at a high risk to develop lung cancer / COPD and is a population based study. [8, 9] The trial was approved by the Dutch Ministry of Health and by the ethics committee of each participating hospital. Written informed consent of each participant was obtained. Detailed information on smoking habits (duration of smoking, number of cigarettes per day and) and smoking status (current/ former smoker) were collected through questionnaires at baseline. Participants meeting the inclusion criteria of having smoked a minimum of 16 cigarettes/day for 25 years or 11 cigarettes/day for 30 years and fit enough to undergo surgery were invited to participate. Those with moderate or poor self-reported health status or unable to climb two flights of stairs were therefore excluded. Only males were included in the study based on the high risk to develop lung cancer/ COPD as fewer women in the Dutch population have accumulated a long-term exposure to cigarettes compared to men. 8 While heavy smokers participating in a lung cancer screening trial are also at high risk to develop COPD, participants of the NELSON trial also underwent spirometry to assess the prevalence and severity of airflow obstruction in the study population.

CT Scanning and Quantification of emphysema

All participants received low-dose CT without intravenous contrast injection at baseline and after 3-year follow-up. At both screening sites 16-detector MDCT scanners were used (Mx8000 IDT or Brilliance 16P, Philips Medical Systems, Cleveland, OH, or Sensation-16 Siemens Medical Solutions, Forchheim, Germany). Scan data were obtained in spiral mode, with 16 x 0.75mm collimation and in full inspiration. No spirometric gating was applied since it has been reported that this does not improve repeatability of lung density measurements. [10, 11] Axial images were reconstructed with 1.0mm thickness at 0.7mm increment. All scans were reconstructed with a soft reconstruction filter (Philips B, Siemens B30f) at a 512x512 matrix. All scans were in spiral mode with 16 × 0.75mm

collimation and 15mm table feed per rotation, pitch=1.3. Exposure settings were 30mAs at 120kVp or 140kVp, depending on participant's weight resulting in CTDIvol values of 1.6mGy and 3.2mGy. Effective dose was <0.9mSv and 1.6<mSv, respectively. This low-dose CT protocol was applied in order to reduce the risk of inducing a neoplasm due to radiation and has previously been used to quantify emphysema in COPD patients and heavy smokers. [12-14]

All CT scans were automatically analyzed with in-house developed software.[15] The software allows for automatic, high-precision 3-dimensional image analysis by segmentation of the lungs with an algorithm based on region growing and morphological processing. [15] Airways were excluded to ensure that only lung parenchyma was analyzed. Air calibration is critical in multicenter lung densitometry studies and incorporation of a correction factor is essential for quantitative image analysis. [16] Therefore, CT-examinations were recalibrated using air in the trachea to ensure comparability between the two centres.

Severity of emphysema was estimated based on the 15th percentile (Perc15) technique. This technique provides the Hounsfield Units (HU) point below which 15% of the voxels are distributed, see Figure S1. The lower the Perc15 values are, i.e. closer to -1000 HU, the more emphysema is present. This method of emphysema quantification has been validated against pathology [17] and has been applied in multiple studies. [18] A secondary analysis was done using the <-950 HU approach and the outcome is reported in the supplementary files. The Perc15 is preferred to the <-950 HU measurement, which is defined as the proportion of low density voxels below -950 HU, because the Perc15 is the most robust measurement of emphysema and its progression. [19] See Figure E3.

Spirometry

Spirometry included forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), FEV_1/FVC ratio and maximum expiratory flow at 50% of FVC (MEF_{50}). Bronchodilator response was not assessed. Spirometry was performed on the same day as the screening CT and was repeated after three years of follow-up. All participants with an FEV_1/FVC <70% at baseline were labelled as having airflow obstruction.

Statistical evaluation

Mean and standard deviation (SD) values were calculated for normally distributed data and median and interquartile range for non-normally distributed data. Student's t-tests and chi-square tests were used to test differences between groups as appropriate.

Pearson's correlations were used to establish associations between normally distributed variables at baseline.

Previous research showed that lung function decline is linear over a time span of 3 years [20] FEV_1, FEV_1/FVC, and MEF_{50} values over time were therefore analyzed via a random intercept, random slope linear mixed model analysis. Time of observation / intercept was chosen as random parameters, all other parameters were considered to be fixed. The choice of the covariance matrix fell on the unstructured one, based on a comparison of the -2 restricted log likelihood values. The results of the analyses for FEV_1/FVC and MEF_{50} are presented in the supplementary files. Baseline low attenuation area at CT (Perc15) was the main explanatory factor. We adjusted for packyears smoked, smoking status, centre, height, BMI and age to obtain corrected lung function parameters. We inserted interactions between Perc15 and absence / presence of airflow obstruction, between Perc15 and smoking status and between Perc15 and packyears to test whether the association between Perc15 and lung function decline was also dependent on the presence of baseline obstruction or on tobacco exposure. [21] Decline was calculated by subtracting corrected t=3 years lung function values from the baseline ones.

The secondary analysis using the <-950 HU as main explanatory factor followed the same approach (results are shown in the supplementary files).

Via logistic regression analysis a sub analysis was done in participants with an FEV_1/FVC > 70% at baseline in order to examine the association between Perc15 and the probability of showing airflow obstruction after follow-up.

P-values ≤ 0.05 were considered significant. All statistical analyses were performed using SPSS 18 for Windows (SPSS, Chicago, Illinois, USA).

Results

Baseline demographics

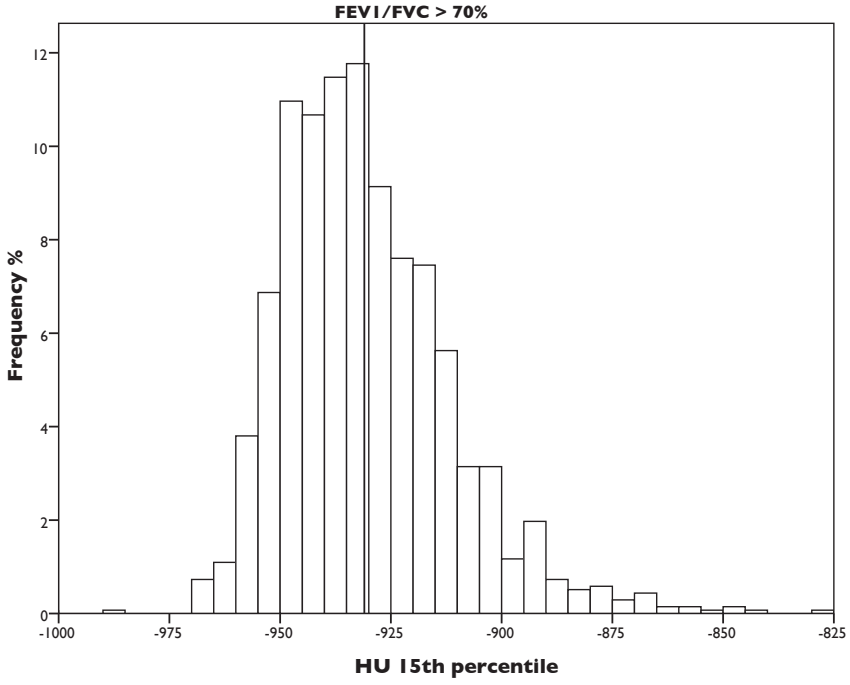
Baseline spirometry and CT-scanning data were available in 3,084 participants. A random sample of 2,254 out of these 3,084 participants underwent follow-up spirometry. There were no relevant differences in baseline spirometry and Perc15 values between participants with baseline spirometry only and with follow-up spirometry (see supplemental files). Out of these 2,254 participants 169 were excluded because the soft-ware failed to measure the Perc15 and/ or co-variates information was missing.

Table 1: Participants demographics at baseline for the cohort, broken down by obstruction status. Mean and standard deviation are provided. * median (interquartile range)

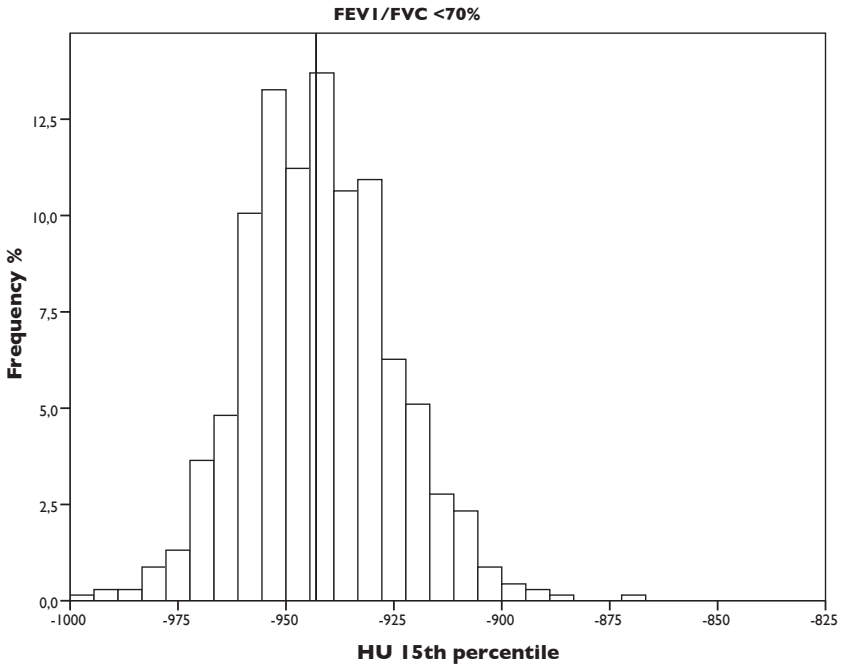
	Total cohort	FEV_1/FVC >70%	FEV_1/FVC <70%
Number of participants	2,085	1,391	694
Age years	59.8 (5.3)	59.4 (5.2)	60.5 (5.5)
Height meters	1.78 (0.06)	1.79 (0.06)	1.78 (0.06)
Weight kilograms	85.4 (12.3)	87.0 (12.0)	82.5 (12.3)
BMI (kilograms*meter^{-2})	26.9 (3.4)	27.2 (3.2)	26.2 (3.5)
Years in study*	3.0 (2.9 - 3.1)	3.0 (2.9 - 3.1)	3.0 (2.9 – 3.1)
Packyears smoking	40.3 (17.8)	39.4 (18.2)	42.0 (16.7)
Current smokers (%)	54.9	53.2	58.4
GOLD classification			
Normal (%)	66.4	100	0
Stage I (%)	21.5	0	64.5
Stage II (%)	10.5	0	30.8
Stage III (%)	1.6	0	4.7
Emphysema severity (Perc15)	-934.9 (19.5)	-931.0 (19.2)	-942.5 (17.7)

Figure 1: Histograms of Perc15 at baseline for participants with (A) and without (B) airflow obstruction. The vertical line indicates the mode in both histograms. Although the COPD participants (FEV_1/FVC <70%) had significantly lower Perc15 scores, a large overlap in baseline Perc15 values was found between participants with and those without COPD.

A. with airflow obstruction



B. without airflow obstruction



In total 2,085 participants were included in the current study. Mean (SD) age was 59.8 (5.3) years and mean packyears smoking 40.3 (17.8). The majority of participants had an FEV_1/FVC >70% (66.4%). Further participants' demographics are provided in Table 1. Median (interquartile range) follow-up time between the first and the last lung function test was 3 years (2.9 – 3.1).

Baseline CT-quantified emphysema (Perc15)

Overall mean (SD) baseline Perc15 was -934.9 HU (19.5). Participants with an FEV_1/FVC <70% had a mean (SD) Perc15 of -942.6 HU (17.6), and participants with FEV_1/FVC >70% -931.0 HU (19.2) ($p < 0.001$) (fig 1.) Increasing age was correlated to lower Perc15 values ($r = -0.21$, $p<0.001$). Current smokers had on average higher Perc15 values than former smokers, respectively -929.7 HU (19.5) and -941.3 HU (17.3), ($p<0.001$). There was no significant correlation between baseline Perc15 and the number of packyears smoking ($p=0.17$). Baseline demographic characteristics stratified by quintiles of baseline Perc15 are presented in Table 2.

Table 2: Participants demographics at baseline stratified by quintiles of baseline Perc15. Mean and standard deviation are provided.

* median (interquartile range), quintiles are based on the number of scanned cases.

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
Emphysema severity (Perc15)	-906.5 (14.2)	-927.3 (3.5)	-937.0 (2.5)	-945.9 (2.6)	-959.2 (9.0)
Number of participants	422	420	413	418	412
Age years	58.0 (4.7)	59.0 (4.7)	60.4 (5.5)	60.4 (5.3)	61.3 (5.8)
Height meters	1.78 (0.07)	1.79 (0.06)	1.79 (0.06)	1.79 (0.06)	1.79 (0.06)
Weight kilograms	85.0 (13.01)	85.7 (12.2)	84.8 (12.4)	86.7 (11.9)	84.3 (11.8)
BMI (kilograms*meter^{-2})	26.9 (3.3)	27.0 (3.3)	26.6 (3.3)	26.9 (3.3)	26.9 (3.3)
Years in study*	3.0 (2.9 - 3.1)	3.0 (2.9 - 3.1)	3.0 (2.9 - 3.1)	2.9 (2.9 - 3.1)	3.0 (2.9 - 3.1)
Packyears smoking	40.1 (16.6)	40.4 (18.9)	40.3 (18.0)	40.5 (17.2)	40.2 (18.1)
Current smokers (%)	78.0	67.4	49.2	43.5	34.1
FEV_1 L	3.49 (0.62)	3.46 (0.63)	3.44 (0.66)	3.45 (0.78)	3.21 (0.89)
FEV_1/FVC %	76.6 (6.8)	73.6 (7.7)	73.0 (7.7)	71.1 (9.0)	66.16 (11.6)
GOLD classification					
Normal (%)	83.4	71.4	69.3	63.9	43.7
Stage I (%)	11.4	21.4	20.4	23.9	31.3
Stage II (%)	5.2	6.4	9.3	10.0	20.5
Stage III (%)	0	0.7	1.0	2.2	4.3

Baseline lung function

Mean baseline FEV_1 was 3.4 L (0.77), which is 98.5% (18.5) of predicted and FEV_1/FVC was 72.2% (9.4). Participants with lower Perc15 values had lower lung function values: FEV_1 ($r=0.12$, $p<0.001$) and FEV_1/FVC ($r=0.39$, $p<0.001$).

Lung function decline

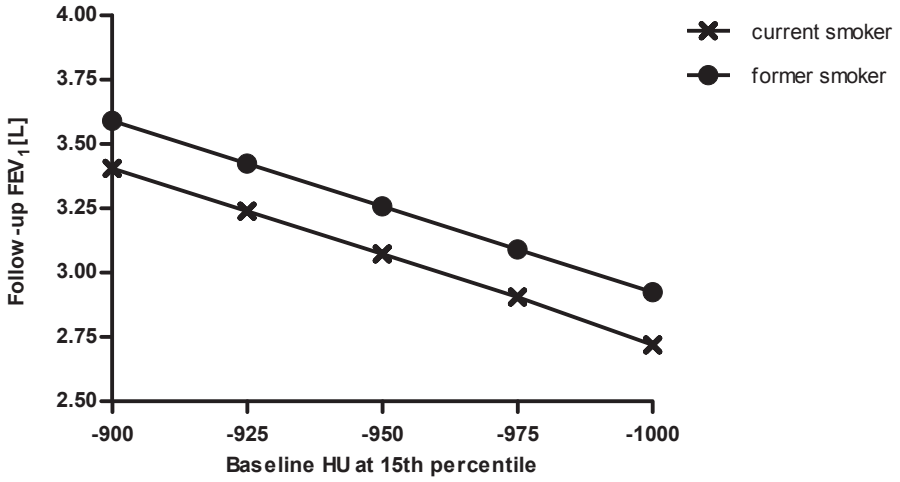
Mean FEV_1 after the follow-up period was 3.21 L (0.72) or 95.4% (18.9) of predicted and mean FEV_1/FVC was 69.3% (9.4). The average FEV_1-decline in the total cohort during the follow-up was 0.19 L (0.07) or 5.9% (2.1) of predicted.

Lower Perc15 values at baseline are associated with significantly lower FEV_1 ($p=<0.0001$) levels at follow-up. The effect sizes of Perc15 and of the adjustment factors are shown in Table 3 and can be illustrated as follows: when two individuals show an identical baseline FEV_1, the one with a 10 HU lower baseline Perc15 will show a 47.5 mL (CI95% 33.0 to 61.0 mL) lower in FEV_1 after one year, for further details see Figure 2.

Table 3: Results linear mixed models analysis for the cohort. Drop in FEV_1 per unit change in covariable. P-values and 95% confidence interval (CI95%) are provided.

Estimated effects of specified increments in covariables: changes in FEV_1 mL				
Covariate	**Increment or Comparison**	**Drop in FEV_1 per unit change in covariable**	**CI 95%**	**P-value**
Study centre	Utrecht vs. Groningen	+43.5	-100 – 13.8	0.137
Years in study	Plus 1 year	-65.6	- 69.4 – -61.6	<0.001
Smoking status	current vs. former	-186.7	-241.0 – -132.2	<0.001
Age years	plus 1 year	-36.2	-41.3 – -31.1	<0.001
Height cm	plus 1 cm	+38.6	34.7 – 42.7	<0.001
Packyears	plus 1 year	-5.16	-6.62 – -3.68	<0.001
HU 15th percentile at CT	decrease of 1 HU	-4.75	-3.30 – -6.10	<0.001

Figure 2: An example of the FEV_1 after 3-year follow-up in a former and a current smoker. Baseline Perc15 value is depicted on the x-axis and the FEV1 level at the end of 3-year follow-up on the y-axis. The reference is a subject with a starting age of 60 years, a height of 179 cm and 40 packyears smoking, being the mean values of the cohort. As an illustration: a Perc15 value of -925 HU at baseline associates with a follow-up FEV_1 of 3.23 L in a current smoker, while a Perc15 value of -975 HU at baseline associates with a follow-up FEV_1 of 2.91 L, reflecting an additional decline of 0.32 L, despite similar age, height and packyears smoking levels of these individuals at baseline.



The interaction between Perc15 and absence / presence of airflow obstruction was not significant (p= 0.276) which indicates that the association between Perc15 and FEV_1-decline did not differ between those with and without baseline airflow obstruction. The interaction between Perc15 and smoking status was not significant (p=0.704). The interaction between Perc15 and packyears was also not significant (p=0.106): the

association between Perc15 and FEV_1 decline is independent of number of packyears smoked.

The estimated effect of 10 HU difference in Perc15 on lung function was comparable to the estimated effect of 10 additional packyears smoking. The additional FEV_1-reduction elicited by 10 extra packyears smoking was 51.5 mL (CI95% 36.8 to 66.2 mL).

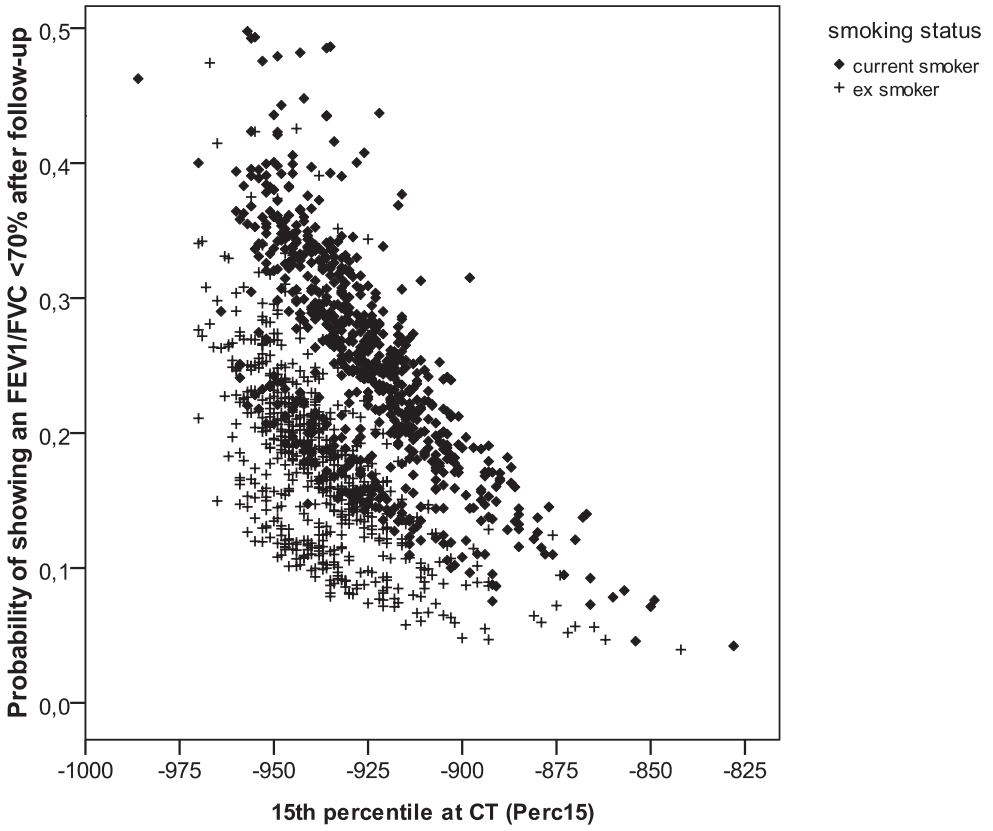
The Perc15 was also significant associated with stronger FEV_1/FVC- and MEF_{50}-reduction (see supplementary files).

Non-obstructive participants who progressed to an FEV_1/FVC <70%.

At baseline, 1,391 participants had an FEV_1/FVC >70%. Of these 1,391 non-obstructive participants, 21.9% (n=305) progressed to an FEV_1/FVC <70% after a mean follow-up of 3 years. This group of participants that had developed airflow obstruction during the study had a mean baseline Perc15 score of -934.2 HU (17.1), which was significantly lower than the baseline Perc15 of the participants who did not develop airflow obstruction: -930.2 HU (19.7), ($p<0.001$).

In the multiple logistic regression analysis a lower Perc15 value at baseline was a significant risk factor for the development of airflow obstruction after follow-up, see Figure 3. A 10 HU lower Perc15 at baseline, i.e. -940 HU instead of -930 HU, resulted in a higher risk (odds ratio 1.20; CI95% 1.10 – 1.30) to develop airflow obstruction after 3-year follow-up. Next to the Perc15 hospital source, age, packyears and smoking status were significant predictors.

Figure 3: Results from logistic regression analysis showing the probability of up of non-obstructive participants (FEV_1/FVC >70%) at baseline to have airflow obstruction (FEV_1/FVC <70%) after follow-according to baseline Perc15. Participants with lower a lower Perc15 show a higher probability of having airflow obstruction after follow-up.



Discussion

We showed that greater extents of baseline low attenuation areas (expressed in Perc15 values) at CT is associated with a stronger lung function reduction, both in non-obstructed and obstructed male heavy smokers. Participants with lower Perc15 values at baseline showed significantly larger lung function declines. In addition we showed that Perc15 is an independent risk factor for the development of airflow obstruction during 3-year follow-up. These results may indicate that CT-quantified emphysema represents a form of sub-clinical COPD and that it may help to identify non-obstructive male smokers with a high risk to develop airflow obstruction.

To our knowledge no other studies assessed low-attenuation areas as a risk factor for developing airflow obstruction. Only a few studies have described the association between low attenuation areas at CT in participants without airway obstruction on the subsequent course of lung function. We expand the results of Yuan et al. who also

suggested that quantitative CT measurements of emphysema predict a stronger decline of lung function in a comparable but smaller cohort of 143 non-obstructive smokers. [6] Here, lung function was re-measured after on average 2.3 years. Over inflated lung areas measured at CT predicted a significantly stronger decline in FEV_1. However, the associations of low attenuation areas were not significant, but they were in the similar direction to the ones in our study. One explanation for the lack of significance could be the smaller sample size. Another longitudinal study visually estimated the extent of emphysema on CT and related it to lung function in a cohort of 111 smoking and non-smoking volunteers. The authors reported a more rapid decline in lung function in participants with emphysema compared to non-emphysematous participants. [7]

Our data are in line with the concept that loss of elastic recoil due to emphysema contributes to airway collapse. [22] The question remains why emphysema does not elicit airway obstruction in every individual. This may be explained by several factors. First, airflow limitation may only become apparent when the severity of emphysema exceeds a certain threshold. Second, lung function must decline below a certain threshold before it is labelled as 'obstructive'. It is evident that individuals with emphysema may have lung function decline, but with FEV_1 and FEV_1/FVC levels yet still within the normal range. Further progression of disease may then give test results below the (GOLD) criteria for COPD. This is exemplified by findings of our study in participants without airway obstruction at baseline who developed airway obstruction at follow-up. This group had significantly lower Perc15 scores on CT than the group that remained non-obstructive. Third, our analysis of the CT-scans focused only on the extent of low attenuation areas and not for example on airway wall thickening. Several cross-sectional studies have reported that lower lung function values associate with CT parameters of more airway wall thickening, more air trapping and lung hyperinflation. [22-24] Hyperinflation and ground glass attenuation have also been reported to be related to a more rapid rate of lung function decline. [6, 21] A composite of CT-derived parameters may therefore offer better lung function associations than measurements of low attenuation areas alone, but such a composite parameter is not (yet) available. Last, FEV_1 is governed by a complicated dynamical process during full and forceful expiration, while we measured the extent of low-attenuation areas during a static full inspiration. Adding measurements performed on expiratory CT has been shown to improve cross-sectional correlations with lung function. [25] This is because low-attenuated areas on expiration scans also reflect air trapping which can be the result of either emphysema or other pathology that causes airflow obstruction, for example obstructive bronchiolitis. [25]

All above effects may reduce the strength of the correlation between CT-quantified emphysema and lung function results. Still, we were able to show that the extent of low attenuation areas is parameter that, additionally to packyears smoking, has a deleterious effect on lung function.

Interestingly we found more low attenuation areas in participants who had quitted smoking compared to current smokers. This was also found in a previous study evaluating COPD subjects and controls. [26] A possible explanation offered is that more chronic inflammation with increased mucus production is present in current smokers. The presence of inflammation may lead to higher densities measured in the lung parenchyma resulting in higher Perc15 scores. In ex-smokers, this chronic inflammation will diminish over time resulting in lower overall lung densities. Another potential explanation is the 'healthy smoker effect', i.e. those with more advanced disease are more likely to reduce or quit smoking and those without symptoms are more likely to continue smoking.

One of the strengths of this study is the large number of participants included and the fact that all were selected from the general population and not after hospital-referral. This enabled us to study the role of low attenuation areas in the development of airflow obstruction in participants who do not (yet) show signs of this. Participants were current or former heavy smokers and thus had a high risk of developing airflow obstruction as shown by the low mean FEV_1/FVC of 72.2% at baseline. The mean rate of lung function decline during follow-up was also much higher than reference values fitting a general population of the same age which may be largely due to the relatively high number of packyears smoked of the cohort. [27] Mean yearly decline of FEV_1 in our cohort was 65 mL, compared to reference values of 29 mL/ year.

We have carefully measured lung function. The data reported here are based on lung function values obtained according to ATS/ERS guidelines and the spirometry equipment used in both centres is interchangeable. [28] The extent of low attenuation areas in the two centres was estimated by the same software package to avoid software bias, and each CT-scan was recalibrated on air to avoid scanner bias. Nonetheless, a hospital factor was inserted in the statistical analysis to remove any residual bias, but this proved to be non-significant as well as all interactions containing the centre factor. Our study was limited by the fact that spirometry was performed without prior bronchodilatation and this could have led to an overestimation of the prevalence of airflow obstruction in our cohort. However, this is not likely to affect the lung function decline over time: all baseline and follow-up tests were performed without bronchodilatation. Another limitation was that, due to the inclusion criteria, only males participated. Previous

studies showed that females have less emphysema [26], and future studies should include females to examine whether emphysema is associated with lung function decline. We were unable to report in how many participants follow-up spirometry was missing due to death. Of importance, there were no relevant differences in spirometry and Perc15 values between participants with baseline spirometry only and with follow-up spirometry. Furthermore, one should bear in mind that the study population included only heavy (former) smokers with a high exposure to tobacco smoking. On the other hand, this is the group of subjects most at risk for lung function decline and development of airflow obstruction.

In conclusion, the results of this study provide new insights in the relationship between smoking related emphysema, i.e, low attenuation areas at CT, and the course of lung function over time in current and former male heavy smokers. Our results show that a greater extent of low attenuation areas at baseline is related to a stronger lung function decline. The large majority of study participants had either no or only mild to moderate airflow obstruction. This suggests that CT-detected emphysema can identify early manifestations of airflow obstruction in patients with a (still) normal lung function. The quantification of low attenuation areas may thus help to identify subjects without airway obstruction who are at risk to develop airflow obstruction.

Reference List

1. Lopez AD, Shibuya K, Rao C, et al. Chronic obstructive pulmonary disease: current burden and future projections. *Eur Respir J* 2006; 27: 397-412.
2. Waterer GW, Wan JY, Kritchevsky SB, et al. Airflow limitation is underrecognized in well-functioning older people. *J Am Geriatr Soc* 2001; 49: 1032-1038.
3. Fletcher C, Peto R. The natural history of chronic airflow obstruction. *Br Med J* 1977; 1: 1645-1648.
4. Rabe KF, Hurd S, Anzueto A, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med* 2007; 176: 532-555.
5. Omori H, Nakashima R, Otsuka N, et al. Emphysema detected by lung cancer screening with low-dose spiral CT: prevalence, and correlation with smoking habits and pulmonary function in Japanese male subjects. *Respirology* 2006; 11: 205-210.
6. Yuan R, Hogg JC, Pare PD, et al. Prediction of the rate of decline in FEV(1) in smokers using quantitative Computed Tomography. *Thorax* 2009; 64: 944-949.
7. Remy-Jardin M, Edme JL, Boulenguez C, et al. Longitudinal Follow-up Study of Smoker's Lung with Thin-Section CT in Correlation with Pulmonary Function Tests. *Radiology* 2002; 222: 261-270.
8. van Iersel CA, de Koning HJ, Draisma G, et al. Risk-based selection from the general population in a screening trial: selection criteria, recruitment and power for the Dutch-Belgian randomised lung cancer multi-slice CT screening trial (NELSON). *Int J Cancer 2007*; 120: 868-874.
9. van Klaveren RJ, Oudkerk M, Prokop M, et al. Management of lung nodules detected by volume CT scanning. *N Engl J Med* 2009; 361(23):2221-9.
10. Gierada DS, Yusen RD, Pilgram TK, et al. Repeatability of Quantitative CT Indexes of Emphysema in Patients Evaluated for Lung Volume Reduction Surgery. *Radiology* 2001; 220: 448-454.
11. Newell JD, Jr., Hogg JC, Snider GL. Report of a workshop: quantitative computed tomography scanning in longitudinal studies of emphysema. *Eur Respir J* 2004; 23: 769-775.
12. Mair G, Miller JJ, McAllister D, et al. Computed tomographic emphysema distribution: relationship to clinical features in a cohort of smokers. *Eur Respir J* 2009; 33: 536-542.
13. Shaker SB, Maltbaek N, Brand P, et al. Quantitative computed tomography and aerosol morphometry in COPD and alpha1-antitrypsin deficiency. *Eur Respir J* 2005; 25: 23-30.
14. Sverzellati N, Calabro E, Randi G, et al. Sex differences in emphysema phenotype in smokers without airflow obstruction. *Eur Respir J* 2009; 33: 1320-1328.
15. van Rikxoort EM, de Hoop B, Viergever MA, et al. Automatic lung segmentation from thoracic computed tomography scans using a hybrid approach with error detection. *Med Phys* 2009; 36: 2934-2947.
16. Parr DG, Stoel BC, Stolk J, et al. Influence of calibration on densitometric studies of emphysema progression using computed tomography. *Am J Respir Crit Care Med* 2004; 170: 883-890.

17. Gould GA, MacNee W, McLean A, et al. CT measurements of lung density in life can quantitate distal airspace enlargement--an essential defining feature of human emphysema. *Am Rev Respir Dis* 1988; 137: 380-392.
18. Parr DG, Stoel BC, Stolk J, et al. Validation of computed tomographic lung densitometry for monitoring emphysema in {alpha}1-antitrypsin deficiency. *Thorax* 2006; 61: 485-490.
19. Parr DG, Sevenoaks M, Deng C, et al. Detection of emphysema progression in alpha 1-antitrypsin deficiency using CT densitometry; methodological advances. *Respir Res* 2008; 9:21.
20. Tashkin DP, Celli B, Senn S, et al. A 4-Year Trial of Tiotropium in Chronic Obstructive Pulmonary Disease. *N Engl J Med* 2008; 359: 1543-1554.
21. Scanlon, P. D., Connett, John E., Waller, L. A. et al. Smoking Cessation and Lung Function in Mild-to-Moderate Chronic Obstructive Pulmonary Disease . The Lung Health Study. Am.J.Respir.Crit.Care Med. 2000; 161: 381-390.
22. Mead J, Turner JM, Macklem PT, et al. Significance of the relationship between lung recoil and maximum expiratory flow. *J Appl Physiol* 1967; 22: 95-108.
23. Lee YK, Oh YM, Lee JH, et al. Quantitative assessment of emphysema, air trapping, and airway thickening on computed tomography. *Lung* 2008; 186: 157-165.
24. Nakano Y, Muller NL, King GG, et al. Quantitative assessment of airway remodeling using high-resolution CT. *Chest* 2002; 122: 271S-275S.
25. Gevenois, P. A., De Vuyst, P., Sy, M. et al. Pulmonary emphysema: quantitative CT during expiration. Radiology 1996; 199: 825-829.
26. Grydeland TB, Dirksen A, Coxson HO, et al. Quantitative computed tomography: emphysema and airway wall thickness by sex, age and smoking. *Eur Respir J* 2009; 34: 858-865.
27. Quanjer PH, Tammeling GJ, Cotes JE, et al. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Respir J Suppl* 1993; 16: 5-40.
28. Munnik P, Zanen P, Lammers JW. A comparison of lung function equipment with emphasis on interchangeability and methods. *Physiol Meas* 2006; 27: 445

Supplement Chapter 3

CT-quantified emphysema in male heavy smokers: association with lung function decline

Table E1: Spirometry and Perc15 values of participants who underwent baseline spirometry only at baseline and those who also underwent follow-up spirometry.

	single observation subjects (n=830)	repeated observation subjects (n=2,085)	95% CI of difference
Perc15	-934.075	-934.838	-0.827 – 2.353
FEV1 (corrected for age/height)	3.304	3.389	-0.137 – -0.032
FEV1/FVC (corrected for age/height)	71.777	72.009	-1.096 – 0.451
MEF50 (corrected for age/height)	3.083	3.176	-0.204 – 0.017

Results of FEV_1/FVC and MEF_{50} decline

Mean FEV_1/FVC after the follow-up period was 69.3% (9.4 and mean MEF_{50} was 2.88 L/s (1.33) or 64.2% (29.1) of predicted. The average decline during 3-year of follow-up was in FEV_1/FVC 2.9% (1.2) and in MEF_{50} 0.32 L/s (0.29) or 5.5% (15.1) of predicted.

A lower Perc15 value at baseline is associated with significantly lower FEV_1/FVC ($p<0.001$) and MEF_{50} ($p<0.001$). The effect sizes of emphysema and the adjustment factors are shown in Table E2 and can be illustrated as follows: considering two individuals with an identical baseline lung function values, the individual with a 10 HU lower baseline Perc15 will experience an additional 2.1% (CI95% 1.9 to 2.3 mL) decline in FEV_1/FVC/ year follow-up. The additional MEF_{50} will be 115.6 mL/s (CI95% 94.1 to 137.1 mL/s). Also see Figures E1 and E2.

Figure S1: An example of the FEV_1/FVC after 3-year follow-up in a former and a current smoker. Baseline Perc15 value is depicted on the x-axis and the FEV_1/FVC level at the end of 3-year follow-up on the y-axis. The reference is a subject with a starting age of 60 years, a height of 179 cm and 40 packyears smoking, being the mean values of the cohort. As an illustration: a Perc15 value of -925 HU at baseline associates with a follow-up FEV_1/FVC of 71.6% L in a current smoker, while a Perc15 value of -975 HU at baseline associates with a follow-up FEV_1/FVC of 59.3%, despite similar age, height and packyears smoking levels of these individuals at baseline.

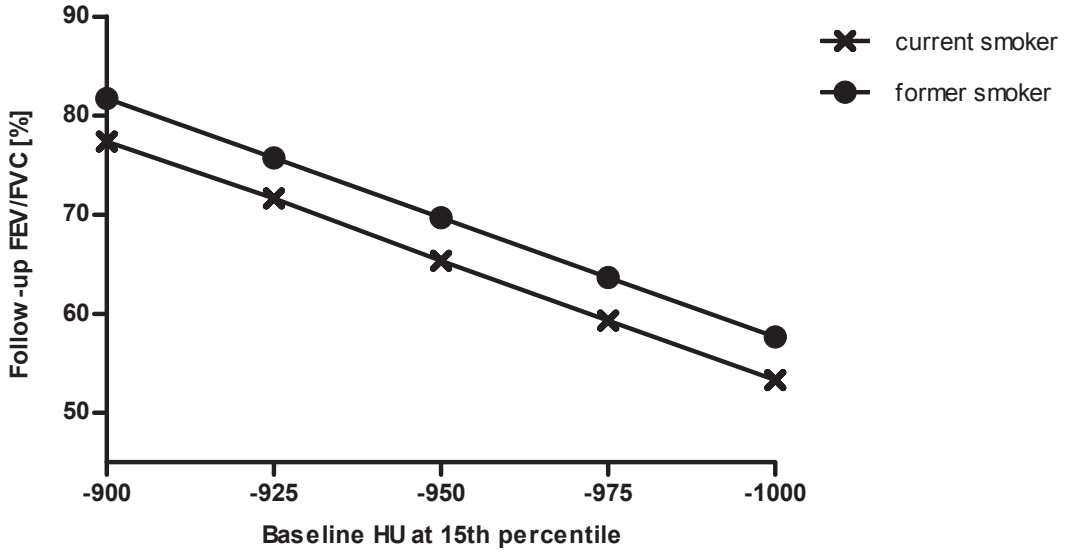


Figure E2: An example of the MEF_{50} after 3-year follow-up in a former and a current smoker. Baseline Perc15 value is depicted on the x-axis and the MEF_{50} level at the end of 3-year follow-up on the y-axis. The reference is a subject with a starting age of 60 years, a height of 179 cm and 40 packyears smoking, being the mean values of the cohort. As an illustration: a Perc15 value of -925 HU at baseline associates with a follow-up MEF_{50} of 3.10 L/s in a current smoker, while a Perc15 value of -975 HU at baseline associates with a follow-up MEF_{50} of 1.86 L/s, despite similar age, height and packyears smoking levels of these individuals at baseline.

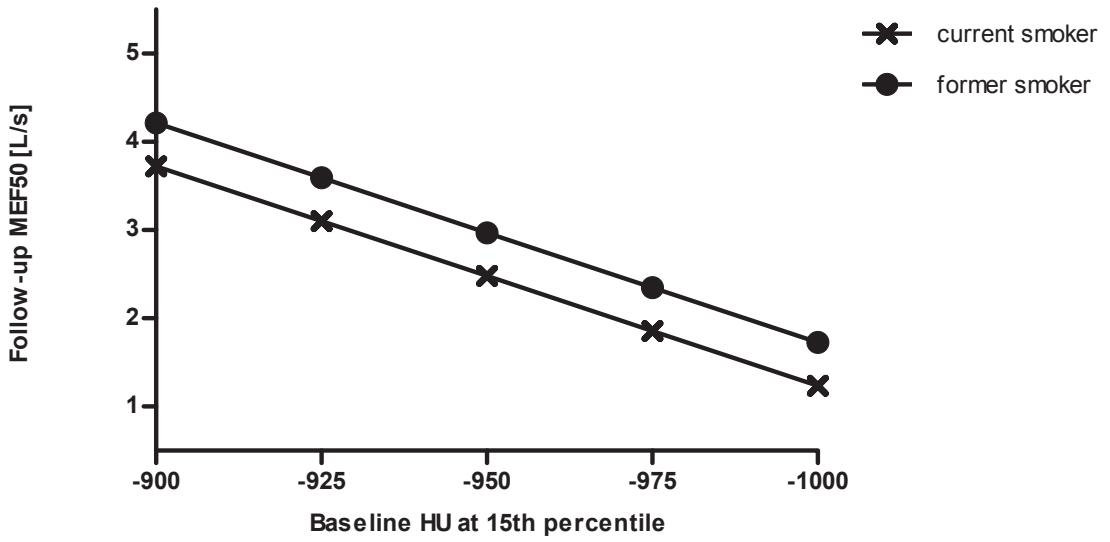


Table E2: Results of linear mixed models. Change in FEV_1/FVC, FEV_1 and MEF_{50} per unit changein covariables. P-values and 95% confidence interval (CI95%) are provided.

Estimated effects of specified increments in covariables: changes in FEV_1/FVC [%]				
Covariate	**Increment or Comparison**	**Drop in FEV_1/ FVC per unit change in covariable**	**CI95%**	**P-value**
Study center	Utrecht vs. Groningen	+0.69	-0.10 –1.49	0.088
Smoking status	current vs. former	-3.89	-4.64 – -3.13	<0.001
Years in study	plus 1	-0.97	-1.03 – -0.90	<0.001
Age [years]	plus 10 years	-1.05	-1.76 – -0.35	0.003
Height [cm]	plus 10 cm	+0.13	-0.42 – 0.70	0.639
Pack years	plus 10 years	-0.48	-0.68 – -0.27	<0.001
HU 15th percentile at CT	decrease of 10 HU	-2.1	-1.9 – -2.3	<0.001

Estimated effects of specified increments in covariables : changes in MEF50 [mL/s]				
Covariate	**Increment or Comparison**	**Drop in MEF_{50} per unit change in covariable**	**CI95%**	**P-value**
Study center	Utrecht vs. Groningen	+19.0	-96.3 – 134.3	0.747
Smoking status	current vs. former	-387.2	-495.5 – -278.9	<0.001
Years in study	plus 1	-64.6	-77.4 – -51.8	<0.001
Age [years]	plus 10 years	-44.4	-54.6 – -34.1	<0.001
Height [cm]	plus 10 cm	+0.73	225.4 – 392.3	<0.001
Pack years	plus 10 years	-92.6	-122.4 – -62.8	<0.001
HU 15th percentile at CT	decrease of 10 HU	-115.6	-137.1 – -94.1	<0.001

Results of analyses with <-950 as emphysema severity measurement at CT

Baseline emphysema (<-950 HU)

Baseline median (Q1-Q3) <-950 HU was 9.1 (5.4 – 14.1) COPD participants had significantly (p<0.001) higher median (Q1-Q3) <-950 HU thresholds than non-COPD participants, 11.6 (7.2 – 17.6) and 7.9 (4.6 – 12.6), respectively. Increasing age was significantly (p<0.001) correlated to lower <-950 HU values (r =0.19). Current smokers had significantly (p<0.001) lower median<-950 HU scores than former smokers, 7.1 (4.2 – 11.5) and 11.5 (7.5 16.7), respectively. Participants with higher <-950 HU had significantly lower lung function values: FEV_1/FVC (r=-0.42), FEV_1 (r=-0.16) and MEF_{50} (r=-0.28), all p-values <0.001.

Lung function after follow-up

A higher <-950 HU thresholds at baseline induces significantly lower lung function levels. The effect sizes of emphysema (<-950 HU) and the adjustment factors are shown in Table E3 and can be illustrated as follows: considering two individuals with an identical baseline lung function value, the individual with 5% higher <-950 HU compared to the second person will experience an additional 0.34% (CI95% -0.50 – -0.18) decline in FEV_1/FVC/ year. The additional FEV_1-decline will be 13.1 mL (CI95% 4.4 to 21.8) and the additional MEF_{50} will be 40 mL/s (CI95% 18 – 61). The effect of 5% difference in <-950 HU was larger compared to the effect of 10 additional pack years of smoking.

Non-obstructive participants who progressed to COPD

At baseline, 1,391 participants had an FEV_1/FVC >70%. Of these 1,391 non-obstructive participants, 21.9% progressed to an FEV_1/FVC <70% after a mean follow-up of 3 years. This group of participants that had developed COPD during the study had a median (Q1-Q3) <-950 HU score of 9.2 (5.4 – 13.1) at baseline. This thresholds was significantly ($p<0.001$) higher, those of who did not develop COPD: 7.7 (4.4 – 12.2)

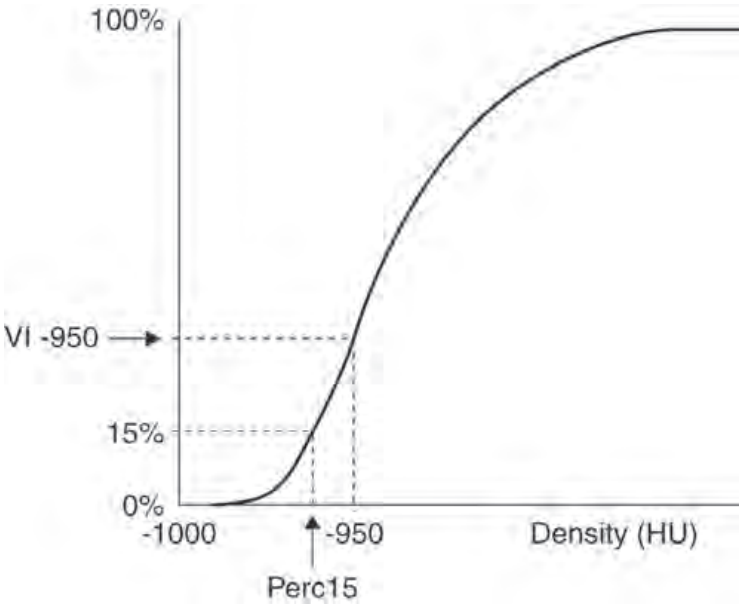
Table E3: Results of linear mixed models using <-950 HU as emphysema severity measurement. Change in FEV_1/FVC, FEV_1 and MEF_{50} per unit change in covariables. P-values and 95% confidence interval (CI95%) are provided.

Estimated effects of specified increments in covariates: changes in FEV_1/FVC [%]				
Covariate		**Change in FEV_1/FVC per unit change in covariable**	**CI95%**	**P-value**
Study centre	Utrecht vs. Groningen	+0.30	-0.49 - 1.1	0.456
Smoking status	current vs. former	-3.0	-3.74 – -2.25	<0.001
Years in study	plus 1	-0.59	-0.68 – -0.50	<0.001
Age [years]	plus 10 years	-1.6	-2.3 – -0.93	<0.001
Height [cm]	plus 10 cm	+0.35	-0.92 – 0.22	0.230
Pack years	plus 10 years	-0.41	-0.62 – -0.21	<0.001
%950 HU at CT	plus 1%	-0.36	-0.40 – 0.32	<0.001

Estimated effects of specified increments in covariates: changes in FEV_1 [mL]				
Covariate		**Change in FEV_1 per unit change in covariable**	**CI95%**	**P-value**
Study centre	Utrecht vs. Groningen	+61.0	-118.5 – 284.0	0.035
Smoking status	current vs. former	-162.8	-216.1– -109.5	<0.001
Years in study	plus 1	-56	-61.2 – -50.9	<0.001
Age [years]	plus 10 years	-368.0	-418.4 – -317.7	<0.001
Height [cm]	plus 10 cm	+400	358.7 – 440.3	<0.001
Pack years	plus 10 years	-49.7	-64.4 – -35.0	<0.001
%950 HU at CT	plus 1%	-8.2	-10.7 – -5.7	<0.001

Estimated effects of specified increments in covariates : changes in MEF_{50} [mL/s]				
Covariate		**Change in MEF_{50} per unit change in covariable**	**CI95%**	**P-value**
Study centre	Utrecht vs. Groningen	+60.5	-107.4 – 12.2	0.900
Smoking status	current vs. former	-385.4	-493.4 – -277.4	<0.001
Years in study	plus 1	-61.0	-73.9 – -51.8	<0.001
Age [years]	plus 10 years	-45.5	-55.7 – -35.3	<0.001
Height [cm]	plus 10 cm	+29.5	-21.2 – -37.8	<0.001
Pack years	plus 10 years	-90.5	-120.2 – -60.7	<0.001
%950 HU at CT	plus 1%	-31.1	-40.0 – -25.4	<0.001

Figure E3: The 15th percentile point (Perc15) is defined as the cut-off value in HU below which 15% of all voxels are distributed and, as a true measure of density, this parameter consequently decreases with worsening emphysema. The <-950 HU is defined as the percentage of lung voxels below a threshold of -950 HU. Adapted from Parr et al., Respiratory Research 2008, 9:21doi:10.1186/1465-9921-9-21.




Chapter
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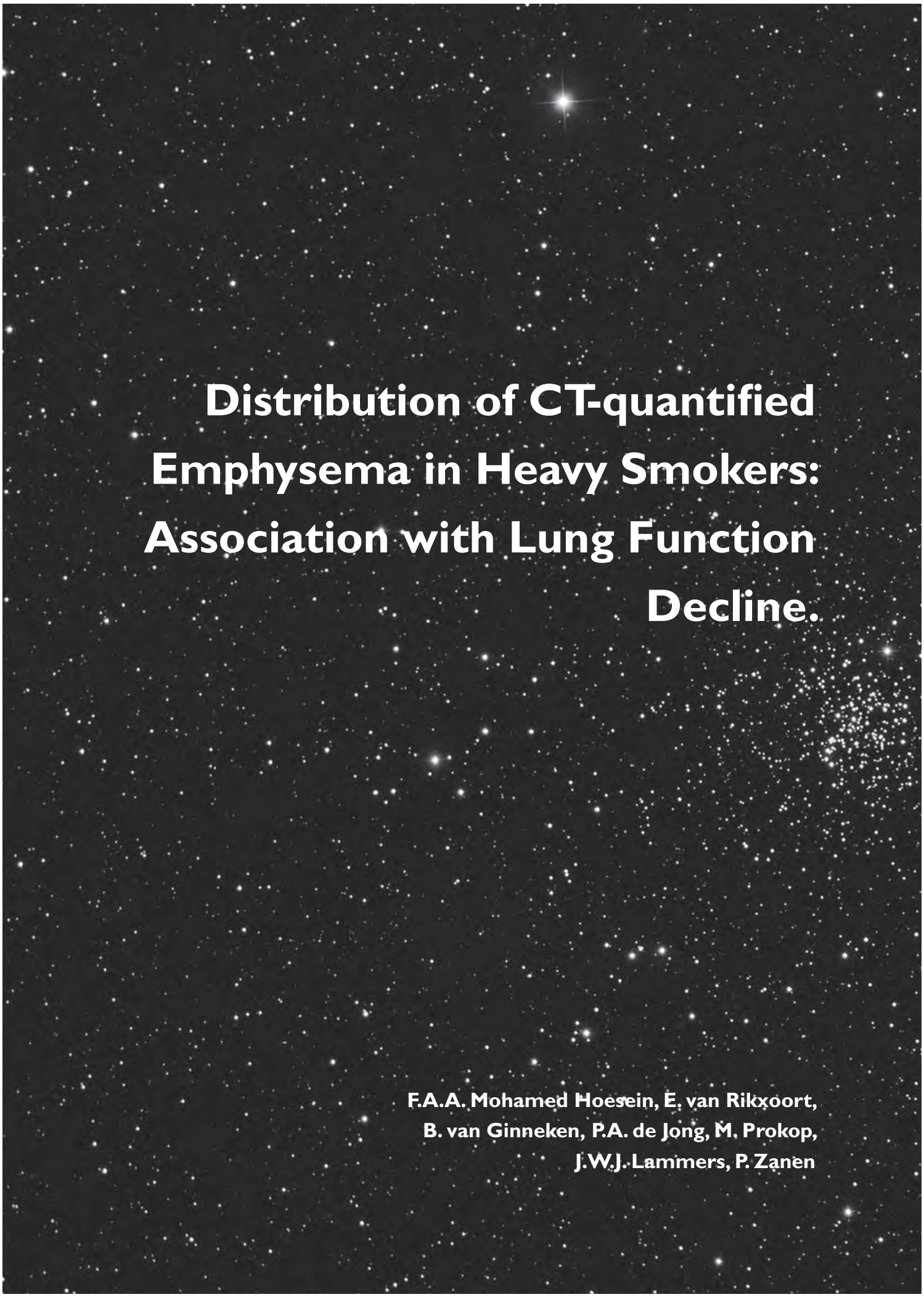

Distribution of CT-quantified Emphysema in Heavy Smokers: Association with Lung Function Decline.

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Abstract

Purpose To assess the association between CT-quantified emphysema distribution (upper / lower lobe) and lung function decline in heavy current and former smokers participating in a lung cancer screening trial.

Methods In this medical ethical approved study, 587 participants underwent CT-scanning of the lungs and pulmonary function testing at baseline and after a median (interquartile range) follow-up of 2.9 (2.8-3.0) years. The lungs were automatically segmented based on anatomically defined lung lobes. Severity of emphysema was automatically quantified per anatomical lung lobe and was expressed as the 15th percentile; (HU point below which 15% of the low attenuation voxels are distributed (Perc15)). The emphysema distribution was based on principal component analysis. Linear mixed models, correcting for age, height, BMI packyears and smoking status, were used to assess the association of emphysema distribution and FEV_1/FVC-decline.

Results Mean (SD) age was 60.2 (5.4) years, mean baseline FEV_1/FVC was 71.6 (9.0) % and overall mean Perc15 was -908.5 (20.9) HU. Participants with upper lobe predominant emphysema had a lower FEV_1/FVC after follow-up compared to participants with lower predominant emphysema (p=0.001), independent of the total extent of emphysema

Conclusion Heavy current and former smokers with upper lobe predominant emphysema have a more rapid decrease in FEV_1/FVC than those with lower lobe predominant emphysema, independent of the overall extent of emphysema. These results seem to indicate that upper and lower lobe predominant emphysema may be different phenotypes.

Introduction

Chronic obstructive pulmonary disease (COPD) is one of the major causes of morbidity and mortality world wide. [1] COPD consists of chronic bronchitis and emphysema, which both may lead to airflow obstruction. Emphysema is defined as an abnormal and permanent enlargement of the air spaces distal to the terminal bronchioles and destruction of bronchial walls, which in the majority of cases is caused by tobacco smoking. Although emphysema is a pathological diagnosis it may also be assessed by quantitative computed tomography CT) measuring low-attenuation areas (LAAs) of the lung. This technique has been validated against pathology [2] and has been used in multiple studies. [3 4 5]

Since lung cancer and COPD share smoking as a mutual risk factor participants of lung cancer screening trials provide the unique opportunity to study the relationships between CT-quantified emphysema and lung function decline in relatively healthy smokers.[6] The results may be useful to select participants in need for more aggressive smoking cessation therapies to prevent further lung function deterioration at a fairly early stage of the disease.

Several studies have shown that subjects with similar degrees of low-attenuation areas, but with different locations within the lung show different degrees of airflow obstruction. [7 8] However, those studies were cross-sectional and the effects of the emphysema distribution on disease progression, i.e. lung function decline, were not assessed. In subjects with α1-anti-trypsin (AAT)-deficiency for instance it was shown that emphysema distribution was associated with lung function decline. [4]

Recent advances enable automatic anatomical-based segmentation of the lungs allowing estimation of the extent of low-attenuation areas per lung lobe, in stead of per e.g. top or lower one-third of the lung. [9]

We hypothesize that, like in AAT-deficiency, distribution of low-attenuation areas in heavy smokers is associated with lung function decline. The aim of the present study was therefore to assess the effect of emphysema distribution, based on anatomically defined lung lobes, on lung function decline in current and former smokers participating in a lung cancer screening trial.

Methods

Participants

The study was conducted among those current and former heavy smokers taking part in the Dutch Belgian Lung Cancer Screening Trial (NELSON). In the current study only participants who underwent CT-scanning and pulmonary function tests at the University Medical Center Utrecht were included. The inclusion criteria have been described in detail elsewhere. [10 11] In brief, the NELSON study is a population based CT-screening trial for lung cancer that studies current and former heavy smokers fit enough to undergo surgery. Both the Dutch ministry of health and the Medical Ethics Committee of the hospital approved the study protocol and informed consent was obtained from all participants. The NELSON trial is registered at www.trialregister.nl with trial number ISRCTN63545820. For this sub study, original approval and informed consent allowed use of data for future research. Participants meeting the inclusion criteria of having smoked a minimum 20 pack years were invited to participate. As fewer women in the Dutch population show the same long-term exposure to cigarettes as men, only males were included. Baseline details on smoking habits were gathered through questionnaires which included questions about duration of smoking habit, number of packyears smoked and smoking status (current or former smoker).

Pulmonary function tests

Pulmonary function tests (PFT) were performed with standardized equipment according to European Respiratory Society (ERS) and American Thoracic Society (ATS) guidelines and included forced expiratory volume in one second (FEV_1) and FEV_1/ forced vital capacity (FVC). [12] Participants with an FEV_1/FVC <70% were regarded as having airflow obstruction. Broncho dilatation was not applied [13].

CT scanning

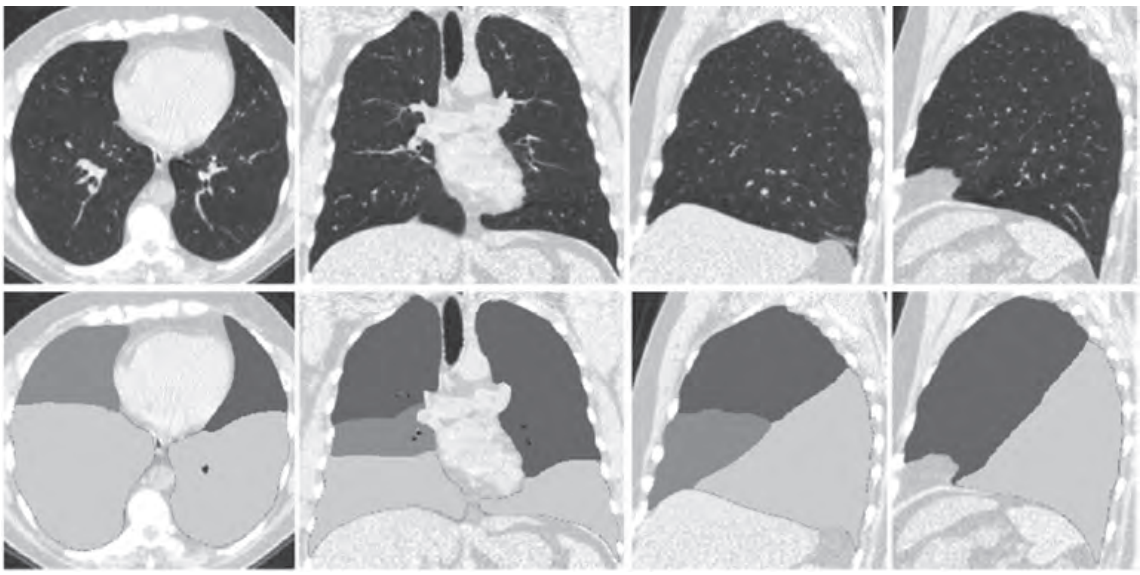
All participants received low-dose CT, with 16-detector MDCT scanners (Mx8000 IDT or Brilliance 16P, Philips Medical Systems, Cleveland, OH). Scan data were obtained in spiral mode, with 16 x 0.75mm collimation and in full inspiration. No spirometric gating was applied since this does not improve repeatability of lung density measurements. [14;15] Axial images were reconstructed with 1.0mm thickness at 0.7mm increment. All scans were reconstructed with a soft reconstruction filter (Philips B) at a 512x512 matrix. Exposure settings were 30mAs at 120kVp or 140kVp, depending on participant's weight,

≤80 kilograms and >80 kilograms, respectively. This low-dose CT protocol has previously been used to quantify emphysema in COPD patients and heavy smokers.[6;16;17] [18] The vast majority of subjects was scanned on the Brilliance 16P scanner. However, a very small fraction was scanned on the Mx8000 IDT scanner, which was used a back-up scanner. We repeated the analyses with exclusion of subjects scanned on the Mx8000 IDT scanner and found no significant differences.

Segmentation of lungs and lobes

In all CT scans, the lungs and lobes were automatically segmented using previously developed and evaluated software. [9] [19] Segmentation of the lungs was performed using an algorithm based on region growing and morphological processing. Segmentation failures, for instance in case of incomplete fissures, were automatically detected based on statistical deviations from volume and shape measurements. In the cases for which failures were detected, an algorithm based on multi-atlas registration was applied to obtain the correct result. The lung segmentation software was previously evaluated on 100 scans from the same screening and performed with accuracy similar to human observers. [19] The software further subdivided the lungs into the anatomical lobes. Two lobes were segmented in the left lung (upper and lower lobe) and three in the right lung (upper, middle, and lower lobe). Lobe segmentation was initiated with a segmentation of the pulmonary fissures. Next, each voxel in the lung was assigned to one of the lobes based on its position inside the lung and relative to the fissures.

Figure 1: Illustration of a random CT scan with the lobe segmentation as it was performed by the software. The top row shows the original scan and the lower row the segmentation of the anatomical lobes.



Emphysema quantification

Emphysema severity was automatically computed for the entire lungs and separately per lung lobe. The airways were automatically excluded to ensure that only lung parenchyma was analyzed.[20] Severity of emphysema was calculated using the 15th percentile (Perc15) technique.[21 22 23] Perc15 provides the Hounsfield units (HU) point below which 15% of all voxels are distributed. The lower the Perc15 values are, i.e. closer to -1000 HU, the more emphysema is present. The use of Perc15 for emphysema quantification has been validated against pathology [24] and applied in multiple studies .[4 6] A secondary analysis was done using the %950 HU as emphysema severity measure, which is defined as the proportion of low density voxels below -950 HU. The results of these analyses are reported in the supplemental files.

Statistical analysis

Mean and standard deviation (SD) were calculated for normally distributed data and median and interquartile range for non-normally distributed data. Students't-test was used to compare means of normally distributed variables and Chi-square tests for categorical variables. Correlations between the Perc15 values per lung lobe were assessed by Pearson's *r*.

The Perc15 value per lobe is expected to be highly correlated with that of the other lobes of individual participants, resulting in multicollinearity issues. Therefore, principal component analysis (PCA) with a varimax rotation was performed first to obtain uncorrelated variables.

PCA is a well-known data reduction technique and is often used to convert a set of correlated variables into an uncorrelated set.[25] Multicollinearity issues are also solved by PCA. The new variables, called components, are linear combinations of the original variables. There is a superficial resemblance with linear regression: the 'regression coefficients' in PCA are called 'scores'. Every component is linked to a characteristic of the original set of variables. The first component is often a mean of the original variables and therefore explains the greatest proportion of variance. The second component explains another characteristic not present in the first component and this is often a contrast in the original set of variables. The percentage of variance explained by this second component will be less than by the first component. This procedure goes on until all variance is explained, however each next component will explain a smaller proportion. Higher components can be ignored as the percentage of additional variance explained is minimal. Only components explaining more than 5% were retained in this case.

The components from the PCA were incorporated in a random intercept, random slope linear mixed model with FEV_1/FVC per time point as primary endpoint. Three separate models were created and compared. The first model contained the variables years in study, height, BMI, age, packyears smoked and smoking status (current/ former smoker). In a second model component 1 was added and in a third model component 1 and 2 were both added. Likelihood ratio tests were performed to evaluate if by inserting the new components the fit of the model significantly improved. P-values ≤0.05 were considered as significant. All statistical analyses were performed using SPSS 18 (SPSS, Chicago, Illinois, USA).

Results

Baseline demographics and lung function

A total of 609 participants underwent baseline and follow-up CT-scanning and PFT. After exclusion of 22 participants because of software failure to segment the lung lobes, 587 participants were included in the current study. Mean (SD) baseline FEV_1 was 97.7 (18.1) % of predicted and FEV_1/FVC was 71.6% (9.0). Further baseline demographics and lung function values are presented in Table 1.

Table 1: Baseline participants' demographics for the total cohort. Mean and standard deviations (SD) are provided. * median (interquartile range) # The severity of emphysema isexpressed as the 15th percentile: HU point below which 15% of the low attenuation areas voxels are distributed. The lower the Perc15 values are, i.e. closer to -1000 HU, the moreemphysema is present.

	Total cohort n=587
Age [years]	60.2 (5.4)
Height [meters]	1.78 (0.07)
BMI [kg* m^{-2}]	26.9 (3.6)
Years in study*	2.9 (2.8 - 3.0)
Packyears smoking	41.2 (18.7)
Current smokers (%)	304 (49.9%)
FEV_1 [L]	3.36 (0.73)
FEV_1 %pred	97.7 (18.1)
FEV_1/FVC [%]	71.6 (9.0)
FEV_1/FVC <70%	218 (35.8%)
Emphysema severity for total lung (Perc15)#	-908.5 (20.9)

Smoking status

Mean (SD) packyears smoked was 41.2 (18.7) years. At enrolment of the study 305 (50.1%) participants had quit smoking and 304 (49.9%) participants were current

smokers. The number of packyears smoked did not significantly differ between current and former smokers, 38.8 and 41.1 years respectively (p=0.251).

Baseline CT-quantified emphysema: results from Principal Component Analysis

Overall mean (SD) baseline Perc15 was -908.5 (20.9) HU. Perc15 per lung lobe is given in Table 2. The Perc15 values between the five lung lobes were highly correlated (*r* ranging from 0.75 to 0.950, all p<0.0001). For that reason, principal component analysis was performed to obtain uncorrelated variables. Two components were retained, which in total explained 94% of the variance: component 1 86.1% and component 2 7.9%. Table 2 shows the scores of the two components. Component 1 characterized the overall mean Perc15 (emphysema score (ES)). Component 2 characterized upper / lower lobe emphysema predominance (distribution score (DS)). The positive values for component 2 in Table 2 relates to the upper left / right lobes, the negative scores to the lower left / right lobes. The low score for the right middle lobe indicates a minor influence of that part of the lung.

Table 2: Mean (SD) Perc15 [HU] per lung lobe (column 2) and results from principal components analysis: component scores (columns 3 and 4). The two new components explained 94% of the total variance. Component 1 (emphysema score): mean Perc15 value of all lobes; component 2 (distribution score): difference between upper and lower lobe Perc15,i.e. lower / upper lobe emphysema predominance. The component scores can be interpreted ina similar way as the regression coefficients (beta's) from multiple linear regression analysis.

Lung lobe	Mean (SD) Perc15	Scores component 1 (emphysema score)	Scores component 2 (distribution score)
Left upper lobe	-912.1 (21.2)	0.218	0.743
Right upper lobe	-906.6 (23.0)	0.212	0.937
Right middle lobe	-915.5 (18.5)	0.214	-0.234
Left lower lobe	-900.5 (24.7)	0.216	-0.680
Right lower lobe	-899.9 (24.2)	0.217	-0.759
Variance explained		**86.15%**	**7.93%**

Association CT-quantified emphysema distribution and lung function after follow-up

Median (interquartile range) follow-up was 2.9 (2.8 - 3.0) years. The two components derived from the principal component analysis (ES and DS) were inserted in the linear mixed model together with the other adjustment factors. The fit of the model significantly improved when ES and DS were inserted respectively (p all <0.001). Both ES and DS

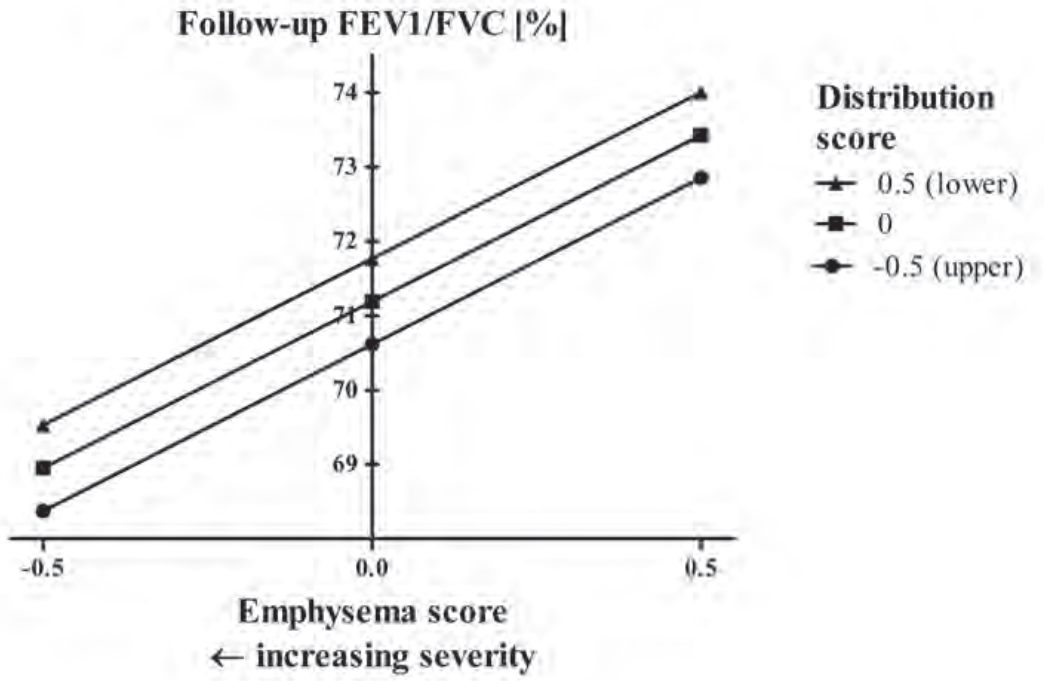
were significantly ($p<0.001$) associated with a lower FEV_1/FVC after follow-up, see Table 3. A 1 point decrement in the ES resulted in a 4.28% lower FEV_1/FVC after 1 year of follow-up. A 1 point decrement in the DS resulted in 1.19% lower FEV_1/FVC after 1 year of follow-up. This shows that lower Perc15 values, i.e. more low-attenuation areas, and an upper lobe predominant emphysema distribution are independently associated with a significantly lower FEV_1/FVC after follow-up. The effects of ES and DS on FEV_1/FVC after follow-up are illustrated in Figure 2. The effect sizes of the other covariates in the model (age, years in study, height, BMI, packyears and smoking status) are also presented in Table 3.

Table 3: Results of the linear mixed models analysis: the model in the table depicts the effectof a change in each parameter on the FEV_1/FVC value after follow-up. P-values and 95% confidence intervals (CI95%) of these effects are provided. Significant values are in bold. The FEV_1/FVC after a certain period of follow-up can be calculated by the following general equation: FEV_1/FVC= constant + $(\beta_1 * x_1) + (\beta_2 * x_2) + (\beta 1 * x_3) + (\beta_i * x_i)$. This results in to the following equation for the FEV_1/FVC after follow-up= 105 + (age*-0.43) + (height*0.03) +(BMI*0.18) + (year in study*-1.65) + (packyears*-0.05) - 4.54 (if current smoker) +(emphysema score*-4.28) + (distribution score*-1.19).

Estimated effects of changes in parameters on FEV_1/FVC [%]				
Parameter	**Change in parameter**	**Resulting change in FEV_1/FVC [%]**	**CI 95%**	**P-value**
Age [years]	plus 1 year	-0.43	-0.43 – -0.26	**<0.001**
Height [cm]	plus 1 cm	+0.03	-0.12 – 0.07	0.606
BMI [kg* m^{-2}]	plus 1 kg* m^{-2}	0.18	-0.02 – 0.38	0.072
Years in study	plus 1 year	-1.65	-3.31 – 0.01	0.052
Packyears	plus 1 year	-0.05	-0.08 – -0.01	**0.005**
Smoking status	current vs. former	-4.54	-5.81 – -3.27	**<0.001**
Emphysema score	minus 1 point	-4.28	3.63 – 4.93	**<0.001**
Distribution score	minus 1 point	-1.19	0.56 – 1.83	**<0.001**

Using the %950 HU approach as measure of emphysema severity yielded similar results as using the Perc15 (see supplemental files).

Figure 2: An illustration of the FEV_1/FVC [%] after 3-year of follow-up in a participant with a starting age of 60 years, height of 1.78 meters and 41 packyears, being the mean values of the cohort. The emphysema score is depicted on the x-axis and the FEV1/FVC [%] after 3 years on the y-axis. It can be seen that a lower value of the emphysema score results in a lower FEV_1/FVC [%] after follow-up. The graph is stratified by the value of the distribution score: -0.5; 0 and 0.5, being the first, second and third quartile, respectively. It shows that a lower value of the distribution score results in a lower FEV_1/FVC [%] after follow-up.



Discussion

In the present study we showed that upper lobe distribution of CT-quantified emphysema is associated with a lower lung function after follow-up in a large cohort of 587 former and current heavy smokers participating in a lung cancer screening trial. Knowledge of the distribution of CT-quantified emphysema thus is important with regards to the course of lung function in former and current heavy smokers.

We used a sophisticated approach, i.e. principal component analysis, to solve the problem of the high correlation between the Per15 values per lung lobe within an individual. This approach delivered two new variables (components 1 and 2). Component 1 (emphysema score) characterized the total extent of emphysema, while component 2 (distribution score) characterized the difference between upper and lower lobe emphysema. The

effect size of the emphysema distribution pattern (-1.19%) is substantially higher compared to the expected normal decline of FEV_1/FVC (-0.18%) in age matched healthy individuals. This shows that the results are of clinical importance. Ignoring the individual distribution of emphysema provides a less precise estimation of lung function decline.

The present longitudinal study is an extension from the previous performed cross-sectional study investigating the association between emphysema distribution and lung function. [8] However, two important methodological differences exist. Firstly, in the current study no distinction was made between mild and severe emphysema, instead the Perc15 was used as a continuous measure for emphysema severity. We preferred this measure because it was shown to be the most robust measure for the progression of low attenuation areas. [5] Secondly, in the former study segmentation of the lungs was based on a division of the lung based on volumes: top one-third and lower one-third. Other studies also used such an approach to separate upper from lower lung fields, for instance by dividing the total lung volume in two parts. [26] Inevitably parts of the anatomical lower lobe will be allocated to the upper lobe and our lobe segmentation, based on anatomical information, avoids this problem. Therefore, in the present study, three-dimensional data and the natural boundaries of the lung were used which enabled a reliable separation of the upper lung lobes from the lower lung lobes. Furthermore, the software used has shown to have an accuracy comparable to that of an independent human observer.[19]

In AAT-deficiency subjects longitudinal studies have been performed showing that lower lobe predominant emphysema was associated with a greater decline of lung function. [4] In COPD patients, without AAT-deficient only cross-sectional studies have been performed investigating the association of emphysema distribution with lung function. Gurney et al. found that a lower lobe predominant emphysema distribution was associated with lower total lung capacity (TLC) values, however these associations were only significant for subjectively quantified emphysema and not when objectively quantified. [27] Three studies are of special interest because they quantified emphysema automatically, based on the percentage of low-attenuation areas below a specified threshold. Saitoh et al. reported that the FEV_1/FVC ratio showed the strongest correlation with lower lobe emphysema distribution, but that the carbon monoxide transfer factor (TLco) showed the strongest correlation with upper lobe emphysema distribution [28] Mair et al. showed that upper zone distribution of emphysema in COPD subjects was associated with a higher total score on the St. George's Respiratory Questionnaire. [29] A higher score on the St.

George's Respiratory Questionnaire indicates more severe respiratory impairment. De Torres et al. failed to find a correlation between the distribution of emphysema and lung function parameters in subjects with mild to moderate COPD. [30] It was concluded that in mild COPD emphysema distribution is not associated with lung function. However, it should be taken in account that the sample sizes might be too small (n=115) to allow sufficient power for detecting true associations. Furthermore, in all studies classification of the emphysema distribution was not anatomically based which might have influenced the findings.

Less is known about why it is that smokers differ in emphysema distribution pattern. Like in AAT-deficiency a genetic susceptibility may play a role. [31] [32] Candidate gene studies in the National Emphysema Treatment Trial (NETT) showed that upper lobe predominant emphysema was associated with polymorphisms in two enzymes playing a role in the detoxification of smoke metabolites . [31] The authors posed that these polymorphisms alter the normal detoxification of cigarette metabolites contributing to the distribution of emphysema. Future genome-wide association studies may further elucidate the association between genetic susceptibility and emphysema distribution in heavy smokers.

There are a number of strengths to our study. Firstly, we included a large number of participants and therefore could extensively correct for confounding factors, like age, BMI, smoking status, pack years etc., unlike most other studies. Secondly, we included relatively healthy, but heavy smoking subjects, at a high risk for developing airflow obstruction. Most previous studies examining the effects of emphysema distribution included subjects with more severe COPD only. Thirdly, all CT-scans were performed in one single center excluding possible scanner bias due to different algorithms used by different types of CT-scanners. Lastly, we used anatomical defined borders to segment the lungs which might be more accurate than using lung volumes to segment the lungs or by a visual assessment. Visual assessment has been reported to be less reliable than when determined automatically. [33] Furthermore, the severity of emphysema was calculated automatically which eliminates intra-observer variability between different readers of CT-scans.

The main limitation of our current study is that only males were included which is especially unfortunate because the prevalence of COPD in females is rising. Our results may not be extrapolated directly on females because it is known that males have more emphysema [34] and that sex is independently associated with upper or lower lung predominant emphysema patterns. [29] Future studies should also include females

to examine the association of emphysema distribution and lung function decline. Furthermore, as we included relatively healthy, but heavy smoking participants and participants with COPD GOLD stage 1, the results may not be extrapolated to more severe COPD participants straightforwardly.

In conclusion, the distribution of CT-quantified emphysema is an additional parameter, besides the total extent of CT-quantified emphysema, in predicting lung function decline. Upper lobe predominant emphysema is significantly associated with stronger lung function decline compared to lower lobe predominance emphysema in former and current heavy smokers.. These findings may be of importance because they may be useful to identify subjects with greater declines in lung function and probably eligible for more intensive smoking cessation counseling.

Reference List

1. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. Lancet 1997 May 24;349(9064):1498-504.
2. Gould GA, MacNee W, McLean A, Warren PM, Redpath A, Best JJ, et al. CT measurements of lung density in life can quantitate distal airspace enlargement--an essential defining feature of human emphysema. Am Rev Respir Dis 1988 Feb;137(2):380-92.
3. Gould GA, Redpath AT, Ryan M, Warren PM, Best JJ, Flenley DC, et al. Lung CT density correlates with measurements of airflow limitation and the diffusing capacity. Eur Respir J 1991 Feb;4(2):141-6.
4. Parr DG, Stoel BC, Stolk J, Stockley RA. Validation of computed tomographic lung densitometry for monitoring emphysema in alpha1-antitrypsin deficiency. Thorax 2006 Jun;61(6):485-90.
5. Parr DG, Sevenoaks M, Deng C, Stoel BC, Stockley RA. Detection of emphysema progression in alpha 1-antitrypsin deficiency using CT densitometry; methodological advances. Respir Res 2008;9:21.
6. Mohamed Hoesein FA, de Hoop B, Zanen P, Gietema H, Kruitwagen CL, van Ginneken B, et al. CT-quantified emphysema in male heavy smokers: association with lung function decline. Thorax 2011 Apr 7.
7. Parr DG, Stoel BC, Stolk J, Stockley RA. Pattern of emphysema distribution in alpha1-antitrypsin deficiency influences lung function impairment. Am J Respir Crit Care Med 2004 Dec 1;170(11):1172-8.
8. Gietema HA, Zanen P, Schilham A, van Ginneken B, van Klaveren RJ, Prokop M, et al. Distribution of emphysema in heavy smokers: impact on pulmonary function. Respir Med 2010 Jan;104(1):76-82.
9. van Rikxoort EM, de Hoop B, Viergever MA, Prokop M, van Ginneken B. Automatic lung segmentation from thoracic computed tomography scans using a hybrid approach with error detection. Med Phys 2009 Jul;36(7):2934-47.
10. van Iersel CA, de Koning HJ, Draisma G, Mali WP, Scholten ET, Nackaerts K, et al. Risk-based selection from the general population in a screening trial: selection criteria, recruitment and power for the Dutch-Belgian randomised lung cancer multi-slice CT screening trial (NELSON). Int J Cancer 2007 Feb 15;120(4):868-74.
11. van Klaveren RJ, Oudkerk M, Prokop M, Scholten ET, Nackaerts K, Vernhout R, et al. Management of lung nodules detected by volume CT scanning. N Engl J Med 2009 Dec 3;361(23):2221-9.
12. Miller MR, Crapo R, Hankinson J, Brusasco V, Burgos F, Casaburi R, et al. General considerations for lung function testing. Eur Respir J 2005 Jul;26(1):153-61.
13. Hunninghake GM, Cho MH, Tesfaigzi Y, Soto-Quiros ME, Avila L, Lasky-Su J, et al. MMP12, lung function, and COPD in high-risk populations. N Engl J Med 2009 Dec 31;361(27):2599-608.

14. Gierada DS, Yusen RD, Pilgram TK, Crouch L, Slone RM, Bae KT, et al. Repeatability of quantitative CT indexes of emphysema in patients evaluated for lung volume reduction surgery. Radiology 2001 Aug;220(2):448-54.

15. Newell JD, Jr., Hogg JC, Snider GL. Report of a workshop: quantitative computed tomography scanning in longitudinal studies of emphysema. Eur Respir J 2004 May;23(5):769-75.

16. Shaker SB, Maltbaek N, Brand P, Haeussermann S, Dirksen A. Quantitative computed tomography and aerosol morphometry in COPD and alpha1-antitrypsin deficiency. Eur Respir J 2005 Jan;25(1):23-30.

17. Sverzellati N, Calabro E, Randi G, La VC, Marchiano A, Kuhnigk JM, et al. Sex differences in emphysema phenotype in smokers without airflow obstruction. Eur Respir J 2009 Jun;33(6):1320-8.

18. Gietema HA, Schilham AM, van Ginneken B, van Klaveren RJ, Lammers JW, Prokop M. Monitoring of smoking-induced emphysema with CT in a lung cancer screening setting: detection of real increase in extent of emphysema. Radiology 2007 Sep;244(3):890-7.

19. van Rikxoort EM, de Hoop B, van der Vorsy, Prokop M, van Ginneken B. Automatic segmentation of pulmonary segments from volumetric chest CT scans. IEEE Trans Med Imaging 2009 Apr;28(4):621-30.

20. van Rikxoort EM, Prokop M, de Hoop B, Viergever MA, Pluim JP, van Ginneken B. Automatic segmentation of the pulmonary lobes from fissures, airways, and lung borders: evaluation of robustness against missing data. Med Image Comput Comput Assist Interv 2009;12(Pt 1):263-71.

21. Coxson HO. Quantitative chest tomography in COPD research: chairman's summary. Proc Am Thorac Soc 2008 Dec 15;5(9):874-7.

22. Coxson HO. Quantitative computed tomography assessment of airway wall dimensions: current status and potential applications for phenotyping chronic obstructive pulmonary disease. Proc Am Thorac Soc 2008 Dec 15;5(9):940-5.

23. Newell JD, Jr., Hogg JC, Snider GL. Report of a workshop: quantitative computed tomography scanning in longitudinal studies of emphysema. Eur Respir J 2004 May;23(5):769-75.

24. Gould GA, MacNeeW, McLean A, Warren PM, Redpath A, Best JJ, et al. CT measurements of lung density in life can quantitate distal airspace enlargement--an essential defining feature of human emphysema. Am Rev Respir Dis 1988 Feb;137(2):380-92.

25. Joliffe IT, Morgan BJ. Principal component analysis and exploratory factor analysis. Stat Methods Med Res 1992;1(1):69-95.

26. Mair G, Miller JJ, McAllister D, Maclay J, Connell M, Murchison JT, et al. Computed tomographic emphysema distribution: relationship to clinical features in a cohort of smokers. Eur Respir J 2009 Mar;33(3):536-42.

27. Gurney JW, Jones KK, Robbins RA, Gossman GL, Nelson KJ, Daughton D, et al. Regional distribution of emphysema: correlation of high-resolution CT with pulmonary function tests in unselected smokers. Radiology 1992 May;183(2):457-63.

28. Saitoh T, Koba H, Shijubo N, Tanaka H, Sugaya F. Lobar distribution of emphysema in computed tomographic densitometric analysis. Invest Radiol 2000 Apr;35(4):235-43.

29. Mair G, Miller JJ, McAllister D, Maclay J, Connell M, Murchison JT, et al. Computed tomographic emphysema distribution: relationship to clinical features in a cohort of smokers. Eur Respir J 2009 Mar;33(3):536-42.

30. de Torres JP, Bastarrika G, Zagaceta J, Saiz-Mendiguren R, Alcaide AB, Seijo LM, et al. Emphysema presence, severity, and distribution has little impact on the clinical presentation of a cohort of patients with mild to moderate COPD. Chest 2011 Jan;139(1):36-42.

31. Demeo DL, Hersh CP, Hoffman EA, Litonjua AA, Lazarus R, Sparrow D, et al. Genetic determinants of emphysema distribution in the national emphysema treatment trial. Am J Respir Crit Care Med 2007 Jul 1;176(1):42-8.

32. Ito I, Nagai S, Handa T, Muro S, Hirai T, Tsukino M, et al. Matrix metalloproteinase-9 promoter polymorphism associated with upper lung dominant emphysema. Am J Respir Crit Care Med 2005 Dec 1;172(11):1378-82.

33. Hersh CP, Washko GR, Jacobson FL, Gill R, Estepar RS, Reilly JJ, et al. Interobserver variability in the determination of upper lobe-predominant emphysema. Chest 2007 Feb;131(2):424-31.

34. Sverzellati N, Calabro E, Randi G, La VC, Marchiano A, Kuhnigk JM, et al. Sex differences in emphysema phenotype in smokers without airflow obstruction. Eur Respir J 2009 Jun;33(6):1320-8.

Supplement Chapter 4

Distribution of CT-quantified Emphysema in Heavy Smokers: Association with Lung Function Decline

Results when using the %950 HU as emphysema measure

Median (interquartile range (Q_1-Q_3)) %950 HU was 0.59 (0.28-1.33). In Table S1 the %950HU per lung lobe is provided. The results from the principal component analysis are found in Table 2. Two components were retained explaining 85.7% of the total variance.

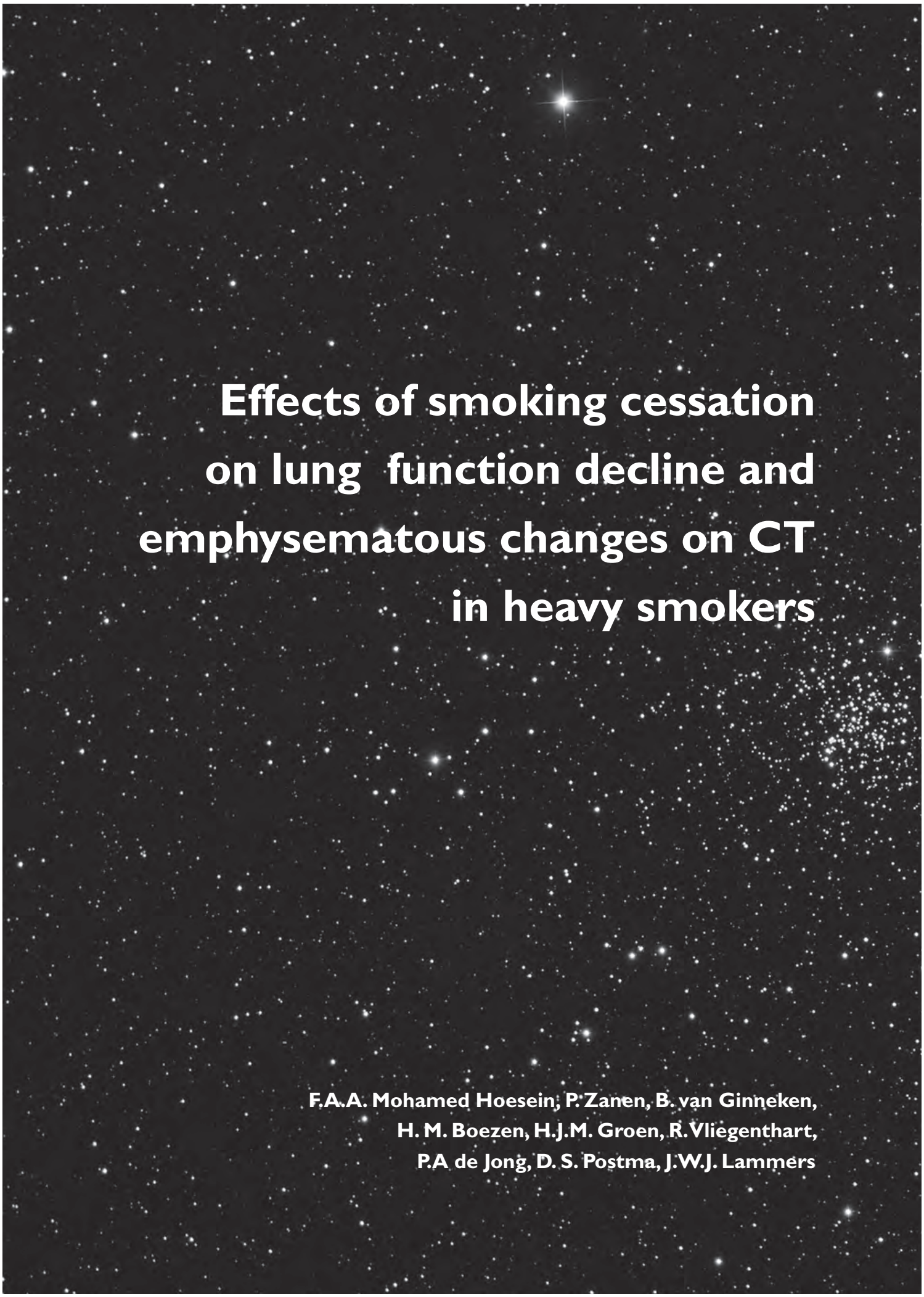
Table S1. Median (Q_1-Q_3) %950 HU per lung lobe (column 2) and results from principal components analysis: component scores (columns 3 and 4). The two new components explained 85.7% of the total variance. Component 1: median %950HU value of all lobes (emphysema score); component 2: difference between upper and lower lobe %950HU, i.e. lower / upper lobe emphysema predominance (distribution score). The component scores can be interpreted in a similar way as the regression coefficients (beta's) from multiple linear regression analysis.

Lung lobe	Median (Q_1-Q_3) %950 HU	Scores component 1 (emphysema score)	Scores component 2 (distribution score)
Left upper lobe	0.79 (0.36 - 1.67)	0.247	-0.550
Right upper lobe	0.45 (0.19 - 1.14)	0.257	-0.591
Right middle lobe	0.33 (0.11 - 1.08)	0.241	-0.355
Left lower lobe	0.59 (0.28 - 1.37)	0.234	0.416
Right lower lobe	0.31 (0.10 – 0.82)	0.239	0.371
Variance explained [%]		**67.35**	**18.37**

Table S2. Results from the linear mixed model analysis when using the component scores based on the %950HU measure for emphysema severity. P-values and 95% confidence intervals (CI95%) of these effects are provided.

Estimated effects of changes in parameters on FEV_1/FVC [%]				
Parameter	**Change in parameter**	**Resulting change in FEV_1/FVC**	**CI 95%**	**P-value**
Emphysema score (median overall %950 HU)	minus 1 point	-4.45%	3.85 – 5.10	**<0.001**
Distribution score (difference between upper and lower lobe %950 HU values)	minus 1 point	-0.741%	0.12 – 1.36	**0.023**


Chapter
5



Effects of smoking cessation on lung function decline and emphysematous changes on CT in heavy smokers

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Abstract

Purpose The relationship between the duration of smoking cessation and lung function decline and emphysematous changes in heavy smokers is not well understood. We investigated the effects of the duration of smoking cessation on lung function decline and the course of CT-quantified emphysema.

Methods Over a 3-year period, 2,003 current and former heavy smokers with a mean (SD) of 40.2 (17.6) pack-years underwent two pulmonary function tests and two CT scans. Smoking status at enrolment, including whether the individual was a current smoker or the number of years of smoking cessation, was assessed. The 15th percentile point (Perc15) method was used to quantify the severity of emphysema on CT. Changes in lung function and emphysema severity were analysed using multiple linear regression.

Results The mean (SD) age at the baseline was 59.8 (5.3) years, the FEV_1/FVC was 72.1% (9.4), the FEV_1 was 3.4 L (0.73) or 98.5% (18.5) of predicted, and the Perc15 was -934.9 HU (19.5). Participants who had ceased smoking for 1-5 years or ≥5 years showed significantly smaller declines in all the tested lung function parameters ($p<0.05$) than did current smokers. However, individuals who had ceased smoking for less than 1 year was not significantly different from the group of current smokers. The Perc15 decline was significantly slower in the group that had quit between 1 and 5 years previously ($p<0.05$).

Conclusion Smoking cessation stabilised lung function decline and emphysema development after a ≥4 year cessation (>1 year at enrolment and 3-years of follow-up).

Introduction

Chronic obstructive pulmonary disease (COPD) is a common ailment with an estimated worldwide prevalence of approximately 8.9%. [1] COPD is a heterogeneous condition that is characterised by airway remodelling and emphysema, which lead to airflow obstruction. Airflow obstruction is easily diagnosed by spirometry, but diagnosing emphysema is more difficult because it requires pathology specimens. However, computed tomography (CT) may also be used to quantify emphysema. This quantification is performed using lung densitometry, which estimates the loss of lung tissue density. [2]

Because the mortality rate of COPD is rising, early recognition and prevention of this disease are of key importance. [3] Currently, smoking cessation is the most important known intervention as it alters the progression of disease. [4 5] However, the effects of the duration of smoking cessation on the rate of lung function decline and emphysema development have not been thoroughly investigated in heavy smokers who are otherwise relatively healthy. Most previous studies have focused only on subjects with airflow obstruction, and these studies used lung function decline or mortality as the primary outcome. [4] One study investigated the effect of the duration of smoking cessation on the progression of emphysema, and the authors described a positive effect of cessation. [6] To our knowledge, no studies have assessed the effect smoking cessation on both lung function decline and emphysematous changes on CT.

The NELSON lung cancer screening trial included relatively healthy heavy smokers who were at risk of developing COPD. [7] This group is of particular interest because an emphasis on smoking cessation may be of the utmost importance in preventing further disease progression in relatively healthy patients. We hypothesised that the beneficial effects of smoking cessation in our population of current and former heavy smokers would depend on the duration of the cessation. Therefore, we investigated the effects of the duration of smoking cessation on the course of lung function decline and CT-quantified emphysematous changes.

Methods

Participants

This study was conducted among the participants of the Dutch-Belgian Lung Cancer Screening Trial (NELSON), which was performed by the University Medical Centres in Utrecht and Groningen, the Netherlands. The population-based NELSON trial included

participants at high risk of developing lung cancer and COPD. [7] The Dutch Ministry of Health and the Medical Ethics Committees of both participating hospitals approved the study protocol, and written informed consent was obtained from all participants.
Individuals with a minimal smoking history of 20 pack-years were invited to participate. Only males were included, based on their higher risk of developing lung cancer / COPD. Relatively few women in the Dutch population have the same level of long-term tobacco exposure as do Dutch men. Individuals with a moderate or poor self-reported health status or those who were unable to climb two flights of stairs were excluded.
Detailed information on smoking habits was collected through questionnaires at baseline, including information regarding the duration of smoking or the duration of smoking cessation at the time of enrolment in the study (i.e., ≥5 years, 1-5 years or ≤1 year). At the beginning of the study, we determined that this research provided a unique opportunity to assess lung function in all the participants and to investigate the results in relation to the CT findings. Therefore, lung function was assessed by spirometry in all individuals.

Pulmonary function tests

Pulmonary function tests (PFT) were performed with standardised equipment according to the European Respiratory Society (ERS) guidelines. The tests included forced expiratory volume in one second (FEV_1), FEV_1/ forced vital capacity (FVC), and maximum expiratory flow at 50% of the FVC (MEF_{50}). [8] Airflow obstruction was considered to be present if FEV_1/FVC <70%. [9] Bronchodilation was not applied. The equipment used at both centres was interchangeable. [10]

CT Scanning

The CT protocol was performed as previously described. [11] In brief, all participants underwent low-dose CT without the intravenous injection of contrast agents at the baseline and after 3 years of follow-up. At both screening sites, 16-detector MDCT scanners were used (Mx8000 IDT or Brilliance 16P, Philips Medical Systems, Cleveland, OH or Sensation-16 Siemens Medical Solutions, Forchheim, Germany). Scan data were obtained at full inspiration in spiral mode, with 16 x 0.75-mm collimation, 15-mm table feed per rotation, and pitch=1.3. No spirometric gating was applied because it has not been shown to improve the repeatability of lung density measurements. [12;13] Axial images were reconstructed with a 1.0-mm thickness at 0.7-mm increments. All scans were reconstructed using a soft reconstruction filter (Philips B, Siemens B30f) with a 512x512

matrix. The exposure settings were 30mAs at either 120kVp or 140kVp, depending on the participant's weight, which resulted in CTDIvol values of 1.6mGy and 3.2mGy. The effective doses were <0.9mSv and <1.6mSv, respectively. This low-dose CT protocol has been previously used to quantify emphysema in COPD patients and heavy smokers. [14-16]

Emphysema quantification

All CT scans were automatically analysed as previously described. [17 11] In brief, the airways were excluded to ensure that only the lung parenchyma was analysed. Air calibration is critical in multi-centre lung densitometry studies, and the incorporation of a correction factor is essential for quantitative image analysis [18]. Therefore, the CT examinations were recalibrated using air in the trachea to ensure comparability between the two centres. Emphysema was quantified by the 15th percentile. This method provides the point in Hounsfield units (HU), below which 15% of all voxels are distributed. When the Perc15 values are lower, i.e., the closer to -1,000 HU, the more emphysema is present. This method of emphysema quantification has been validated against pathology samples [2] and has been applied in multiple studies. [11 19] Because the Perc15 is the most robust measurement of emphysema and its progression, [20] it was used in the present study instead of the <-950 HU measurement (defined as the proportion of low-density voxels below -950 HU).

Statistical analyses

Mean and standard deviation (SD) values were calculated for normally distributed data and median values and interquartile ranges (Q1-Q3) were calculated for non-normally distributed data. Previous research has shown that lung function decline can be assumed to be linear over a time span of 3 years.[21] End-observation lung function parameters (FEV_1/FVC, FEV_1, MEF_{50}) and Perc15 at the end of the observation period were analysed using multiple linear regression. Smoking status at enrolment in the study (current smoker or quit ≥5 years, 1-5 years or ≤1 year prior) was the main explanatory factor. The group of current smokers was used as the reference. At the beginning of the study, we adjusted for lung function / Perc15, pack-years, centre, height, body mass index (BMI), and age to obtain the adjusted end observation values. Declines in lung function and Perc15 were calculated by subtracting the adjusted end-observation values from the baseline. P-values less than 0.05 were considered significant. All statistical analyses were performed using SPSS 18 for Windows (SPSS, Chicago, Illinois, USA).

Results

Baseline demographics, lung function and Perc15

In total, 2,003 participants underwent PFT and CT scanning twice with a median (Q1-Q3) follow-up time of 3.0 (2.9-3.1) years and these were included in the current study. The mean (SD) age of the subjects was 59.8 (5.3) years, with a mean of 40.2 (17.6) pack-years. The majority of the participants, 1,093 out of 2,003 (54.6%), were current smokers. Only 339 (16.9%) had stopped for ≥5 years; 406 (20.3%) had stopped for 1-5 years, and 165 (8.2%) had stopped for ≤1 year.

Table 1. Baseline demographics status at baseline for the total cohort. *median (inter quartile range)

	Total N = 2,003
Age	59.8 (5.3)
Pack years	40.2 (17.6)
Years in study*	3.0 (2.9-3.1)
BMI [kilograms*meter^{-2}]	26.9 (3.4)
FEV_1/FVC <70%	33%
FEV_1/FVC [%]	72.2 (9.4)
FEV_1 [L]	3.4 (0.73)
FEV_1 %pred	98.5 (18.5)
MEF_{50} [L/s]	3.2 (1.4)
Perc15 (HU)	-934.9 (19.5)

Lung function parameters for the entire study population and the classifications according to smoking status are provided in Tables 1 and 2, respectively. We determined that 1,343 (67%) of the patients had an FEV_1/FVC >70%. There was no difference in the prevalence of FEV_1/FVC <70% between the four smoking groups (p= 0.233). There were no significant differences in FEV_1, FEV_1/FVC or MEF_{50} values between the four smoking groups (p= 0.42, 0.19 and 0.49, respectively).

Table 2. Baseline demographics according to smoking status at baseline. *median (inter quartile range)

	Quit ≥5years N=339	Quit 1 – 5 years N=406	Quit ≤1 year N=165	Current smoker N= 1,093
Age	62.3 (6)	60.6 (5.3)	59.5 (5.1)	58.9 (4.8)
Pack years	40.7 (19.5)	41.6 (19.7)	41 (17.6)	39.4 (16)
Years in study*	3.0 (2.9-3.1)	3.0 (2.9-3.1)	3.0 (2.9-3.2)	3.0 (2.9-3.1)
BMI [kilograms*meter^{-2}]	27.8 (3.2)	27.4 (3.2)	27.4 (3.7)	26.2 (3.3)
FEV_1/FVC <70%	29%	32%	31%	34%
FEV_1/FVC [%]	72.8 (9)	72.8 (9.1)	71.8 (10.2)	71.9 (9.4)
FEV_1 [L]	3.4 (0.8)	3.4 (0.7)	3.5 (0.8)	3.4 (0.7)
FEV_1 % pred	99.9 (20)	100 (18)	97.1 (19.4)	97.6 (17.9)
MEF_{50} [L/s]	3.2 (1.4)	3.3 (1.4)	3.2 (1.5)	3.2 (1.4)
Perc15 (HU)	-941.4 (18.1)	-942.1 (17)	-939.1 (16.6)	-929.6 (19.6)

The overall mean (SD) baseline Perc15 was -934.9 HU (19.4). Emphysema severity (Perc15) varied significantly according to the duration of smoking cessation ($p<0.001$) (Table 2).

Effects of the duration of smoking cessation on lung function decline

The mean (SD) FEV_1/FVC at the end of the follow-up period was 69.3% (9.9), the mean FEV_1 was 3.21 L (0.72) or 95.4% (19.0) of predicted, and the mean (SD) MEF_{50} was 2.88 L/s (1.33) or 64.2% (29.1) of predicted. During a median (Q1-Q3) follow-up period of 3.0 (2.9-3.1) years, the adjusted mean (SD) decline in FEV_1/FVC was 2.9% (1.2), the decline in FEV_1 was 0.21 L (0.07) or 3.2% (2.1) of predicted and the decline in the MEF_{50} was 0.32 L/s (0.29) or 5.5% (15.1) of predicted. The statistical models explained 90%, 81% and 80% of the variance in FEV_1, FEV_1/FVC and MEF_{50} after follow-up, respectively (R^2 = 0.90, 0.81 and 0.80, respectively).

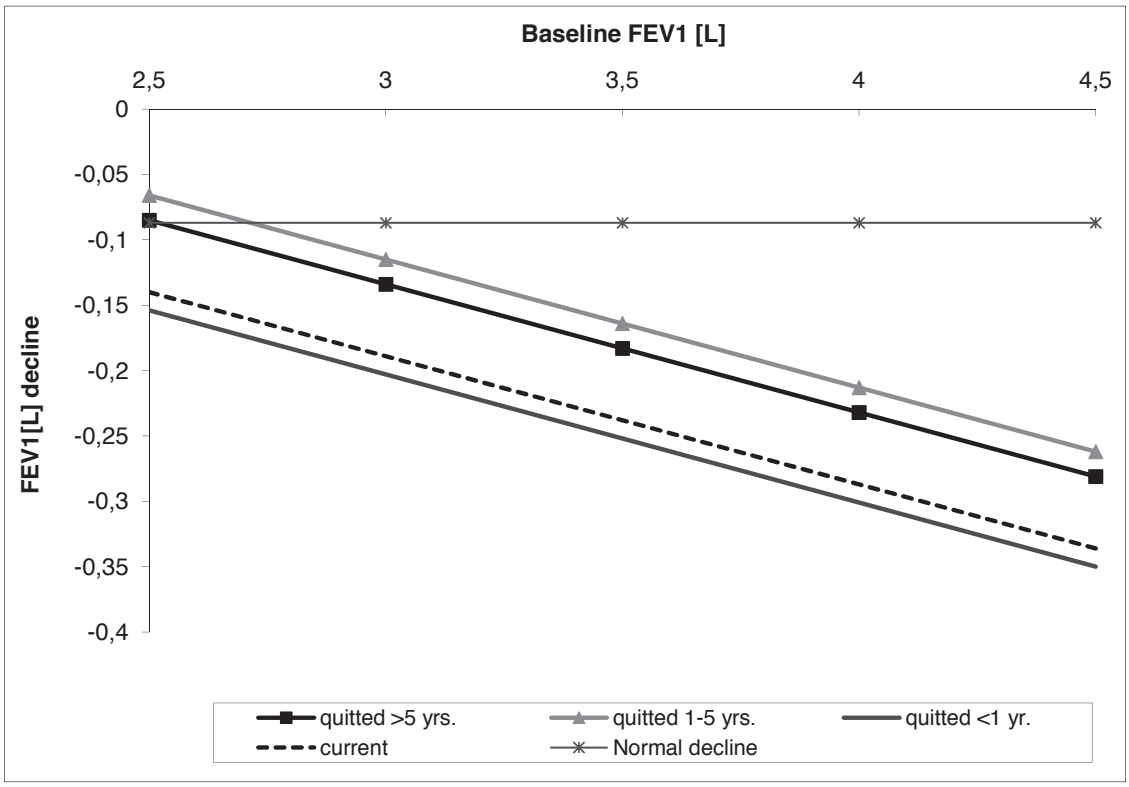
All three lung function parameters were significantly higher in the groups of participants who had quit for ≥5 years or between 1 and 5 years at the time of enrolment in the study when compared with the current smokers. For participants who had quit for ≤1 year at enrolment, there were no significant differences in lung function decline when compared with continuing smokers. The effects of smoking status on changes in FEV_1, FEV_1/FVC and MEF_{50} values during follow-up are listed in Table 3 and depicted in Figure 1. The estimated effects of the other significant covariates in the model are supplied in the online supplement.

Table 3. Estimates for duration of smoking cessation at start study are listed for comparison. Significant differences in changes in lung function parameters and Percentile 15 compared to the current smokers group are in bold. The estimates for duration of smoking cessation in relation with baseline lung function and/ baseline emphysema severity are visualized in Figures 1 and 2.

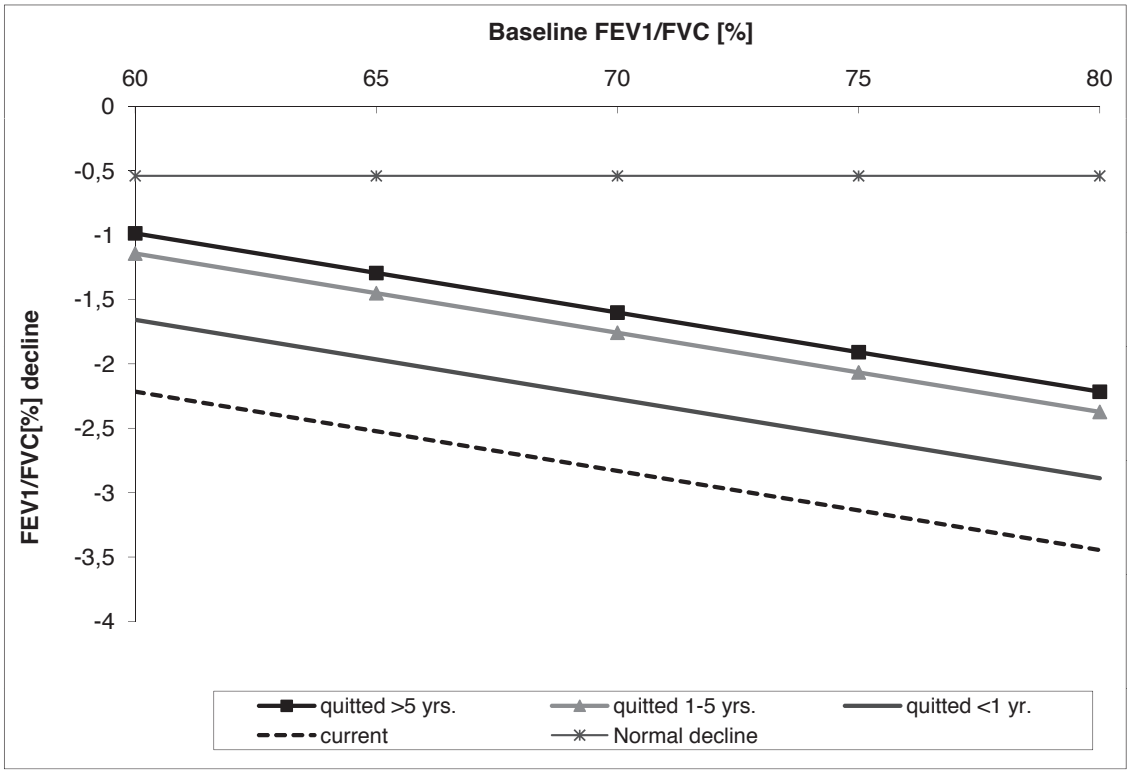
	Comparison	Estimated Effect	Confidence interval 95%	p Value
FEV_1 [mL]	**>5 versus 0**	+55	27– 82	**<0.001**
	≥1 - ≤5 versus 0	+71	45 – 96	**<0.001**
	≤1 versus 0	-9	-46 – 28	0.621
FEV_1/FVC [%]	**>5 versus 0**	+1.18	0.67 – 1.69	**<0.001**
	≥1 - ≤5 versus 0	+1.1	0.61 – 1.56	**0.004**
	≤1 versus 0	+0.63	-0.048 – 1.31	0.068
MEF_{50} [mL/s]	**>5 versus 0**	+160	89 – 231	**<0.001**
	≥1 - ≤5 versus 0	+150	83 – 215	**0.03**
	≤1 versus 0	+70	-28 – 161	0.65
Perc15 (HU)	**>5 versus 0**	-1.47	-2.90 – -0.03	**<0.045**
	≥1 - ≤5 versus 0	-1.73	-3.08 – -0.38	**<0.012**
	≤1 versus 0	-1.87	-3.76 – .03	0.053

Figure 1.

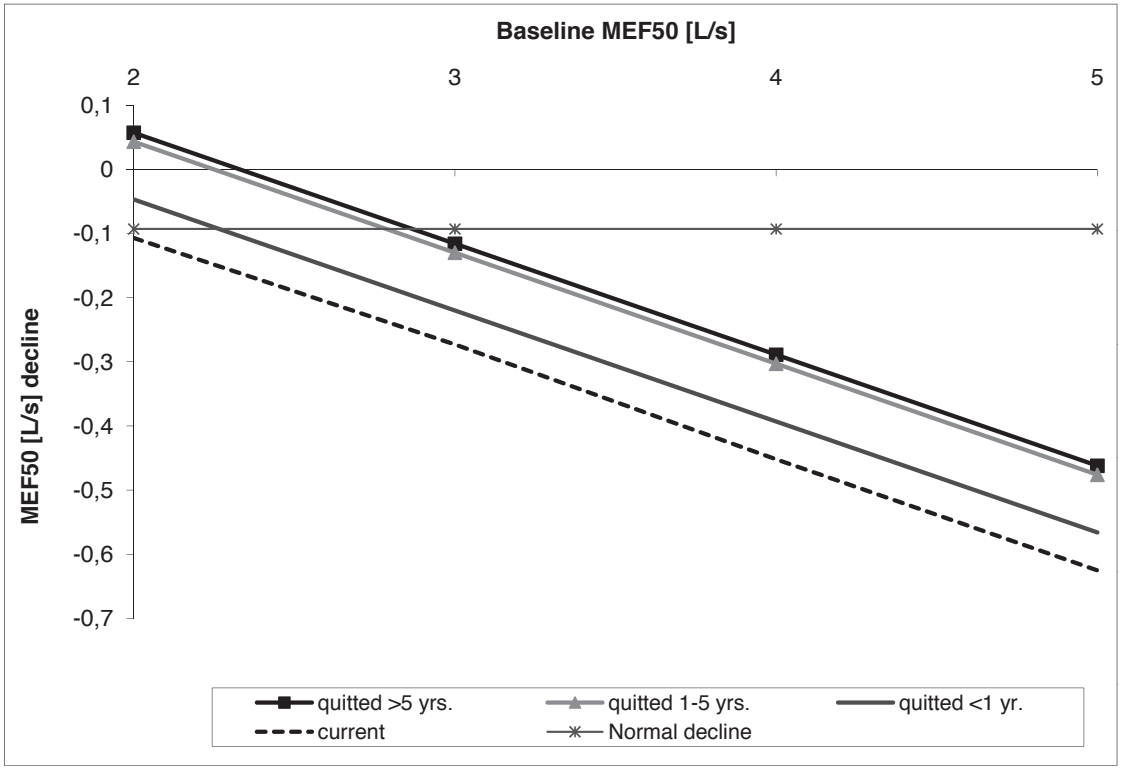
A. Adjusted FEV_1 decline according to smoking status and baseline FEV_1 [L]. These situations apply for subjects with a starting age = 60 years, height = 179 cm, pack years smoking = 40 years.



B. Adjusted FEV_1/FVC decline according to smoking status and baseline FEV_1/FVC [%]. These situations apply for subjects with a starting age = 60 years, height = 179 cm, pack years smoking = 40 years.



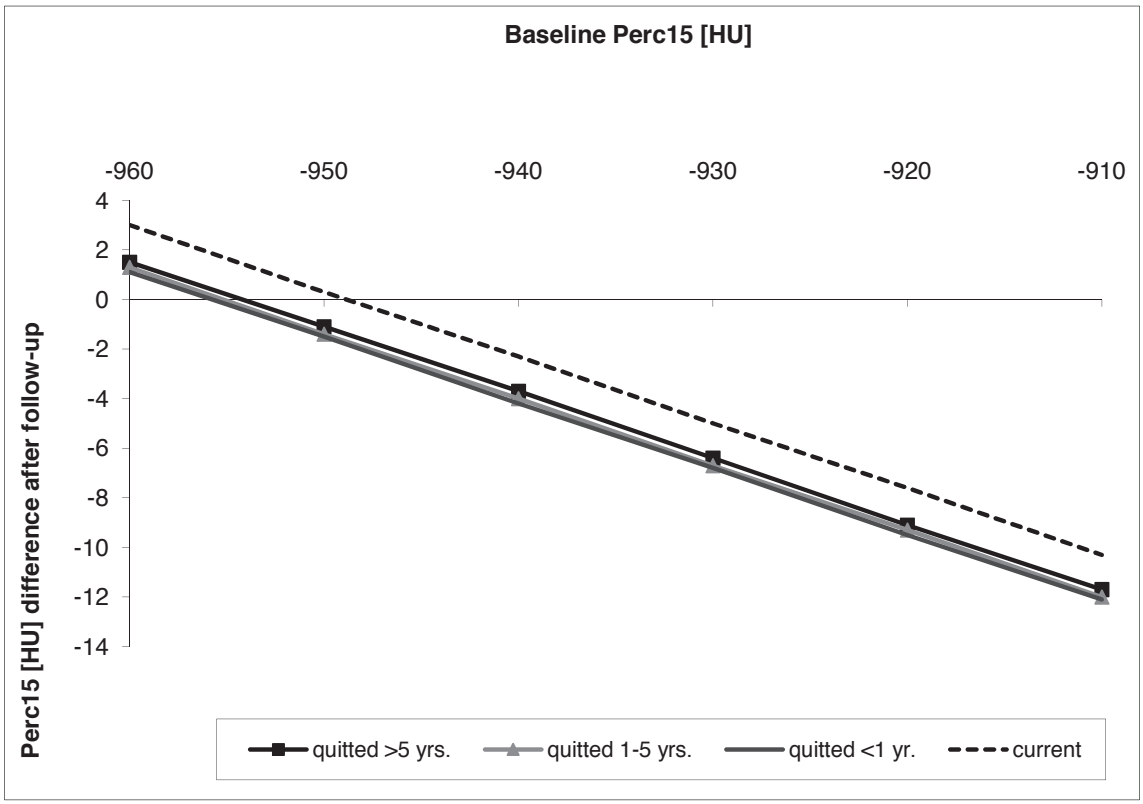
C. Adjusted $MEF5_{50}$ decline according to smoking status and baseline MEF_{50} [L]. These situations apply for subjects with a starting age = 60 years, height = 179 cm, pack years smoking = 40 years.



Effects of the duration of smoking cessation on Perc15 progression

The mean (SD) Perc15 at the end of the follow-up period was -938.1 (19) HU. The statistical model explained 63% of the variance in Perc15 (R^2 = 0.63). The adjusted effects of the duration of smoking on the course of Perc15 are listed in Table 3. The estimated effects of all of the significant covariates in the model are supplied in the online supplement. Perc15 at follow-up was modestly but significantly lower in the groups that had quit smoking for either 1-5 years or ≥5 years at the time of enrolment (p=0.045 and 0.012, respectively) when compared with the Perc15 of the continuing smokers. The Perc15 at follow-up in the groups that had only quit for ≤1 year did not significantly differ from that of the continuing smokers (p=0.053). The Perc15 values according to smoking status and baseline emphysema severity are depicted in Figure 2.

Figure 2. Adjusted difference in Perc15 [HU] during follow-up according to smoking status and baseline Perc15 value. These situations apply for subjects with a starting age = 60 years, height = 179 cm, pack years smoking = 40 years.



Discussion

Our results show that smoking cessation for ≥4 years, i.e., >1 year at the time of enrolment in the study and for the 3-year median follow-up, reduced the decline in lung function in this cohort of current and former heavy smokers. Furthermore, smoking cessation significantly reduced the progression of Perc15 assessed by CT scans, which is a measure of emphysema. However, these effects were small. Taken together, the results indicate that the duration of smoking cessation has differential effects on the conducting airways and on the extracellular matrix, effects which are expressed as Perc15 values. This result is consistent with the hypothesis that COPD consists of small airway disease and emphysema.

Effects of smoking cessation on lung function decline

Other longitudinal studies have examined the effects of smoking cessation on lung function decline in population-based cohorts and have generally shown that smoking cessation was beneficial. [22;23] However, only a few studies have addressed the effects of the duration of smoking cessation. Our results expand on the outcomes of the Lung Health Study (LHS), a landmark study that investigated the effect of smoking cessation in COPD patients. [24] The LHS study demonstrated that quitters had a considerably lower FEV_1 decline than continuous smokers, with values of 31 and 62 mL/year, respectively. There are some important differences between our study and the LHS. First, the LHS included subjects with COPD, whereas the majority of our subjects had no COPD. The population studied in the LHS was relatively young, with a mean age below 50 years, but with a similar number of packyears smoked as in our population, 40 pack years. The LHS therefore included smokers with a high susceptibility to COPD. In contrast, we also included a large number of participants without COPD (67%). The LHS investigators reported that after 11 years of follow-up, the rate of FEV_1 decline in former smokers did not differ from that in never-smokers.

Because we did not include never-smokers in our study, we cannot compare the decline in former smokers to that in never-smokers. However, when comparing the rate of FEV_1 decline in our group of participants who had for quit ≥5 years at enrolment (55 mL/ year) to the 'normal' decline according to the reference values of the European Community for Coal and Steel (29 mL/ year), it is evident that FEV_1-decline had not yet normalised. [25]

The investigators of the Honolulu Heart Program (HHP) found that individuals who

quit smoking during the first two years of follow-up had almost the same decline in FEV_1 as the continuing smokers, 32 ml/ year and 34 ml/ year, respectively.[22] Subjects who had quit for >2 years had nearly the same decline as never-smokers, 19 ml/ year and 21 ml/ year, respectively. In contrast, >4 years of smoking cessation was required in our study before the lung function decline stabilised. An explanation for this difference could lie in the study population we selected, all of whom were current or former heavy smokers. The HHP also included never-smokers. Unfortunately, our study design did not include never-smokers. Therefore, it is impossible to compare the rates of decline that we observed with the rates of decline in never-smokers.

After smoking cessation, pathological processes, such as those leading to accelerated FEV1 decline, apparently need time to arrest or disappear. Cross-sectional studies have demonstrated that the percentage of CD4+ lymphocytes normalised after two years of smoking cessation. 26 27 Lapperre et al. found that long-term ex-smokers who had quit for ≥3.5 years exhibited significantly lower CD8 cell numbers than those who had quit for <3.5 years.. 28 Furthermore, Lapperre et al. also reported that epithelial remodelling in bronchial biopsies from COPD subjects was only reduced in subjects who had quit smoking for ≥3.5 years when compared with current smokers. [29] Notably, all the subjects included in these studies had GOLD stage II/III COPD. There is an unfortunate lack of longitudinal studies on the effects of the duration of smoking cessation on inflammatory processes with longer follow-up periods and larger numbers of subjects with and without overt COPD. Nonetheless, the results of the above studies suggest that pathological processes require time to be silenced, and the effect of the duration of smoking cessation on inflammation is consistent with the approximately 4 years required to stabilise the FEV_1 decline in our cohort of current and former heavy smokers with and without COPD.

Effects of smoking cessation on Perc15 as marker of emphysema

The effect of ≥4 years of smoking cessation on Perc15 progression in the current work was, although significant, modest. Only a few studies to date have examined the effects of smoking cessation on the progression of CT-quantified emphysema. Bellomi et al. reported that current smokers had a higher risk of emphysema deterioration (OR 1.6, 95% CI 1.1-2.4), which was defined as an increase of 30% in % <-950 HU, than former smokers after 2 years of follow-up. [5] The design of that study was comparable to ours in that the subjects participated in a lung cancer screening trial and underwent low-dose CT scans. Unfortunately, because the lung function values of the participants were not

obtained, the investigators did not correct for lung function differences between former and current smokers. This omission may help explain some of the differences in the emphysema increases between the two groups. Furthermore, the duration of smoking cessation in their study ranged from 0 to 11 years, but the effect of the duration of smoking cessation was not considered. Soejima et al. reported no significant differences in CT-quantified emphysema progression between former and current smokers after a 5-year follow-up, and they concluded that smoking cessation does not alter emphysema progression. However, their sample was small, consisting of only 35 current and 12 former smokers, and they did not correct for the ages of the study participants and the duration of smoking cessation. These factors may have diluted the effects of smoking cessation. Ashraf et al. examined a study population that was comparable to ours and demonstrated that after 2 years of smoking cessation, no significant changes in CT-quantified emphysema could be detected. [30] These results are consistent with our findings, and we expanded on their results by employing a longer follow-up period of a median of 3 years, compared to only 1 year of follow-up in their study.

However, the question remains: why is there only a modest effect of duration of smoking cessation on Perc15 progression? It is possible that the participants in our cohort exceeded a threshold with respect to the effects of smoking on emphysema development because the subjects had an average smoking exposure of approximately 40 pack-years. This theory is compatible with the observations of Wright et al., who reported that smoking cessation after a smoking exposure of four months halted the emphysematous changes in guinea pigs but did not reverse them. [31] Those results imply that the animals exposed to smoking for four months passed a point of no return. Moreover, we did not find a significant effect of pack-years smoked on Perc15 progression. Because we assume that smoking caused in increase in emphysema rates in this group of heavily smoking subjects, the lack of influence of pack-years on Perc15 progression signifies that emphysema progression in this group was independent of additional smoking exposure, which is more evidence that these patients passed a point of no return.

Strengths and limitations

A major strength of this study is the large number of participants (n=2,003) recruited from the general population but with a high risk of developing COPD. This strength makes our results applicable to the vast number of 'healthy smokers'. This group is of particular interest because smoking cessation is the most effective intervention for disease progression. Second, both lung function tests and CT-scans were prospectively

collected before and after 3-year follow-up. Second, both lung function tests and CT scans were prospectively performed before and after 3 years of follow-up. A previous study assessed the effect of short-term smoking cessation on Perc15 progression alone, but the authors did not report any significant consequences for lung function. [30] This outcome was probably due to their short follow-up. Third, the quantification of emphysema was fully automated and employed the same software packages in the two hospitals, making it free from interrater variability. Each CT scan was recalibrated using tracheal air to reduce scanner bias. Nonetheless, a hospital factor was inserted in the statistical analysis to remove any residual bias. Failure to inspire at the same levels at baseline and follow-up could influence the emphysema quantification; however, in the current study, no significant differences existed in inspiratory CT volumes at baseline and follow-up.

This study has some limitations that deserve comment. First, smoking cessation was self-reported and not objectively confirmed by exhaled carbon monoxide or cotinine tests. Second, some participants could have resumed smoking during the observation period. However, this occurrence would only have diluted the reported effects of the duration of smoking cessation, therefore causing an underestimation of the true effects. Third, spirometry was performed without prior bronchodilation, which could possibly lead to an overestimation of airflow obstruction. However, because both the baseline and follow-up lung function tests were performed without bronchodilation, this omission is not likely to have influenced the lung function decline over time. Lastly, no females were included in our study, which is unfortunate because COPD prevalence in women is rising. Females have lower rates of CT-quantified emphysema when compared to males. [32] Therefore, future studies should also include female subjects.

In conclusion, we showed that smoking cessation for ≥4 years reduced lung function decline in former heavy smokers, but it did not elicit clinically relevant changes in the development of emphysema (assessed using CT). Nonetheless, our results demonstrate that smoking cessation also provides benefits to heavy smokers.

Reference List

1. Halbert, R. J., J. L. Natoli, A. Gano, E. Badamgarav, A. S. Buist, and D. M. Mannino. 2006. Global burden of COPD: systematic review and meta-analysis. *Eur.Respir J* 28:523-532.
2. Gould, G. A., W. MacNee, A. McLean, P. M. Warren, A. Redpath, J. J. K. Best, D. Lamb, and D. C. Flenley. 1988. CT measurements of lung density in life can quantitate distal airspace enlargement - an essential defining feature of human emphysema. *AM.REV. RESPIR.DIS.* 137:380-392.
3. Lopez, A. D., K. Shibuya, C. Rao, C. D. Mathers, A. L. Hansell, L. S. Held, V. Schmid, and S. Buist. 2006. Chronic obstructive pulmonary disease: current burden and future projections. *Eur.Respir.J.* 27:397-412.
4. Godtfredsen, N. S., T. H. Lam, T. T. Hansel, M. E. Leon, N. Gray, C. Dresler, D. M. Burns, E. Prescott, and J. Vestbo. 2008. COPD-related morbidity and mortality after smoking cessation: status of the evidence. *Eur.Respir.J.* 32:844-853.
5. Willemse, B. W., D. S. Postma, W. Timens, and N. H. ten Hacken. 2004. The impact of smoking cessation on respiratory symptoms, lung function, airway hyperresponsiveness and inflammation. *Eur.Respir J* 23:464-476.
6. Bellomi, M., C. Rampinelli, G. Veronesi, S. Harari, F. Lanfranchi, S. Raimondi, and P. Maisonneuve. 2010. Evolution of emphysema in relation to smoking. *Eur.Radiol.* 20:286-292.
7. van Iersel, C. A., H. J. de Koning, G. Draisma, W. P. Mali, E. T. Scholten, K. Nackaerts, M. Prokop, J. D. Habbema, M. Oudkerk, and R. J. van Klaveren. 2007. Risk-based selection from the general population in a screening trial: selection criteria, recruitment and power for the Dutch-Belgian randomised lung cancer multi-slice CT screening trial (NELSON). *Int.J.Cancer* 120:868-874.
8. Miller, M. R., R. Crapo, J. Hankinson, V. Brusasco, F. Burgos, R. Casaburi, A. Coates, P. Enright, C. P. van der Grinten, P. Gustafsson, R. Jensen, D. C. Johnson, N. MacIntyre, R. McKay, D. Navajas, O. F. Pedersen, R. Pellegrino, G. Viegi, and J. Wanger. 2005. General considerations for lung function testing. *Eur.Respir J* 26:153-161.
9. Rabe, K. F., S. Hurd, A. Anzueto, P. J. Barnes, S. A. Buist, P. Calverley, Y. Fukuchi, C. Jenkins, R. Rodriguez-Roisin, W. C. van, and J. Zielinski. 2007. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am.J Respir Crit Care Med* 176:532-555.
10. Munnik, P., P. Zanen, and J. W. Lammers. 2006. A comparison of lung function equipment with emphasis on interchangeability and methods. *Physiol Meas.* 27:445-455.
11. Mohamed Hoesein, F. A., H. B. de, P. Zanen, H. Gietema, C. L. Kruitwagen, G. B. van, I. Isgum, C. Mol, R. J. van Klaveren, A. E. Dijkstra, H. J. Groen, H. M. Boezen, D. S. Postma, M. Prokop, and J. W. Lammers. 2011. CT-quantified emphysema in male heavy smokers: association with lung function decline. *Thorax*.
12. Gierada, D. S., R. D. Yusen, T. K. Pilgram, L. Crouch, R. M. Slone, K. T. Bae, S. S. Lefrak, and J. D. Cooper. 2001. Repeatability of Quantitative CT Indexes of Emphysema in Patients Evaluated for Lung Volume Reduction Surgery. *Radiology* 220:448-454.

13. Newell, J. D., Jr., J. C. Hogg, and G. L. Snider. 2004. Report of a workshop: quantitative computed tomography scanning in longitudinal studies of emphysema. *Eur Respir J* 23:769-775.

14. Mair, G., J. J. Miller, D. McAllister, J. Maclay, M. Connell, J. T. Murchison, and W. MacNee. 2009. Computed tomographic emphysema distribution: relationship to clinical features in a cohort of smokers. *Eur Respir J* 33:536-542.

15. Shaker, S. B., N. Maltbaek, P. Brand, S. Haeussermann, and A. Dirksen. 2005. Quantitative computed tomography and aerosol morphometry in COPD and alpha1-antitrypsin deficiency. *Eur Respir J* 25:23-30.

16. Sverzellati, N., E. Calabro, G. Randi, V. C. La, A. Marchiano, J. M. Kuhnigk, M. Zompatori, P. Spagnolo, and U. Pastorino. 2009. Sex differences in emphysema phenotype in smokers without airflow obstruction. *Eur Respir J* 33:1320-1328.

17. van Rikxoort, E. M., de Hoop B, M. A. Viergever, M. Prokop, and B. van Ginneken. 2009. Automatic lung segmentation from thoracic computed tomography scans using a hybrid approach with error detection. *Med.Phys.* 36:2934-2947.

18. Parr, D. G., B. C. Stoel, J. Stolk, P. G. Nightingale, and R. A. Stockley. 2004. Influence of calibration on densitometric studies of emphysema progression using computed tomography. *Am J Respir Crit Care Med.* 170:883-890.

19. Parr, D. G., B. C. Stoel, J. Stolk, and R. A. Stockley. 2006. Validation of computed tomographic lung densitometry for monitoring emphysema in {alpha}1-antitrypsin deficiency. *Thorax* 61:485-490.

20. Parr, D. G., M. Sevenoaks, C. Deng, B. C. Stoel, and R. A. Stockley. 2008. Detection of emphysema progression in alpha 1-antitrypsin deficiency using CT densitometry; methodological advances. *Respir.Res.* 9:21.

21. Tashkin, D. P., B. Celli, S. Senn, D. Burkhart, S. Kesten, S. Menjoge, M. Decramer, and U. S. the, I. 2008. A 4-Year Trial of Tiotropium in Chronic Obstructive Pulmonary Disease. *N Engl J Med* 359:1543-1554.

22. Burchfiel, C. M., E. B. Marcus, J. D. Curb, C. J. Maclean, W. M. Vollmer, L. R. Johnson, K. O. Fong, B. L. Rodriguez, K. H. Masaki, and A. S. Buist. 1995. Effects of smoking and smoking cessation on longitudinal decline in pulmonary function. *Am.J Respir Crit Care Med* 151:1778-1785.

23. Tashkin, D. P., V. A. Clark, A. H. Coulson, M. Simmons, L. B. Bourque, C. Reems, R. Detels, J. W. Sayre, and S. N. Rokaw. 1984. The UCLA population studies of chronic obstructive respiratory disease. VIII. Effects of smoking cessation on lung function: a prospective study of a free-living population. *Am.Rev Respir Dis* 130:707-715.

24. Anthonisen, N. R., J. E. Connett, and R. P. Murray. 2002. Smoking and lung function of Lung Health Study participants after 11 years. *Am.J Respir Crit Care Med* 166:675-679.

25. Quanjer, P. H., G. J. Tammeling, J. E. Cotes, O. F. Pedersen, R. Peslin, and J. C. Yernault. 1993. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur.Respir J Suppl* 16:5-40.

26. de Jong, J. W., d. B.-G. van, G. H. Koeter, and D. S. Postma. 1997. Peripheral blood lymphocyte cell subsets in subjects with chronic obstructive pulmonary disease: association with smoking, IgE and lung function. *Respir Med* 91:67-76.

27. Skold, C. M., E. Blaschke, and A. Eklund. 1996. Transient increases in albumin and hyaluronan in bronchoalveolar lavage fluid after quitting smoking: possible signs of reparative mechanisms. *Respir Med* 90:523-529.

28. Lapperre, T. S., D. S. Postma, M. M. Gosman, J. B. Snoeck-Stroband, N. H. ten Hacken, P. S. Hiemstra, W. Timens, P. J. Sterk, and T. Mauad. 2006. Relation between duration of smoking cessation and bronchial inflammation in COPD. *Thorax* 61:115-121.

29. Lapperre, T. S., J. K. Sont, S. A. van, M. M. Gosman, D. S. Postma, I. M. Bajema, W. Timens, T. Mauad, and P. S. Hiemstra. 2007. Smoking cessation and bronchial epithelial remodelling in COPD: a cross-sectional study. *Respir.Res.* 8:85.

30. Ashraf, H., P. Lo, S. B. Shaker, B. M. de, A. Dirksen, P. Tonnesen, M. Dahlback, and J. H. Pedersen. 2011. Short-term effect of changes in smoking behaviour on emphysema quantification by CT. *Thorax* 66:55-60.

31. Wright, J. L. and J. P. Sun. 1994. Effect of smoking cessation on pulmonary and cardiovascular function and structure: analysis of guinea pig model. *J Appl.Physiol* 76:2163-2168.

32. Grydeland, T. B., A. Dirksen, H. O. Coxson, S. G. Pillai, S. Sharma, G. E. Eide, A. Gulsvik, and P. S. Bakke. 2009. Quantitative computed tomography: emphysema and airway wall thickness by sex, age and smoking. *Eur.Respir.J.* 34:858-865.

Supplement Chapter 5

Effects of smoking cessation on lung function decline and emphysematous changes on CT in heavy smokers

Table S1: Results of multiple linear regression analyses. Change in A) FEV_1/FVC,B) FEV_1 [mL], C) MEF_{50} [mL/s] and D) Perc15 [HU] per unit change in covariate and 95% confidence interval (CI95%) over 3 years follow-up. Only the significant factors remained in this model.

A.

Estimated effects of specified changes in covariates: effects in FEV_1/FVC in % after follow-up			
Covariate	**Increment or Comparison**	**Change in FEV_1/FVC per unit covariate**	**CI95%**
Study center	Utrecht vs. Groningen	1.71	1.31 - 2.11
Length smoking cessation	>5 versus 0	+1.18	0.67 – 1.69
	≥1 - ≤5 versus 0	+1.1	0.61 – 1.56
	≤1 versus 0	+0.63	-0.048 – 1.31
FEV_1/FVC baseline [%]	plus 1 %	0.93	0.91 – 0.95
Age [years]	plus 10 years	-0.57	-0.92 – -0.35
Perc15 baseline [HU]	minus 10 HU	-0.18	-0.19 – -0.07

B.

Estimated effects of specified changes in covariates: effects in FEV_1 in mL after follow-up			
Covariate	**Increment or Comparison**	**Change in FEV_1 per unit change in covariate**	**CI95%**
Study center	Utrecht vs. Groningen	23	47 – 0.00
Length smoking cessation	>5 versus 0	+55	27– 82
	≥1 - ≤5 versus 0	+71	45 – 96
	≤1 versus 0	-9	-46 – 28
FEV_1 baseline [mL]	plus 1 mL	0.91	0.89 – 0.93
Age [years]	plus 10 years	-40.0	-60.0 – -20.0
Height [cm]	plus 10 cm	25	10 – 40
Pack years	plus 10 years	-10	-15 – -5
Perc15 baseline [HU]	decrease of 10 HU	-8.3	-14.6 – -2.1

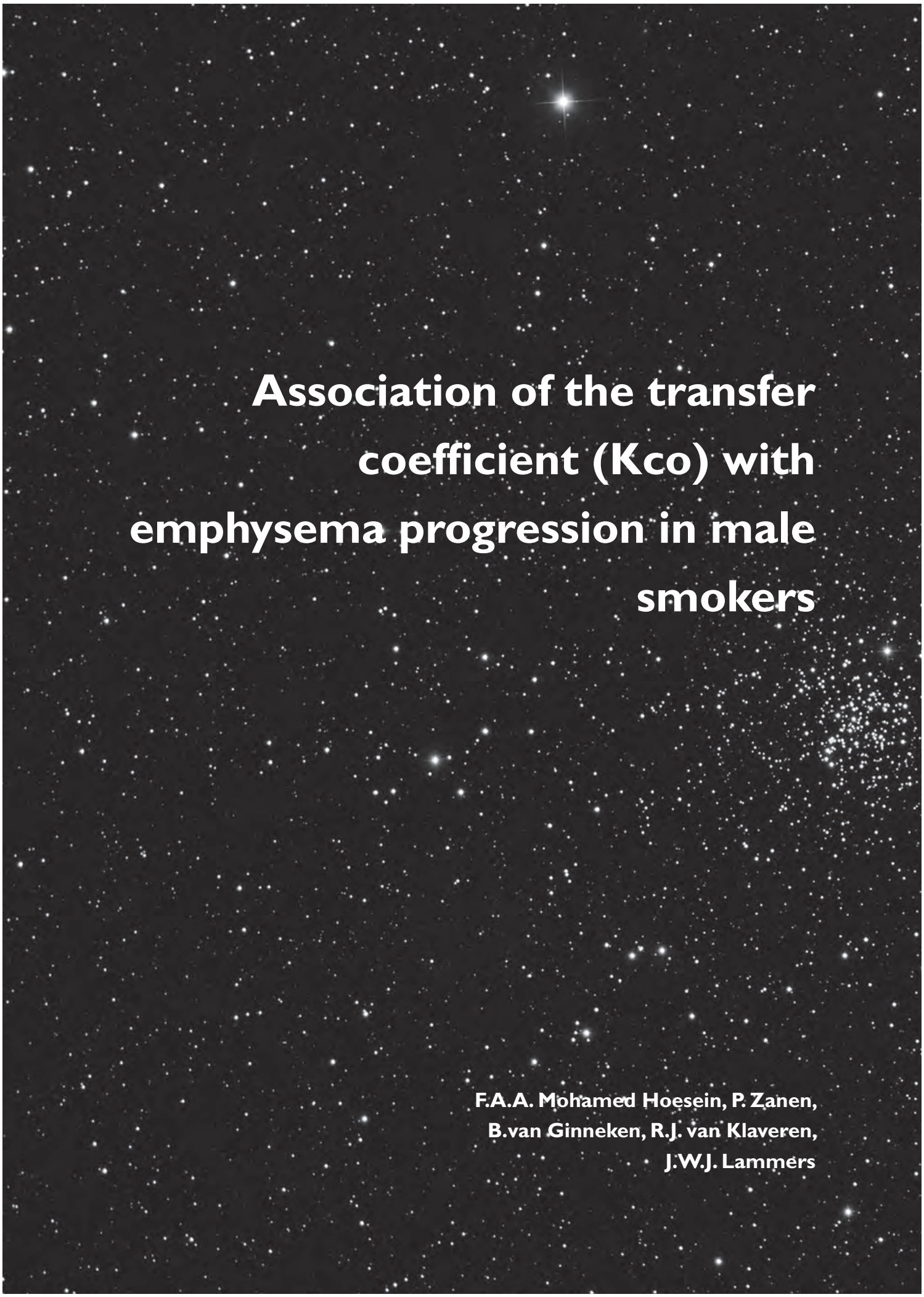
C.

Estimated effects of specified changes in covariates : effects in MEF_{50} in mL/s after follow-up			
Covariate	**Increment or Comparison**	**Change in MEF_{50} per unit change in covariate**	**CI95%**
Length smoking cessation	>5 versus 0	+160	89 – 231
	≥1 - ≤5 versus 0	+150	83 – 215
	≤1 versus 0	+70	-28 – 161
MEF_{50} baseline [mL/s]	plus1 mL/s	0.84	0.82 – 0.86
Age [years]	plus 10 years	-100	-150 – -50
Pack years	plus 10 years	-20	-30 – -10
Perc15 baseline [HU]	decrease of 10 HU	-30	-42 – -12

D.

Estimated effects of specified changes in covariates : effects in Perc15 in HU after follow-up			
Covariate	**Increment or Comparison**	**Change in Perc15 per unit change in covariate**	**CI95%**
Study center	Utrecht vs. Groningen	-4.86	-6.00 – -3.76
Length smoking cessation	>5 versus 0	-1.47	-2.90 – -0.03
	≥1 - ≤5 versus 0	-1.73	-3.08 – -0.38
	≤1 versus 0	-1.87	-3.76 – .03
Perc15 baseline [HU]	decrease of 1 HU	-0.74	-0.76 – -0.72
Height [cm]	plus 10 cm	+0.10	0.02 – 0.18


Chapter
6



Association of the transfer coefficient (Kco) with emphysema progression in male smokers

F.A.A. Mohamed Hoesein, P. Zanen,
B.van Ginneken, R.J. van Klaveren,
J.W.J. Lammers

Abstract

Purpose A decreased Kco is associated with emphysema. We evaluated whether in heavy smokers, baseline Kco was associated with progression of CT-detected emphysema, and progression of airflow limitation.

Methods Heavy smokers, mean (SD) 41.3 (18.7) pack years, participating in a lung cancer screening trial underwent diffusion testing and CT-scanning of the lungs. CT-scanning was repeated after median (25^{th} – 75^{th} percentile) 2.8 (2.7-3.0) years and emphysema was assessed by lung densitometry using the 15^{th} percentile (Perc15). The association between Kco at baseline with progression of emphysema and lung function decline was assessed by multiple linear regression, correcting for baseline CT-quantified emphysema severity and FEV_1/FVC, age, height, BMI, pack years and smoking status (current / former smoker).

Results 522 participants were included with a mean (SD) age of 60.1 (5.4) years. Mean) Perc15 was -938 (19), absolute FEV_1/FVC was 71.6 % (9) and Kco was 1.23 (0.25), which is 81.8% (16.5) of predicted. By interpolation: a one standard deviation (0.25) lower Kco value at baseline, predicted a 1.6 HU lower Perc15 and a 0.78% lower FEV_1/FVC after follow-up ($p<0.001$).

Conclusion A lower baseline Kco value is independently associated with a more rapid progression of emphysema and airflow limitation in heavy smokers.

Introduction

Chronic obstructive pulmonary disease (COPD) is the only chronic disease with increasing mortality rates and is supposed to be the third leading cause of death by 2020. [1] Since prevention of COPD appears to be more promising than treatment, early recognition of COPD susceptible subjects therefore is pivotal to reduce the increasing burden of this disease.

COPD is characterized by progressive airflow limitation and consists of chronic bronchitis and emphysema. Chronic bronchitis leads to e.g. increased mucus production in the (smaller) airways causing airway obstruction, while emphysema induces airflow obstruction by loss of elastic recoil of lung tissue. Both can coincide, and contribute to a greater degree of airflow obstruction. Currently, in the living subject, emphysema can only be assessed by means of computed tomography (CT)-scans and lung densitometry measurements to quantify the extent of it. However, there are some disadvantages to CT-scanning, most importantly the radiation exposure, the costs and the availability of the equipment. [2]

The diffusion capacity of carbon monoxide is an easy to perform tool to assess the functionality of the alveolar-capillary membrane and is reported as the Kco, the carbon monoxide transfer coefficient. [3] The Kco can be considered as the rate constant for alveolar CO uptake and is lowered in the presence of emphysema. Holme et al. showed that a large proportion of subjects with a lowered Kco, but with normal FEV_1/FVC values, show radiological evidence of emphysema. [4]A decreased Kco therefore supports a diagnosis of emphysema in patients with or without airflow obstruction and may add to spirometry to establish the diagnosis of COPD.[5]

However, little is known about the association between Kco and the natural course of CT-quantified emphysema and FEV_1/FVC in heavy, but relatively healthy smokers. We hypothesized that lower baseline Kco values were associated with a more rapid progression of CT-quantified emphysema and decline in FEV_1/FVC. Therefore, the aim of the present study was to assess the relationship between the Kco at baseline and the progression of CT-quantified emphysema and, secondly the progression of airflow obstruction.

Methods

Participants

The study was conducted among participants of the Dutch-Belgian Lung Cancer Screening Trial (NELSON) who were recruited by the University Medical Center Utrecht, the Netherlands, as only this center included diffusion capacity measurements. The NELSON is a population based CT-screening trial for lung cancer and inclusion criteria have been published before. [6] [7] In short, participants meeting the inclusion criteria of having smoked a minimum 20 pack years and fit enough to undergo potential thoracic surgery were invited to participate. Only males were included based on the high risk to develop lung cancer/ COPD as fewer women in the Dutch population have accumulated a long-term exposure to cigarettes compared to men. [5] Baseline details on smoking habits were gathered through questionnaires which included questions about duration of smoking, number of packyears smoked and smoking status at enrolment (current or former smoker). At the start of the study it was decided that this study provided the unique opportunity to also assess lung function and to investigate this in relation to CT measures. Therefore spirometry was assessed in all individuals.

The NELSON trial was approved by the Dutch Ministry of Health on December 23, 2003 and by the institutional review board of the University Medical Center Utrecht, the Netherlands (approval number 03/040) The NELSON trial is registered at www.trialregister.nl with trial number ISRCTN63545820. Informed consent was obtained from all participants.

Pulmonary Function Testing

Pulmonary function tests (PFT) included forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), alveolar volume (Va), and transfer coefficient for carbon monoxide (Kco), which all were carried out according to current European Respiratory Society guidelines. [8] [9] Reversibility was of airflow obstruction not assessed. PFT was performed on the same day as the CT-scan. Airflow obstruction was defined as an FEV_1/FVC below the lower limit of normal (LL) at baseline. [10]

Kco measurements were performed with a MasterLab Pro (Erich Jaeger GmbH, Wurzburg, Germany), with the single breath maneuver method; the test gas contained CO 0.25%, He 9.17% with balance air. Kco was expressed as mmol/min/kPa/l. A breath holding period of 10 seconds (Jonas and Meade method) and discard / sample volumes 750 mL were adopted[11]. Smokers refrained smoking from 24 hours before the

measurement; no correction for hemoglobin levels was made since this only has a very limited effect. [12] Predicted values and the lower limits of normal were calculated by using appropriate reference values. [10 13] Kco values below the lower limit of normal (LLN) were considered abnormal.

CT Scanning

All participants received low-dose CT, with 16-detector MDCT scanners (Mx8000 IDT or Brilliance 16P, Philips Medical Systems, Cleveland, OH), at baseline and after follow-up. Scan data were obtained in spiral mode, with 16 x 0.75mm collimation and in full inspiration. No spirometric gating was applied since this does not improve repeatability of lung density measurements. [14;15] Axial images were reconstructed with 1.0mm thickness at 0.7mm increment. All scans were reconstructed with a soft reconstruction filter (Philips B, Siemens B30f) at a 512x512 matrix. Exposure settings were 30mAs at 120kVp or 140kVp, depending on participant's weight. This low-dose CT protocol has previously been used to quantify emphysema in COPD patients and heavy smokers [16;17]. All CT scans were automatically analyzed by in-house developed soft-ware. [18] Airways were excluded to ensure that only lung parenchyma was analyzed. [19]

Emphysema quantification

Severity of emphysema was based on the 15th percentile (Perc15) technique. This technique provides the Hounsfield Units (HU) point below which 15% of the voxels are distributed. The lower the Perc15 values are, i.e. closer to -1000 HU, the more emphysema is present. This method of emphysema quantification has been validated against pathology [20] and has been applied in multiple studies [21]. The Perc15 was preferred to the % 950 HU measurement,. [22] However, a secondary analysis was done using the % 950 HU as emphysema severity measure, which is defined as the proportion of low density voxels below -950 HU, and is reported in the supplementary files.

Statistical evaluation

Mean and standard deviation (SD) values were calculated for normally distributed data and median and 25^{th} – 75^{th} percentile (Q_1-Q_3) values for non-normally distributed data. Student's t-tests and chi-square tests were used to test differences between groups as appropriate. Pearson's correlations were used to establish associations between variables at baseline. Emphysema severity (Perc15) and FEV_1/FVC at the end of the observation period were the primary endpoints and were analyzed by multiple linear regression analyses. Kco at

baseline was the main explanatory factor. Adjustments were made for baseline Perc15 and FEV_1/FVC, age, height, BMI, pack years, and smoking status (current / former smoker). Perc15 progression and FEV_1/FVC decline were calculated by subtracting follow-up values adjusted by multiple linear regression analyses from observed baseline values. P-values <0.05 were considered significant. All statistical analyses were performed using SPSS 18 for Windows (SPSS, Chicago, Illinois, USA).

Results

Baseline demographics, lung function and CT-quantified emphysema

A number of 609 participants underwent follow-up CT-scanning and spirometry. Of these 609 participants, 87 participants were excluded due to missing or incomplete baseline Kco values, resulting in 522 participants being included in the current study. There were no significant differences in baseline age, height, BMI, packyears, smoking status, spirometry results or CT-quantified emphysema severity between included participants and excluded due to missing or incomplete Kco values.

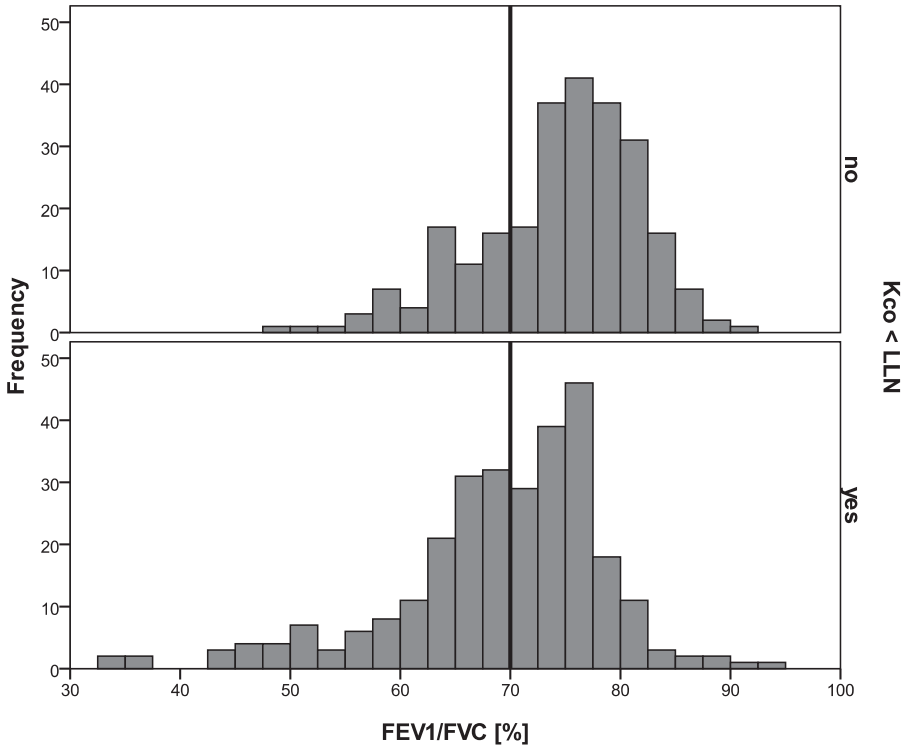
Mean (SD) age was 60.1 (5.4) years and 256 (49.2%) were current smokers. Mean (SD) FEV_1/FVC was 71.6 % (9) of predicted and mean (SD) Kco was 1.23 (0.25) which is 81.8% (16.5) of predicted. Further baseline demographics and lung function parameters for the total study population are provided in Table 1.

Table 1. Baseline demographics. Means and standard deviations (SD) are provided. * Median (Q1-Q3)

N= 522	Mean (SD)
Age [years]	60.1 (5.4)
Height [meters]	1.78 (0.07)
BMI (kilograms*meter^{-2})	26.8 (3.3)
Follow-up* [years]	2.75 (2.7 - 3.0)
Pack years smoking	41.3 (18.7)
Current smokers [%]	48.7
FEV_1 [L]	3.35 (0.72)
FEV_1 % predicted	97.6 (18.2)
FEV_1/FVC absolute [%]	71.6 (9)
Participants with airflow obstruction (FEV_1/FVC <LLN) (%)	98 (18.8%)
Kco [mmol/min/kPa/l]	1.23 (0.25)
Kco %predicted	81.8 (16.5)
Perc15 emphysema score on CT scan [HU]	-937.8 (18.5)

More than half of the participants, 272 (52.3%), had an abnormal Kco at baseline and the baseline Kco was significantly correlated with baseline FEV_1/FVC (r = 0.46, p<0.001). Demographics and lung function parameters stratified by normal and a Kco<LLN are presented in Table 2. The majority of participants, 424 (81.2%), had no airflow obstruction (FEV_1/FVC >LLN). Of the participants with no airflow obstruction, 213 (50.2%) had a lowered Kco. Figure 1 illustrates the baseline FEV_1/FVC stratified by Kco >LLN and <LLN.

Figure 1. Histograms of absolute FEV_1/FVC values for participants stratified by Kco >LLN and <LLN.



The mean (SD) Perc15 was -937.7 HU (18.5). Baseline Kco was significantly correlated with Perc15 at baseline (r = 0.23, p<0.001). Participants with an abnormal Kco (<LLN) had significantly (p=0.002) more CT-quantified emphysema as compared to subjects with a normal Kco, -940.1 HU (19.0) and -935.1 HU (17.6), respectively.

Association of Kco with progression of CT-quantified emphysema

Median (interquartile range) follow-up time was 2.8 (2.7 - 3.0) years. The mean Perc15 HU after follow-up was -944.4 HU (17.9) and the mean (SD) progression of emphysema was 6.3 HU (5). The statistical model explained 68% of the variance in the Perc15 after follow-up (R^2 = 0.68). Baseline values of FEV_1/FVC, Perc15 and Kco and smoking status (current or former smoker) proved to be significant predictive factors for the progression of Perc15, see Table 3.

Table 2. Baseline demographics stratified by normal and Kco <LLN. Means and standard deviations (SD) are provided. * Median (Q1-Q3)

	Normal Kco (n= 250)	Kco < LLN (n=272)	P-value
Age [years]	60.6 (5.4)	59.7 (5.3)	0.067
Height [meters]	1.78 (0.06)	1.78 (0.06)	0.155
BMI (kilograms*meter^{-2})	26.8 (3.3)	26.8 (3.3)	0.256
Follow-up* [years]	2.75 (2.7 - 3.0)	2.75 (2.7 - 3.0)	0.325
Pack years smoking	40.4 (19.1)	42.1 (18.3)	0.326
Current smokers [%]	91 (36.4)	170 (59.4%)	**<0.001**
FEV_1 [L]	3.40 (0.68)	3.30 (0.75)	0.142
FEV_1 %predicted	99.6 (16.6)	95.8 (19.3)	**0.015**
FEV_1/FVC absolute [%]	74.4 (7.4)	69.2 (9.6)	**<0.001**
Participants with airflow obstruction (FEV_1/FVC < LLN) (%)	27 (10.8%)	71 (26.1%)	**<0.001**
Kco [mmol/min/kPa/l]	1.43 (0.15)	1.05 (0.17)	**<0.001**
Kco %predicted	95.6 (9.6)	69.7 (10.8)	**<0.001**
Perc15 emphysema score on CT scan [HU]	-935.1 (17.6)	-940.1 (19.0)	**0.002**

Table 3. Results from the multiple linear regression analyses showing the estimated effects of baseline Kco and the other significant covariateson A) FEV_1/FVC% after follow-up and B) Perc15 HU after follow-up. A) A 0.25 lower Kco at baseline predicts an additionally 0.78% lower FEV_1/FVC after 3-year follow-up. B) A 0.25 lower Kco at baseline predicts an additionally 1.6 HU lower Perc15 after 3-year of follow-up.

A. Estimated effects of changes in parameters on FEV_1/FVC in % after follow-up				
Covariate	**Change**	**Change in FEV_1/FVC [%]**	**CI95%**	**p-value**
Kco	0.25 lower	-0.78%	0.31 – 1.14	<0.001
Baseline FEV_1/FVC absolute [%]	1% lower	-0.90%	0.85 – 0.94	<0.001

B. Estimated effects of changes in parameters on Perc15 in HU after follow-up				
Covariate	**Change**	**Change in Perc15 [HU]**	**CI95%**	**p-value**
Kco	0.25 lower	-1.6 HU	0.59 – 2.60	0.002
Baseline FEV_1/FVC absolute [%]	1% lower	-0.3 HU	0.19 – 0.43	<0.001
Smoking status	Current versus ex-smoker	+3.2 HU	1.30 – 5.1	<0.001
Baseline Perc15 HU	1 HU lower	-0.67 HU	-0.61– -0.72	<0.001

A 0.25 lower baseline Kco (being the standard deviation of Kco in this sample) predicted an additional 1.6 HU lower Perc15 after follow-up (p<0.001). The effect of Kco is illustrated in Figure 3. The effects of the other significant covariates in the model are listed in Table 3. Age, height, BMI and pack years smoked were not significantly associated with Perc15 progression.

An additional analysis was performed to test whether the association of Kco with Perc15 progression was independent of the baseline level of FEV_1/FVC. An interaction term between baseline FEV_1/FVC and baseline Kco was inserted in the statistical model. The baseline FEV_1/FVC value was significantly (p<0.001) associated with progression of Perc15; a 1% lower baseline Kco value predicted an additional 0.3 HU lower Perc15. However, the interaction term was not significant (p=0.099) indicating that the association of baseline Kco was similar in participants with different levels of airflow obstruction.

Using the % 950 HU approach as measure of emphysema severity yielded similar results as using the Perc15 (see supplemental files).

Association of Kco with decline in FEV_1/FVC

Mean absolute (SD) FEV_1/FVC after follow-up was 70.2% (9.4). The statistical model explained 80% of the variance in FEV_1/FVC after follow-up (R^2 = 0.80). Baseline values of FEV_1/FVC and Kco proved to be significant predictive factors for FEV_1/FVC decline as shown in Table 3. Adjusted mean (SD) decline was 1.44 % (0.92) during 3-year follow-up. To put this decline in perspective, the expected 3-year decline in FEV_1/FVC according to appropriate reference values is 0.5%. [10] When a subject showed a 0.25 lower Kco (being one standard deviation) compared to another subject, that subject suffered from an additional 0.78% lower FEV_1/FVC after follow-up (p<0.001), see Table 3 and Figure 2.

Figure 2. Relation of baseline Kco and FEV_1/FVC after follow-up. This situation represents participants with a baseline age of 60.1 years, packyears of 41.3, height of 1.75 meters, FEV_1/FVC of 71.6% and a Perc15 of 937.8 HU, which are the mean values of the study population at baseline. It can be seen that a lower Kco at baseline predicts a larger decline in FEV_1/FVC. As an illustration: a Kco value of 1.2 at baseline associates with a follow-up FEV_1/FVC of 70.07%, while a Kco value of 1.4 at baseline associates with a follow-up FEV_1/FVC of 70.69%, despite similar age, height, packyears smoking and Perc15 levels of these individuals at baseline.

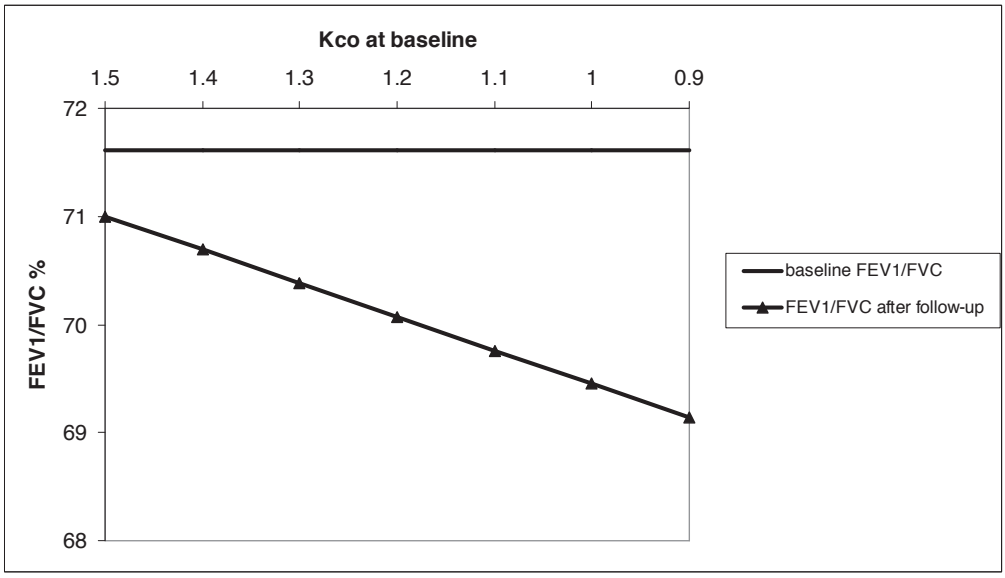
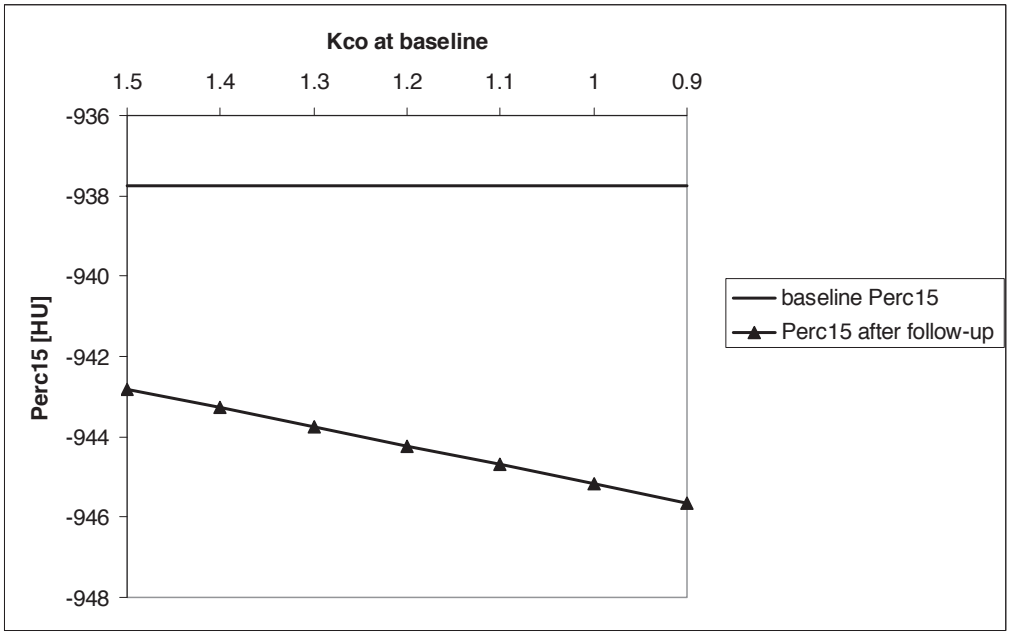


Figure 3. Relation of baseline Kco and Perc15 after follow-up. This situation represents participants with a baseline age of 60.1 years, packyears of 41.3, height of 1.75 meters, FEV_1/FVC of 71.6% and a Perc15 of 937.8 HU, which are the mean values of the study population at baseline. It can be seen that a lower Kco at baseline predicts a more rapid decline of Perc15, e.g. worsening of CT-quantified emphysema. As an illustration: a Kco value of 1.2 at baseline associates with a follow-up Perc15 of -944.2 HU, while a Kco value of 1.4 at baseline associates with a follow-up Perc15 of -943.3 HU, despite similar age, height, packyears smoking and Perc15 levels of these individuals at baseline.



The analysis with insertion of an interaction term between baseline FEV_1/FVC and baseline Kco showed that the association of Kco with FEV_1/FVC decline was independent of the baseline FEV_1/FVC value as the interaction term was not significant (p=0.133).

Discussion

In the present study we showed that a lower Kco value is associated with an increase of CT-quantified emphysema and a larger decline in FEV_1/FVC during a three year follow-up of heavy male smokers. This association proved to be independent of the level of FEV_1/FVC. Kco, a simple and patient friendly measurement, therefore may help to detect current and former smokers who are susceptible for a more rapid progression of CT-quantified emphysema and decline in lung function independently of their FEV_1/FVC level. Parameters like the FEV_1/FVC do not reflect the presence or the severity of emphysema accurately. [23] Mild emphysema does not always lead to a FEV_1/FVC <70% (or <LLN), and thus COPD can be missed if only spirometry is performed. However, in daily practice the evaluation of subjects at risk for (or with established) COPD is usually based on spirometry. [10] Unfortunately, spirometry fails to discriminate between chronic bronchitis and emphysema, the latter may be assessed by CT-scanning. A disadvantage of CT-scanning is that it exposes subjects to radiation and is relatively expensive and therefore CT-scanning is not performed on a regular basis, which is also true for repeatedly performed low-dose CT-scans. An advantage of CT-scanning is the additional information which is obtained on the distribution of emphysema, but it is questionable whether this information is clinically relevant. [24] On the other hand, diffusion testing is harmless, less expensive, and thus can be applied routinely and more frequently than CT-scanning. This is strengthened by the finding that of the 272 participants with a Kco below the LLN only 71 had an FEV_1/FVC below the LLN. This finding again illustrates that in an at-risk population with high smoking history only performing spirometry may miss a large degree of subjects with abnormal diffusion tests results.

The association of a lower baseline Kco with progression of emphysema and decline of FEV_1/FVC was independent of the level of baseline FEV_1/FVC as there were no significant interactions between them. This is an important finding because it illustrates that it is useful to perform Kco measurements in heavy smokers, independently of their FEV_1/FVC. Only taking in account the FEV_1/FVC, and not the Kco, in the evaluation of heavy

smokers may result in missing subjects who will suffer from a stronger progression of Perc15. The assessment of Kco thus may have important prognostic implications.

To our knowledge, there are no longitudinal studies examining the predictive value of Kco on FEV_1/FVC decline. One study did examine the predictive value of DLco on FEV_1-decline and showed that DLco differentiates smokers who will experience a rapid FEV_1-decline. [25] The included subjects were comparable to our population. They were also relatively healthy, but slightly younger. Although these authors measured the DLco instead of the Kco, their results support our findings that the Kco may help to identify subjects with a more rapid lung function decline.

As for the association between Kco and lung function decline, literature evaluating the predictive value on emphysema progression is scarce. Cross-sectional studies have shown that the Kco is lower in subjects with pathologically defined as well as with CT-detected emphysema [26] [27] [28]. We confirm these findings by showing that there was a significant correlation between Kco and Perc15 at baseline (r = 0.23). The correlation however was not as strong as previously reported, which is most probably due to the fact that the included subjects were relatively healthy and without severe emphysema.

There are a number of strengths to our study. Firstly, emphysema scores were automatically quantified which eliminates interobserver variability known to be present in the visual assessment of emphysema. Secondly, the study was performed in one center and only one type of CT-scanner was used, excluding possible scanner bias due to different algorithms used by different types of CT-scanners. Thirdly the same diffusion testing equipment was used. This is especially important since it is known that large variability may exist in Kco measurements between different lung function laboratories. [29] Fourthly, only heavy smoking, but relatively healthy participants were included. This makes the results especially applicable to subjects who are at risk for progression of emphysema and airflow obstruction. The earlier mentioned cross-sectional studies were almost all restricted to (severe) COPD subjects. Finally, because of the large sample size we could extensively correct for potential confounding factors like age, packyears smoked and smoking status. This makes our reported results more precise.

This study also has some limitations. Firstly, only pre-bronchodilator spirometry was obtained, which could have resulted in lower measured FEV_1/FVC values in our study. As a result, the percentage of participants without airflow obstruction could actually be lower. However, because we treated FEV_1/FVC as a quantitative treat we do not expects that this has affected our results. Secondly, no females were included, due to

the inclusion criteria of the study. This is unfortunate because the prevalence of COPD is increasing in women. Previous studies showed that emphysema scores in females are lower than in men, and that females also show lesser progression of emphysema after follow-up. [30] [31] [32] Lastly, we performed analyses with both the Perc15 as the %950 HU, the results of the latter are described in the supplemental files. The outcomes of the analyses with %950 as emphysema measurement are in the similar direction as by using Perc15 and underscore our conclusions. It should however be realized that the Perc15 takes in account, not only the regions with markedly reduced density, but the whole lung, while the %950 HU is less sensitive for lung density changes of the whole lung.

In conclusion, we have shown that current and former heavy smokers with lower baseline Kco values show a significantly greater progression of CT-quantified emphysema and decline in FEV_1/FVC. These results show that the Kco may be a useful measurement in the evaluation and follow-up of heavy smoking subjects, with or without airflow obstruction yet.

Reference List

1. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. Lancet 1997; 349(9064):1498-1504.
2. Brenner DJ, Hall EJ. Computed tomography--an increasing source of radiation exposure. N Engl J Med 2007; 357(22):2277-2284.
3. Hughes JM, Pride NB. In defence of the carbon monoxide transfer coefficient Kco (TL/VA). Eur Respir J 2001; 17(2):168-174.
4. Holme J, Stockley RA. Radiologic and clinical features of COPD patients with discordant pulmonary physiology: lessons from alpha1-antitrypsin deficiency. Chest 2007; 132(3):909-915.
5. van der Lee, I, Gietema HA, Zanen P et al. Nitric oxide diffusing capacity versus spirometry in the early diagnosis of emphysema in smokers. Respir Med 2009; 103(12):1892-1897.
6. van Iersel CA, de Koning HJ, Draisma G et al. Risk-based selection from the general population in a screening trial: selection criteria, recruitment and power for the Dutch-Belgian randomised lung cancer multi-slice CT screening trial (NELSON). Int J Cancer 2007; 120(4):868-874.
7. van Klaveren RJ, Oudkerk M, Prokop M et al. Management of lung nodules detected by volume CT scanning. N Engl J Med 2009; 361(23):2221-2229.
8. Miller MR, Hankinson J, Brusasco V et al. Standardisation of spirometry. Eur Respir J 2005; 26(2):319-338.
9. MacIntyre N, Crapo RO, Viegi G et al. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. Eur Respir J 2005; 26(4):720-735.
10. Quanjer PH, Tammeling GJ, Cotes JE et al. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993; 16:5-40.
11. Jones RS, Meade F. A theoretical and experimental analysis of anomalies in the estimation of pulmonary diffusing capacity by the single breath method. Q J Exp Physiol Cogn Med Sci 1961; 46:131-143.
12. Stam H, Hrachovina V, Stijnen T et al. Diffusing capacity dependent on lung volume and age in normal subjects. J Appl Physiol 1994; 76(6):2356-2363.
13. Cotes JE, Chinn DJ, Quanjer PH et al. Standardization of the measurement of transfer factor (diffusing capacity). Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993; 16:41-52.
14. Gierada DS, Yusen RD, Pilgram TK et al. Repeatability of quantitative CT indexes of emphysema in patients evaluated for lung volume reduction surgery. Radiology 2001; 220(2):448-454.
15. Newell JD, Jr., Hogg JC, Snider GL. Report of a workshop: quantitative computed tomography scanning in longitudinal studies of emphysema. Eur Respir J 2004; 23(5):769-775.

16. Shaker SB, Maltbaek N, Brand P et al. Quantitative computed tomography and aerosol morphometry in COPD and alpha1-antitrypsin deficiency. Eur Respir J 2005; 25(1):23-30.
17. Sverzellati N, Calabro E, Randi G et al. Sex differences in emphysema phenotype in smokers without airflow obstruction. Eur Respir J 2009; 33(6):1320-1328.
18. van Rikxoort EM, de Hoop B, Viergever MA et al. Automatic lung segmentation from thoracic computed tomography scans using a hybrid approach with error detection. Med Phys 2009; 36(7):2934-2947.
19. van Rikxoort EM, Prokop M, de Hoop B et al. Automatic segmentation of the pulmonary lobes from fissures, airways, and lung borders: evaluation of robustness against missing data. Med Image Comput Comput Assist Interv 2009; 12(Pt 1):263-271.
20. Gould GA, MacNee W, McLean A et al. CT measurements of lung density in life can quantitate distal airspace enlargement--an essential defining feature of human emphysema. Am Rev Respir Dis 1988; 137(2):380-392.
21. Parr DG, Stoel BC, Stolk J et al. Influence of calibration on densitometric studies of emphysema progression using computed tomography. Am J Respir Crit Care Med 2004; 170(8):883-890.
22. Parr DG, Sevenoaks M, Deng C et al. Detection of emphysema progression in alpha 1-antitrypsin deficiency using CT densitometry; methodological advances. Respir Res 2008; 9:21.
23. Hogg JC, Wright JL, Wiggs BR et al. Lung structure and function in cigarette smokers. Thorax 1994; 49(5):473-478.
24. Gietema HA, Zanen P, Schilham A et al. Distribution of emphysema in heavy smokers: impact on pulmonary function. Respir Med 2010; 104(1):76-82.
25. Cauberghs M, Clement J, Van de Woestijne KP. Functional alterations accompanying a rapid decline in ventilatory function. Am Rev Respir Dis 1993; 147(2):379-384.
26. Morrison NJ, Abboud RT, Ramadan F et al. Comparison of single breath carbon monoxide diffusing capacity and pressure-volume curves in detecting emphysema. Am Rev Respir Dis 1989; 139(5):1179-1187.
27. Gould GA, Redpath AT, Ryan M et al. Lung CT density correlates with measurements of airflow limitation and the diffusing capacity. Eur Respir J 1991; 4(2):141-146.
28. Cerveri I, Dore R, Corsico A et al. Assessment of emphysema in COPD: a functional and radiologic study. Chest 2004; 125(5):1714-1718.
29. Jensen R, Leyk M, Crapo R et al. Quality control of DL,CO instruments in global clinical trials. Eur Respir J 2009; 33(4):828-834.
30. Bellomi M, Rampinelli C, Veronesi G et al. Evolution of emphysema in relation to smoking. Eur Radiol 2010; 20(2):286-292.
31. Grydeland TB, Dirksen A, Coxson HO et al. Quantitative computed tomography: emphysema and airway wall thickness by sex, age and smoking. Eur Respir J 2009; 34(4):858-865.
32. Martinez FJ, Curtis JL, Sciurba F et al. Sex differences in severe pulmonary emphysema. Am J Respir Crit Care Med 2007; 176(3):243-252.

Supplement Chapter 6

Association of the transfer coefficient (Kco) with emphysema progression in male smokers

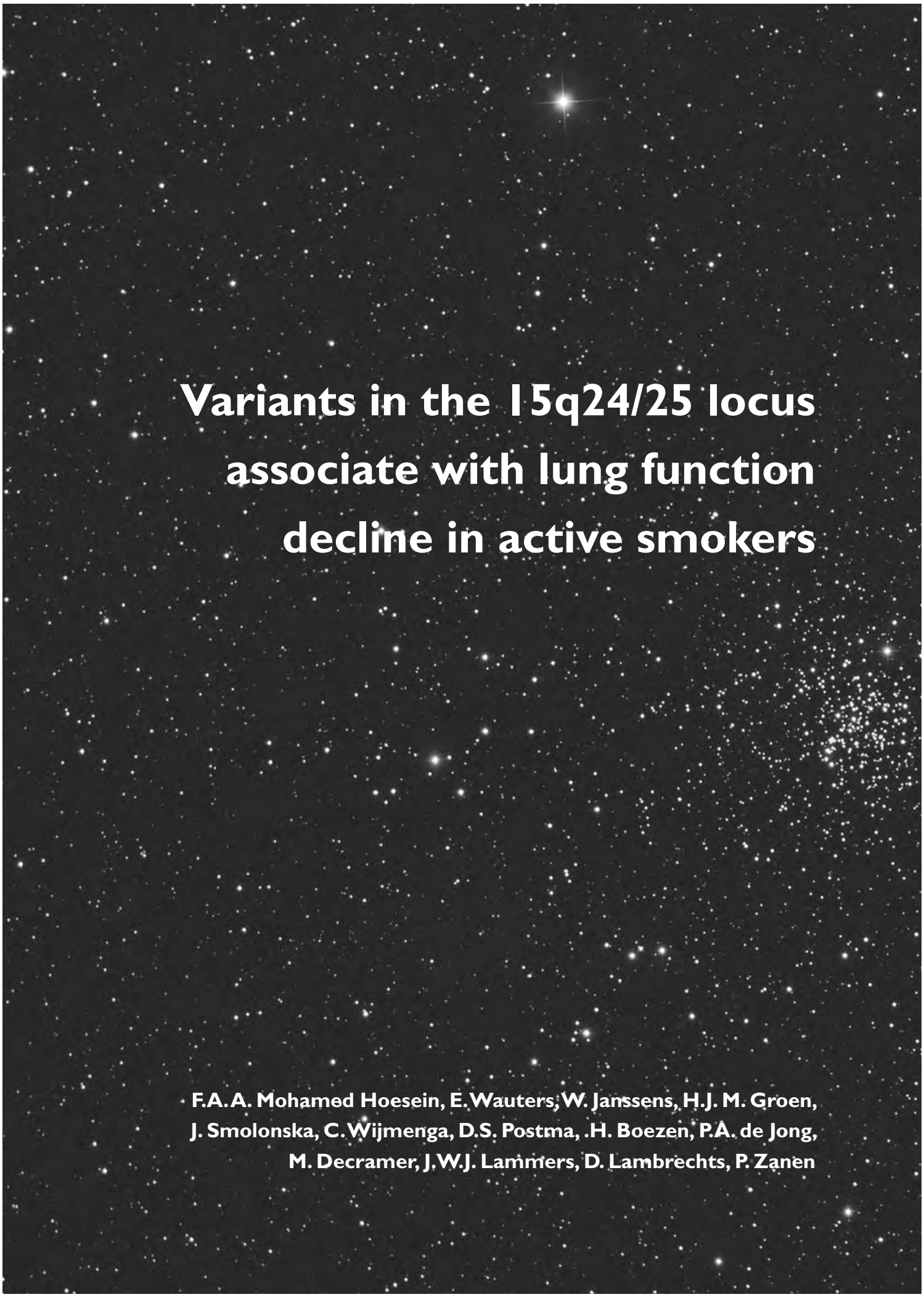
Results with using%950 HU as emphysema severity measurement

Baseline median (inter quartile) %950 was 8.7% (4.5-15). Kco at baseline significantly (p=0.004) correlated with %950 at baseline, r = -0.13. Follow-up median (inter quartile) %950 was 14.23% (7.4-19.7). The statistical model explained 73% of the variance in %950 after follow-up. The significant covariates are listed in Table S1.

Table S1. Results from the multiple linear regression analyses showing the estimated effects of baseline Kco and other significant covariates on %950 after follow-up.

Estimated effects of changes in parameters on %950 in % after follow-up				
Covariate	**Increment or comparison**	**Change in %950 (%)**	**CI95%**	**p-value**
Kco baseline	0.25 decrease	+0.65 %	0.2 – 1.1	0.01
Baseline FEV_1/FVC [%]	1% decrease	+0.14 %	0.08 – 0.19	<0.001
Smoking status	Current versus ex-smoker	-1.2 %	-2.0 – -0.30	0.012
Baseline %950	1% increase	+0.77 %	-0.61– -0.72	<0.001


Chapter
7



Variants in the 15q24/25 locus associate with lung function decline in active smokers

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Abstract

Purpose Genetic variation in nicotinic acetylcholine receptor subunit genes (*nAChRs*) is associated with lung function level and chronic obstructive pulmonary disease (COPD). It is unknown whether these variants also predispose to an accelerated lung function decline. We investigated the association of *nAChR* susceptibility variants with lung function decline and COPD severity.

Methods The rs1051730 and rs8034191 variants were genotyped in a population-based cohort of 1,226 heavy smokers (COPACETIC) and in an independent cohort of 883 heavy smokers, of which 653 with COPD of varying severity (LEUVEN). Participants underwent pulmonary function tests at baseline. Lung function decline was assessed over a median follow-up of 3 years in COPACETIC.

Results Current smokers homozygous for the rs1051730 A-allele or rs8034191 G-allele had significantly greater FEV_1/FVC decline than homozygous carriers of wild-type alleles (3.3% and 4.3%, p=0.026 and p=0.009, respectively). In the LEUVEN cohort, rs1051730 AA-carriers and rs8034191 GG-carriers had a two-fold increased risk to suffer from COPD GOLD IV (p=0.025 and p=0.016, respectively). The same risk alleles conferred, respectively, a five- and four-fold increased risk to be referred for lung transplantation because of end-stage COPD (p=0.004 and p=0.010).

Conclusion Variants in *nAChRs* associate with an accelerated lung function decline in current smokers and with clinically relevant COPD.

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by progressive airflow limitation, as reflected by an accelerated decline in forced expiratory volume in one second (FEV_1).[1] Smoking is by far the most important risk factor.[1] Although cigarette smokers have a larger FEV_1 decline than non-smokers, only 10-20% of them develop COPD.[2] Additional susceptibility factors predictive of an accelerated lung function decline thus need to be identified.

Genetic predisposition to COPD development is actively being explored.[3] For many years, uncommon mutations in the gene encoding the alpha 1-antitrypsin protein represented the only established genetic risk factor for severe, early-onset COPD.[4] Recently, several genome-wide association studies (GWAS) identified multiple novel genetic risk factors.[5-7] One of these at-risk loci is located on chromosome 15q24/25 in a region that contains the nicotinic acetylcholine receptor subunit genes (*nAChR*s).[7] Interestingly, *nAChR* variants have also been associated with smoking addiction,[8-11] peripheral arterial disease,[12] lung cancer [12-14] and emphysema,[15] indicating that the *nAChR* locus is implicated in the development of smoking-related conditions.

Although it is widely accepted that COPD results from a progressive decline in lung function,[16] it has not yet been established whether *nAChR* variants predisposing to COPD are also associated with an accelerated lung function decline. In addition, it is currently unclear how smoking behavior may interact with this genetic locus on disease progression. We therefore assessed whether rs1051730 and rs8034191, two variants in the *nAChR* locus, were associated with the decline in FEV_1, FEV_1/FVC and MEF_{50} over 3 years in subjects from a large population-based cohort (COPACETIC, n=1,226). As proof of concept, we additionally assessed the predictive value of our findings in an independent group of heavy smokers (LEUVEN), consisting of healthy smokers (n=230) and patients diagnosed with COPD of varying stages of severity (GOLD I – IV, n=653).

Methods

Study subjects

The COPACETIC cohort included 1,226 participants from the NELSON lung cancer screening trial recruited by the University Medical Centers of Groningen and Utrecht.[17] In brief, all participants were male heavy smokers (≤ 20 or more pack-years), aged between 50-75 years and fit enough to undergo surgery.

The LEUVEN cohort included 366 Belgian participants of the Dutch-Belgian randomized lung cancer screening trial (NELSON) who were recruited from the general population and who were not previously diagnosed with COPD. In addition, 517 COPD patients were included at the outpatient clinic of the University Hospital Gasthuisberg in Leuven. Of these 517 subjects, 123 were listed for lung transplantation because of disabling end-stage COPD.[18] The 883 LEUVEN participants were all heavy smokers, matched for smoking history (>15 pack-years) and age (>50 years). All LEUVEN participants self-declared Belgian-Flemish ethnicity for three generations.[19] Participants from both COPACETIC and LEUVEN provided written informed consent. The medical ethical committees of the involved hospitals approved the study protocol. The complete inclusion criteria for COPACETIC and LEUVEN are described in detail in the online supplement (supplementary note 1).

Pulmonary function testing

All participants underwent pulmonary function tests with standardized equipment according to the American Thoracic Society (ATS) and European Respiratory Society (ERS) guidelines.[20] Bronchial obstruction was established as a post-bronchodilator FEV_1/FVC ratio of <0.70 in LEUVEN and as a pre-bronchodilator FEV_1/FVC ratio of <0.70 in COPACETIC.[1] Severity was staged by FEV_1 expressed as % predicted according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification.[1]

Genotyping

Two variants in the 15q24/25 locus, rs1051730 and rs8034191, were selected based on previous GWAS for COPD and lung cancer.[7, 12-14] Genotyping was conducted in analogy to a previously published study, in which we established an association between a single SNP, rs1051730, and the risk of COPD and emphysema.[15] In particular, COPACETIC genotypes were extracted from Human610-Quad BeadChip data generated in the COPACETIC GWAS (Illumina Inc., San Diego, CA, USA), whereas LEUVEN participants were genotyped in a blinded manner using iPLEX technology on a MassARRAY Compact Analyser (Sequenom Inc., San Diego, CA, USA). A more detailed description is given in the online supplement (supplementary note 1).

Statistical Analysis

Means and standard deviations (SD) were calculated for normally distributed variables, and medians and interquartile ranges for non-normally distributed variables. Chi-square

tests were used to test for differences in demographics and nicotine-addiction related variables between genotypes. Multivariate regression analyses correcting for age, smoking status and study center were performed to assess the association of the genotypes with smoking behavior. In COPACETIC, multiple regression analyses were performed to test the association of rs1051730 and rs8034191 with changes in FEV_1/FVC, FEV_1 and MEF_{50} over time. To differentiate between genetic variability in lung function decline and a genetically determined lower lung function level, adjustments for baseline FEV_1, FEV_1/FVC and MEF_{50} were made. In particular, adjustments were made for study center, age, height, smoking status (current/former smoker), pack-years, years in study and baseline FEV_1/FVC, FEV_1, MEF_{50}. In addition, we calculated the observed power to discover a significant association of genotypes, smoking status (current versus former) and the genotype*smoking status interaction term with lung function decline. The methods and results of power calculations are provided in the online supplement (supplementary note 2).

In LEUVEN, the relationship between rs1051730 or rs8034191 genotypes and GOLD stage was assessed by chi-square analysis. The association between genotypes and the risk of developing severe COPD (GOLD IV) was confirmed via multinomial logistic regression analysis, while correcting for age, height, sex, pack-years and years-quit for former smokers. In addition, given the extensive heterogeneity in degree of functional impairment among COPD patients, even with the same level of airflow obstruction, we classified the LEUVEN subjects in three clinical subgroups: *(i)* asymptomatic smokers, defined as heavy smokers that do not report respiratory symptoms and are therefore not diagnosed with COPD, *(ii)* ambulatory COPD patients, defined as COPD patients that are routinely visiting the outpatient clinic because of stable respiratory symptoms, and *(iii)* patients listed for lung transplantation because of the severe repercussion of COPD for their daily life activities and life expectancy (<18 months). Differences between these subgroups were assessed using a Pearson's chi-square analysis. A multinomial logistic regression analysis to assess the probability of belonging to any of these clinical subgroups in function of genotypes was performed, while correcting for age, height, sex, pack-years and years-quit for former smokers. The p-value threshold for significance was adjusted for testing three clinical variables (decline in FEV_1/FVC, FEV_1 and MEF_{50}) using the Bonferroni correction method, resulting in a significance threshold of $p<0.0167$. All statistical analyses were performed using SPSS 18 for Windows (SPSS, Chicago, Illinois, USA).

Results

Population Characteristics

In total, 1,226 participants were included in COPACETIC and 883 participants in LEUVEN. Demographics, smoking history, pulmonary function measurements and the prevalence of COPD is shown for all COPACETIC and LEUVEN participants in Table 1.

Table 1. Characteristics for all participants at baseline and after three-year follow-up for COPACETIC.

	COPACETIC		LEUVEN
	Baseline (n=1,226)	**Follow-up (n=1,226)**	**(n=883)**
Demographics			
Age, mean (SD), yr	59.7 (5.3)	62.7 (5.4)	63.9 (7.97)
Male sex, no. (%)	1,226 (100)	1,226 (100)	656 (74.0)
Height, mean (SD), cm	177.5 (6.5)	178.1 (6.3)	169.6 (8.90)
Smoking			
Pack-year history, mean (SD), yr	40.7 (17.4)	N/A	47.4 (24.8)
Current smokers, no. (%)	753 (61.4)	N/A	367 (41.3)
Smoked years, mean (SD), yr	39.6 (8.87)	N/A	41.7 (9.0)
Years quit smokers, median (25^{th}-75^{th} percentiles)	8.1 (3.0-9.0)	N/A	1.0 (0.0-8.0)
Pulmonary function tests, mean (SD)			
FEV_1, L	3.33 (0.73)	3.14 (0.72)	1.92 (1.08)
FEV_1, % predicted	96.5 (18.4)	93.7 (18.8)	65.4 (32.7)
FVC, L	4.70 (0.79)	4.61 (0.81)	3.36 (1.13)
FVC, % predicted	107.13 (14.6)	106.7 (15.3)	91.9 (24.3)
FEV_1/FVC ratio	0.71 (0.10)	0.68 (.10)	0.54 (0.18)
COPD severity, no. (%)			
No Obstruction	682 (55.6)	557 (45.4)	230 (26)
GOLD class I	357 (29.1)	430 (35.1)	123 (13.9)
GOLD class II	162 (13.2)	210 (17.1)	183 (20.7)
GOLD class III	24 (2.0)	23 (1.9)	188 (21.2
GOLD class IV	0	5 (0.4)	159 (18)

FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; COPD: chronic obstructive pulmonary disease; GOLD: global initiative for chronic obstructive lung disease; N/A: non-applicable. Percentages are column percentages.

Of the LEUVEN participants, 653 subjects were diagnosed with COPD (FEV_1/FVC < 0.70). There were no significant differences in pack-years between former and current smokers (p=0.188 and p=0.309 for COPACETIC and LEUVEN, respectively; data not shown). Baseline characteristics for the LEUVEN clinical subgroups are shown in Table 2.

Table 2. Baseline Characteristics for LEUVEN participants stratified for the clinical impact of airflow obstruction.

	Asymptomatic smokers	Ambulatory COPD patients	End-stage COPD patients	p-value
	(n=366)	(n=394)	(n=123)	
Demographics				
Age, mean (SD), yr	62.3 (5.71)	67.6 (8.67)	57.2 (4.69)	<0.001
Male sex, no. (%)	286 (78.1)	305 (77.4)	62 (50.4)	<0.001
Height, mean (SD), cm	172.0 (8.9)	168.6 (8.4)	165.0 (8.5)	<0.001
Smoking				
Pack-years history, mean (SD), yr	45.8 (21.8)	52.5 (27.3)	35.5 (19.8)	<0.001
Current smokers, no. (%)	203 (55.8)	157 (40.5)	5 (15.6)	<0.001
Smoked years, mean (SD), yr	40.6 (7.0)	43.2 (10.4)	36.1 (8.2)	<0.001
Years quit smokers, median (25^{th}-75^{th} percentiles)	0.0 (0.0-7.0)	2.0 (0.0-9.0)	3.0 (1.0-6.0)	<0.001
Pulmonary function tests, mean (SD)				
FEV_1, L	2.91 (0.74)	1.38 (0.63)	0.70 (0.30)	<0.001
FEV_1, % predicted	96.4 (18.3)	49.3 (20.1)	25.5 (9.9)	<0.001
FEV_1/FVC ratio	0.70 (0.09)	0.45 (0.13)	0.33 (0.08)	<0.001
COPD severity, no. (%)				
No Obstruction	218 (59.6)	11 (2.8)	0 (0)	<0.001
GOLD class I	96 (26.2)	27 (6.9)	1 (0.8)	
GOLD class II	46 (12.6)	134 (34.0)	3 (2. 4)	
GOLD class III	6 (1.6)	157 (39.8)	25 (20.3)	
GOLD class IV	0 (0)	65 (16.5)	94 (76.4)	

FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; COPD: chronic obstructive pulmonary disease; GOLD: global initiative for chronic obstructive lung disease; N/A: non-applicable. Percentages are column percentages.

The 366 asymptomatic smokers are population-based participants of the Dutch-Belgian lung cancer screening trial (NELSON). None of them were previously

diagnosed with COPD. However, 148 subjects (40.4%) were found to have an obstructive lung function (based on FEV_1/FVC<0.70) at inclusion. Eleven subjects (2%), that were followed-up at the outpatient clinic because of symptoms compatible with COPD, did not fulfill the criterion of COPD (FEV_1/FVC >0.70).
In COPACETIC, genotyping for both rs1051730 and rs8034191 succeeded in 99% of participants. In LEUVEN genotyping for rs1051730 and rs8034191 succeeded in 99% and 100%, respectively. Genotype frequencies were similar as observed in the HAPMAP databases and other studies.[7, 21] There was strong linkage disequilibrium between rs1051730 and rs8034191 in both cohorts (r^2=0.86 for COPACETIC and r^2=0.88 for LEUVEN). Baseline characteristics for LEUVEN and COPACETIC participants according to rs1051730 and rs8034191 genotypes are shown in Table 3 and 4.

Association of nAChR variants with nicotine addiction-related variables
To assess whether rs1051730 and rs8034191 were associated with nicotine addiction, we tested for association with pack-years, smoking status (current/former smoker), quit-years in former smokers (>5, 1-5, <1 years) and age-started smoking (<14 years, 15-19 years, >20 years) in COPACETIC. No significant associations were found (Table 3). Moreover, multivariate regression analysis, correcting for age, smoking status and study center, showed no significant effect of rs1051730 and rs8034191 on pack-years smoked (p=0.327 and 0.258, respectively).

Genotyping succeeded in 1226 (100%) and 1224 (99.8%) COPACETIC participants, respectively for rs1051730 and rs8034191.
Likewise, no significant association between rs1051730 and rs8034191 genotypes, and smoking status (current/ former) or pack-years was found in LEUVEN (respectively, p=0.144 and p=0.08 for rs1051730, p=0.211 and P=0.103 for rs8034191; Table 4). Multivariate regression analysis, correcting for age, gender and smoking status, showed no significant effects for rs1051730 and rs8034191 on pack-years smoked (p=0.133 and 0.140, respectively; data not shown).

Table 3. Baseline characteristics for the COPACETIC cohort according to rs1051730 and rs8034191 genotypes.

	rs1051730				rs8034191			
	AA (n=134)	GA (n=562)	GG (n=530)	p-value	GG (n=133)	GA (n=560)	AA (n=531)	p-value
Age, mean (SD), yr	59.6 (6.0)	59.6 (5.3)	59.7 (5.4)	0.952	59.6 (5.7)	59.6 (5.2)	59.8 (5.4.)	0.739
Pack year history, mean (SD), yr	39.3 (15.9)	41.4 (17.4)	40.2 (17.7)	0.301	39.7 (17.4)	41.6 (17.6)	39.9 (17.2)	0.209
Current smokers, no (%)	76 (56.7%)	333 (59.3%)	344 (64.9%)	0.093	75 (56.4%)	341 (60.9%)	337 (63.5%)	0.298
Years quit smoking % (no)				0.307				0.356
0	57.8% (78)	59.4% (337)	64.8% (346)		57.0% (75)	60.9% (344)	63.4% (339)	
<1	7.4% (10)	6.4% (35)	7.8% (41)		6.7% (9)	6.5% (35)	7.9 5 (42)	
1-5	18.5% (24)	18.5% (102)	14.3% (74)		19.3% (25)	17.5% (97)	15.2% (79)	
>5	16.3% (22)	15.7% (88)	13.1% (69)		17.0% (23)	15.2% (84)	13.5% (71)	
Age started smoking % (no)				0.491				0.397
<14 years	14.9% (20)	19.2% (118)	17.4% (92)		16.5% (22)	18.8% (111)	16% (87)	
15-19 years	70.2% (94)	65.8% (395)	67.7% (359)		67.0% (91)	65.7% (378)	68.4% (368)	
>20	14.9% (20)	15.0% (48)	14.9% (79)		16.5% (22)	11.8% (71)	13.7% (76)	
Pulmonary function tests, mean (SD)								
FEV_1, L	3.26 (0.76)	3.29 (0.73)	3.41 (0.72)	0.013	3.24 (0.74)	3.29 (0.74)	3.41 (0.71)	0.008
FEV_1, % predicted	94.4 (19.5)	95.1 (18.5)	98.6 (17.9)	0.003	94.1 (18.8)	95.2 (19.0)	98.6 (17.4)	0.002
FEV_1/FVC ratio	0.70 (0.10)	0.71 (0.10)	0.72 (0.107)	0.002	0.71 (0.10)	0.70 (0.11)	0.72 (0.10)	0.002
MEF_{50}, L/s	2.97 (1.44)	2.95 (1.39)	3.25 (1.50)	0.002	2.94 (1.38)	2.96 (1.44)	3.24 (1.47)	0.003
COPD severity, no. (%)				0.004				0.010
No Obstruction	67 (50%)	291 (51.6%)	326 (61.3%)		71 (52.2%)	286 (51.2%)	326 (61.2%)	
GOLD class I	38 (28.4%)	181 (32.2%)	138 (26.0%)		39 (28.4%)	176 (31.4%)	143 (26.9%)	
GOLD class II	27 (20.1)	74 (13.2%)	61 (11.5%)		24 (17.9%)	81 (14.4%)	57 (10.7%)	
GOLD class III	2 (1.5%)	17 (3.0%)	5 (0.9%)		2 (1.5%)	17 (3.0%)	5 (0.9%)	

Table 4. Baseline characteristics for the LEUVEN group according to rs1051730 and rs8034191 genotypes.

	rs1051730				rs81034191			
	AA	AG	GG	P-value	GG	AG	AA	P-value
Age	63.9 (7.8)	63.4 (8.0)	64.7 (8.0)	0.086	63.5 (7.9)	63.6 (8.0)	64.6 (8.0)	0.234
Pack-years history, mean (SD), yr	45.5 (21.6)	49.6 (26.7)	45.9 (23.9)	0.080	46.2 (22.2)	49.4 (26.6)	45.7 (23.5)	0.103
Current smokers, no (%)	44 (38.3)	170 (47.2)	147 (48.8)	0.144	52 (41.3)	167 (45.5)	146 (50.2)	0.211
Pulmonary function tests, mean (SS)								
FEV_1, L, post	1.74 (1.04)	1.93 (1.09)	1.98 (1.07)	0.088	1.77 (1.06)	1.93 (1.08)	1.98 (1.08)	0.146
FEV_1, % predicted, post	59.8 (31.6)	65.5 (32.6)	68.1 (33.1)	0.044	60.7 (32.6)	65.6 (32.0)	67.5 (33.4)	0.111
FEV_1/FVC ratio,	0.51 (0.18)	0.54 (0.18)	0.55 (0.18)	0.054	0.51 (0.18)	0.54 (0.18)	0.55 (0.18)	0.045
COPD severity, no. (%)				0.444				0.385
No bronchial obstruction	30 (13.1)	106 (46.3)	93 (40.6)		31 (13.5)	109 (47.4)	90 (39.1)	
GOLD class I	18 (14.8)	51 (41.8)	53 (43.4)		22 (17.9)	52 (42.3)	49 (39.8)	
GOLD class II	27 (15.0)	89 (49.4)	64 (35.6)		28 (15.3)	93 (50.8)	62 (33.9)	
GOLD class III	27 (14.4)	85 (45.5)	75 (40.1)		29 (15.4)	86 (45.7)	73 (38.8)	
GOLD class IV	33 (21.2)	71 (45.5)	52 (33.3)		36 (22.6)	69 (43.4)	54 (34.0)	
Clinical Category				0.018				0.067
Asymptomatic smokers, no (%)	43 (11.8)	175 (48.2)	145 (39.9)		48 (13.1)	176 (48.1)	142 (38.8)	
Ambulatory COPD patients, no (%)	62 (15.9)	176 (45.1)	152 (39.0)		68 (17.3)	182 (46.2)	144 (36.5)	
Patients with end-stage COPD, no (%)	30 (24.8)	51 (42.1)	40 (33.1)		30 (24.4)	51 (41.5)	42 (34.1)	

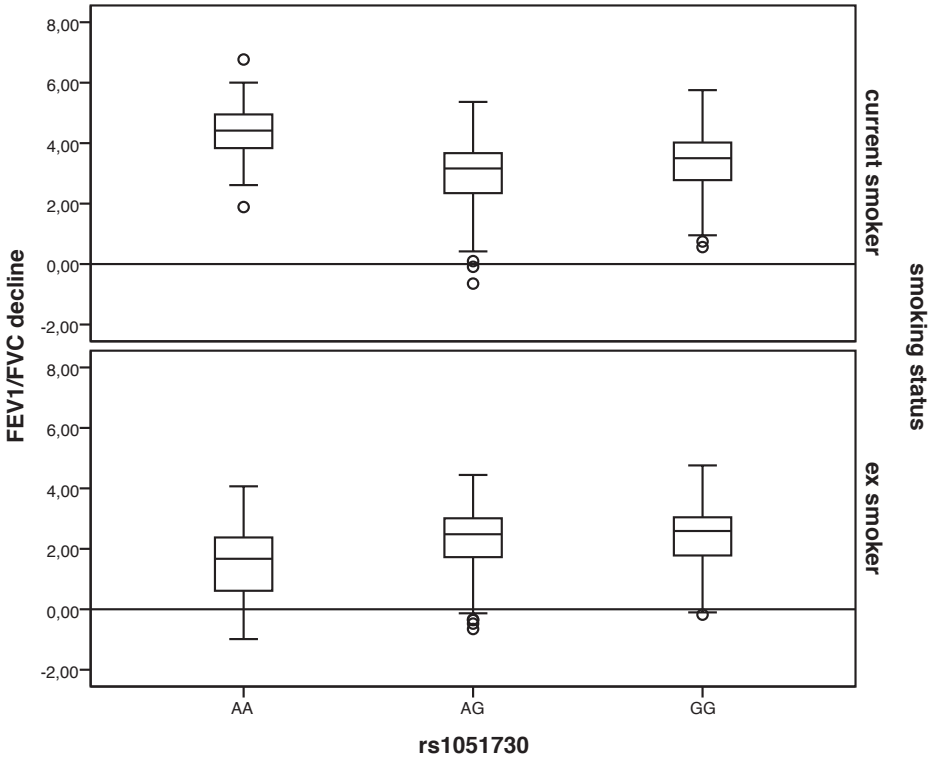
Genotyping succeeded in 874 (99%) and 883 (100%) LEUVEN participants, respectively for rs1051730 and rs8034191.

Association of nAChR variants with lung function decline in COPACETIC

Lung function measurements at baseline and after a median follow-up time of 3 years (interquartile range 2.9-3.1) are provided in Table 1. Mean FEV_1/FVC at baseline was 70.9±10.2 % and 68.0±9.6% at follow-up, representing a mean decrease of 2.9%. Baseline lung function measurements stratified for rs1051730 and rs8034191 are provided in Table 3.

In a multivariate regression analysis to test the association of both genotypes with lung function decline, the interaction between rs1051730 and smoking status was significant (p=0.015 for interaction term). This indicates that the effect of rs1051730 genotypes differed between current and former smokers. Current smokers homozygous for the rs1051730 A-allele had a more pronounced decline of the FEV_1/FVC compared to current smokers homozygous for the G-allele (4.3% and 3.3%, p=0.026; Figure 1). Heterozygotes experienced a decline of 3.0%. In contrast, former smokers carrying the AA genotype had no such stronger decline FEV_1/FVC compared to former smoking GG-carriers (p=0.317).

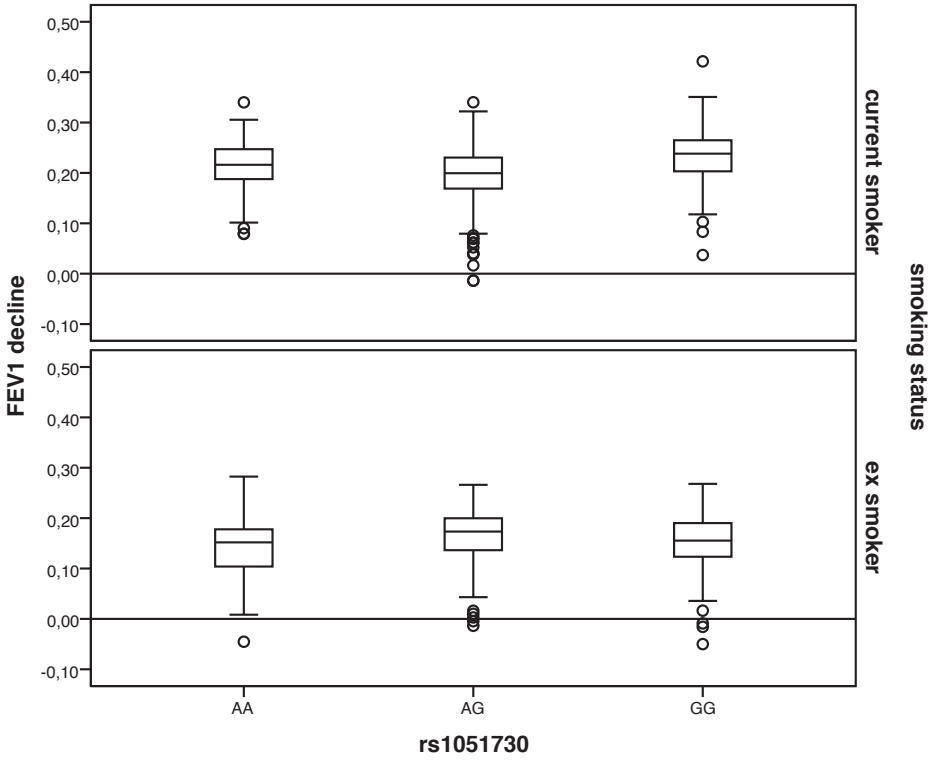
Figure 1. Decline in FEV_1/FVC over 3-year follow-up stratified by smoking status and rs1051730 genotypes. Box plot of FEV_1/FVC decline over a 3-year period split by the genotype. The horizontal bar in the box shows a median. FEV_1: forced expiratory volume in one second; FVC: forced vital capacity.



Similar results were obtained for the interaction between rs8034191 and smoking status (p=0.002 for interaction term). Current smokers homozygous for the rs8034191 G-allele showed a significantly stronger decline of the FEV_1/FVC compared to current smoking participants homozygous for the A-alleles (3.3% and 2.7%, p=0.009). For heterozygotes this decline was 2.9%. As for rs1051730, former smokers homozygous for the rs8034191 G-allele had no significant additional FEV_1/FVC decline compared to former smoker homozygous for the A-allele (p=0.465).

When performing similar analyses for FEV_1 neither of the two SNPs was significantly associated with a lower FEV_1 at follow-up (p=0.964 and 0.857, respectively) and none of the interactions between SNPs and smoking status were significant (p=0.203 and 0.107, respectively; Figure 2).

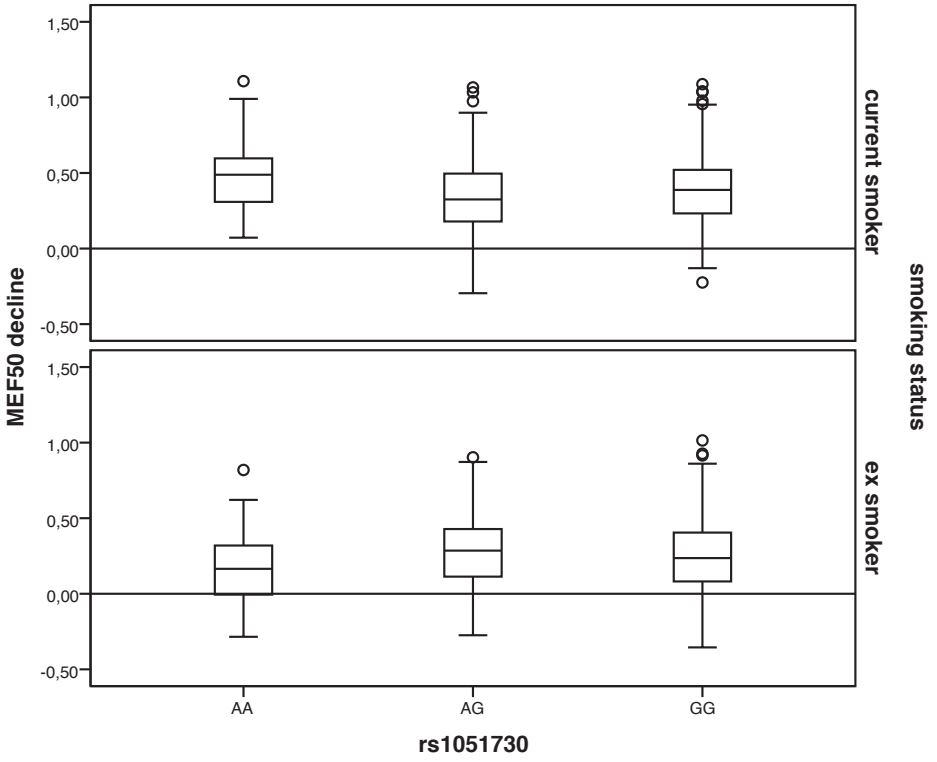
Figure 2. Decline in FEV_1 over 3-year follow-up stratified by smoking status and rs1051730 genotypes. Box plot of FEV_1 decline over a 3-year period split by the genotype. The horizontal bar in the box shows a median. FEV_1: forced expiratory volume in one second.



When analyzing MEF_{50}, which represents the maximal expiratory flow at 50% FVC and may reflect changes in the smaller peripheral airways,20 a similar effect as observed with FEV_1/FVC was noted (p=0.047 and 0.036 for rs105170 or rs8034191, respectively;

Figure 3). Smokers homozygous for the rs1051730 A-allele had a 491 mL/s decline in MEF_{50} compared to a 393mL/s in GG-carriers (p=0.083). Heterozygotes had a decline of 316mL/s. A similar pattern was found for smokers carrying the rs8034191 GG genotype (485 mL/s decline compared to 377 mL/s, p=0.017) in current smokers. In heterozygotes the decline was 314 mL/s. For both SNPs there were no significant differences in former smokers (p=0.280 and p=0.188, respectively for rs1051730 and rs8034191).

Figure 3. Decline in MEF_{50} over 3-year follow-up stratified by smoking status and rs8034191 genotypes. Box plot of MEF_{50} decline over a 3-year period split by the genotype. The horizontal bar in the box shows a median. MEF_{50} maximum expiratory flow when 50% of the FVC has been exhaled.



To additionally demonstrate that the association of rs1051730 and rs8034191 with decline in FEV_1/FVC and MEF_{50} in active smokers was independent of baseline lung function level, we inserted the interaction term baseline FEV_1/FVC*genotype or MEF_{50}*genotype as a covariate in the regression model. As expected, these analyses did not reveal a significant effect for these interaction terms (P=0.225 and 0.310 for FEV_1/FVC and P=0.248 and 0.172 for $MEF_{50,}$ respectively for rs1051730 and rs8034191).

Association between nAChR variants and severity of COPD

To assess the clinical relevance of an accelerated lung function decline due to variation

in *nAChR* genes, we studied the association of rs1051730 and rs8034191 with COPD severity and symptoms in an independent group of heavy smokers (LEUVEN). A multinomial logistic regression analysis was performed to assess the association between both genotypes and the risk of developing severe COPD (GOLD IV). AA-carriers of the rs1051730 genotype had a two-fold increased risk (OR 2.29, 95% confidence interval [CI]=1.11-4.75; p=0.025) of suffering from COPD GOLD IV compared to GG-carriers. Likewise, GG-carriers of rs8034191 (compared to AA-carriers) had a two-fold increased risk for GOLD IV versus no COPD (OR 2.42, 95% [CI]=1.18-4.95; p=0.016).

To establish whether the accelerated lung function decline in at-risk smokers also influences the severity of disease presentation, we classified all the LEUVEN subjects in three clinical categories (Table 2). AA-carriers were twice as frequent in the most severe COPD group (24.0% in patients with end-stage COPD versus 15.9% and 11.8% in ambulatory COPD patients and asymptomatic smokers; p=0.018; Table 4). Moreover, multinomial logistic regression revealed that (compared to GG) AA-carriers of the rs1051730 genotype exhibited an odds ratio of 5.0 (95% confidence interval [CI]=1.68-14.89; p=0.004) for receiving a lung transplantation. Likewise, AA-carriers had a 1.48-fold increased risk of being an ambulatory COPD patient (95% [CI]=0.90-2.42; p=0.12). The latter analysis was not significant presumably because some of the asymptomatic heavy smokers in LEUVEN already developed COPD (respectively 12.5% and 1.6% exhibited COPD with GOLD II and III). Similar data were observed for the rs8034191 SNP, as AA-carriers had a 4-fold increased risk of evolving to end-stage COPD with need of lung transplantation (OR=4.06; 95% [CI]=1.39-11.88; p=0.010) and a 1.56-fold increased risk of belonging to the group of ambulatory COPD patients (OR=1.56; 95% [CI]=0.97-2.51; p=0.07).

Discussion

In the current study, we observed that two common variants in the *nAChR* locus on chromosome 15q24/25 affect lung function decline in a population-based sample consisting of heavy smokers (COPACETIC). To the best of our knowledge, our study is the first to show an association of the 15q24/25 locus with decline in lung function over time. Importantly, this genotype-associated difference in lung function decline was independent of baseline lung function level, indicating that variants in the *nAChR* locus are not merely associated with an inherited lower lung function level, but also with an

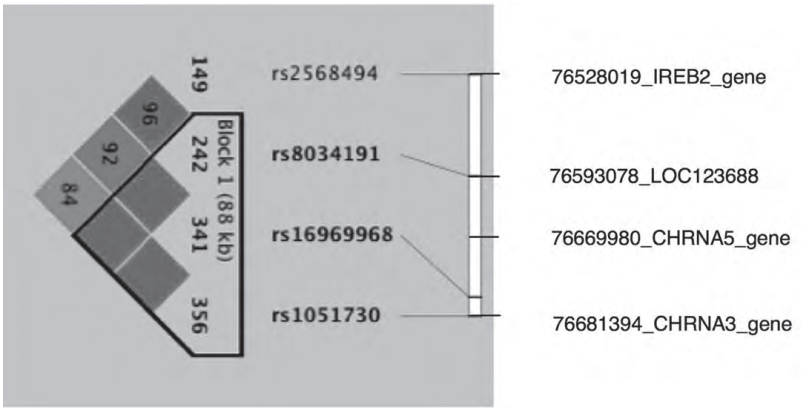
accelerated decline in lung function. Remarkably, no such effect was observed in former smokers. Based on power calculations, which revealed that our study had respectively 81.9% and 88.0% power for rs1051730 and rs8034191 to detect a significant difference in FEV1/FVC decline in former smokers (supplementary note 2), we believe that the absence of genetic variability in lung function decline in former smokers is a true negative finding. Importantly, we also established that the accelerated lung function decline in rs1051730 AA-carriers and rs8034191 GG-carriers is relevant for clinical practice. For instance, compared to GG-carriers, homozygotes for the rs1051730 A-allele showed a two-fold increased risk (OR=2.29) to be diagnosed with severe COPD GOLD IV and exhibited an OR=5.0 to have end-stage COPD with need for lung transplantation.[22] Likewise, GG-carriers of the rs8034191 genotype had a two-fold increased risk (OR=2.42) to have GOLD stage IV disease and a four-fold increased risk (OR=4.06) to be in need of lung transplantation compared to AA-carriers. Overall, these data suggest that current smokers carrying two copies of the at-risk alleles in the *nAChR* genes have an accelerated lung function decline leading to clinically significant COPD.

The observed associations may be biased for differences in smoking behavior between the *nAChR* at-risk and wild type genotypes. Indeed, recent GWA studies have indicated that the *nAChR* risk variants increase the risk of smoking addiction, presumably by mediating addictive effects in the brain, and promote more intense smoking, as reflected by a higher level of tobacco-specific nitrosamines per cigarette smoked in homozygous carriers of the at-risk alleles.[23,24] However, we did not find an association between *nAChR* variants and nicotine addiction related variables in our study population. Furthermore, the observed association between *nAChR* variants and lung function decline withstood correction for smoking status (current versus former smokers), years-quit smoking and pack-years smoked. Nicotinic acetylcholine receptors (*nAChR*s), which are encoded by the *CHRNA* genes, are also widely expressed on airway epithelial cells and immune cells, such as macrophages, and their role in mediating inflammatory processes has been established.[25,26] It is therefore possible that *nAChR*s directly affect lung parenchyma and that genetic variation modulates the inflammatory response upon stimulation of *nAChR*s by its agonists, thereby determining the extent of lung function decline. To completely discriminate between a direct and indirect effect, the relationship between the 15q24/25 locus and COPD could be studied among never-smokers. However, this approach is difficult given the very small number of never-smoking COPD patients. Alternatively, new statistical approaches could be applied, such as for instance mediation analysis. By use of this technique, Wang et al. established a direct association between rs1051730

and COPD risk (P=0.046), but also an indirect effect mediated by the variability in smoking behavior according to rs1051730 genotypes (0.006).[27] These findings are similar to our results: we did not establish an association between the 15q24/25 locus and nicotine addiction related variables. However, we demonstrated that rs1041730 affects lung function decline only in the group of active smokers, suggesting that this SNP exerts a mediating, but not a causal influence of smoking behavior. The hypothesis of a dual association (direct and indirect via smoking behavior) between 15q24/25, lung function decline and COPD should be investigated in future studies.

The biology, by which rs1051730 and rs8034191 contribute to smoking-related disease phenotypes still remains unresolved. The rs1051730 SNP is a synonymous SNP located in exon 5 of the *CHRNA3* gene, which is in strong linkage disequilibrium with a non-synonymous variant rs16969968 in exon 5 of the *CHRNA5* gene [28] and with rs55853698 in the promoter region of CHRNA 5 (all pairwise r^2>0,96; figure 4).[29] The SNP rs16969968 results in an amino acid change (D398N) in the alpha5 receptor subunit protein and has been shown to affect receptor function.30 The location of the rs55853698 variant makes it a candidate for affecting mRNA transcription. On the other hand, rs1051730 and rs8034191 are also strongly linked with rs2568494 in the IREB2 gene (r^2 = 0.692 and r^2 = 0.790, respectively; figure 4).

Figure 4: Linkage Disequilibrium Map for the COPD-associated variants in the 15q24/25 region. Red corresponds to r2 ≥ 0.8. Values for D' are included in the text of boxes. The genomic positions were retrieved from the NCBI dbSNP identifier (NCBI Human genome Build 36 location). The CHRNA 5 gene variant, rs55853698, is not present in the HapMap 21.



Therefore genetic variation in the 15q24/25 region can also result in an altered function of the IREB2 gene. Moreover, the finding of increased IREB2 protein and mRNA in lung-tissue samples from COPD subjects in comparison to controls supports a role of

the IREB2 gene in COPD pathogenesis.[31] Additional functional analyses are therefore required to establish whether the CHRNA3, CHRNA5 or IREB2 genes are involved in COPD development.

Regardless of the mechanisms by which *nAChR* variants predispose to an accelerated lung function decline in smokers, the identification of this genetic locus could have important clinical implications. First of all, this marker would contribute to the prospective identification of a subset of susceptible smokers at high risk for an accelerated loss of lung function. Secondly, since smoking cessation is the most effective way to reduce the rate of lung function decline, this information could be used to convince subjects to quit smoking before symptoms of COPD develop. Remarkably, we did not identify a significant association between genetic variation in the *nAChR* genes and FEV_1 decline. However, as also suggested in another genetic association study on COPD-related phenotypes, it is likely that different genetic loci control FEV_1 and FEV_1/FVC, since both parameters reflect a slightly different functional measure.[32]

The major strength of the present study is that we were able to access a large number of apparently healthy, but heavy smokers, as well as a relatively large cohort of symptomatic COPD patients, including a significant number of patients with end-stage lung disease in need of lung transplantation. This enabled us to study the role of *nAChR* genetic variants in various stages of COPD severity. Furthermore, the large number of study participants allowed extensive corrections for potentially confounding factors such as pack-years and smoking status. Nevertheless, some limitations of the current study need to be acknowledged as well. A first limitation is that COPACETIC only recruited heavy smokers and that we could thus not assess the effects of the 15q24/25 locus in subjects being exposed to less nicotine. Secondly, our follow-up period was limited to three years and we only assessed lung function level at two different time points.

In conclusion, we have demonstrated that heavy smokers carrying homozygous at-risk alleles for rs1051730 and rs8034191 in the *nAChR* locus are characterized by an accelerated decline in lung function, leading to an increased risk of developing severe COPD. We thus provide one of the first genetic markers predictive for lung function decline.

Reference List

1. Rabe KF, Hurd S, Anzueto A, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med* 2007;176:532-55.
2. Pauwels RA, Rabe KF. Burden and clinical features of chronic obstructive pulmonary disease (COPD). *Lancet* 2004;364:613-20.
3. Castaldi PJ, Cho MH, Litonjua AA, et al. The Association of Genome-Wide Significant Spirometric Loci with COPD Susceptibility. *Am J Respir Cell Mol Biol* 2011;Published Online First: 9 June 2011. doi:10.1165/rcmb.2011-0055OC.
4. Silverman EK, Sandhaus RA. Clinical practice. Alpha1-antitrypsin deficiency. *N Engl J Med* 2009;360:2749-57.
5. Wilk JB, Walter RE, Laramie JM, et al. Framingham Heart Study genome-wide association: results for pulmonary function measures. *BMC Med Genet* 2007;8 Suppl 1:S8.
6. Wilk JB, Chen TH, Gottlieb DJ, et al. A genome-wide association study of pulmonary function measures in the Framingham Heart Study. *PLoS Genet* 2009;5:e1000429.
7. Pillai SG, Ge D, Zhu G, et al. A genome-wide association study in chronic obstructive pulmonary disease (COPD): identification of two major susceptibility loci. *PLoS Genet* 2009;5:e1000421.
8. Berrettini W, Yuan X, Tozzi F, et al. Alpha-5/alpha-3 nicotinic receptor subunit alleles increase risk for heavy smoking. *Mol Psychiatry* 2008;13:368-73.
9. Bierut LJ, Madden PA, Breslau N, et al. Novel genes identified in a high-density genome wide association study for nicotine dependence. *Hum Mol Genet* 2007;16:24-35.
10. Li MD. Identifying susceptibility loci for nicotine dependence: 2008 update based on recent genome-wide linkage analyses. *Hum Genet* 2008;123:119-31.
11. Caporaso N, Gu F, Chatterjee N, et al. Genome-wide and candidate gene association study of cigarette smoking behaviors. *PLoS One* 2009;4:e4653.
12. Thorgeirsson TE, Geller F, Sulem P, et al. A variant associated with nicotine dependence, lung cancer and peripheral arterial disease. *Nature* 2008;452:638-42.
13. Amos CI, Wu X, Broderick P, et al. Genome-wide association scan of tag SNPs identifies a susceptibility locus for lung cancer at 15q25.1. *Nat Genet* 2008;40:616-22.
14. Hung RJ, McKay JD, Gaborieau V, et al. A susceptibility locus for lung cancer maps to nicotinic acetylcholine receptor subunit genes on 15q25. *Nature* 2008;452:633-7.
15. Lambrechts D, Buysschaert I, Zanen P, et al. The 15q24/25 susceptibility variant for lung cancer and chronic obstructive pulmonary disease is associated with emphysema. *Am J Respir Crit Care Med* 2010;181:486-93.
16. Fletcher C, Peto R. The natural history of chronic airflow obstruction. *Br Med J* 1977;1:1645-8.
17. van den Bergh KA, Essink-Bot ML, Borsboom GJ, et al. Short-term health-related quality of life consequences in a lung cancer CT screening trial (NELSON). *Br J Cancer* 2010;102:27-34.

18. Janssens W, Bouillon R, Claes B, et al. Vitamin D deficiency is highly prevalent in COPD and correlates with variants in the vitamin D-binding gene. *Thorax* 2009;65:215-20.
19. Janssens W, Nuytten H, Dupont LJ, et al. Genomic copy number determines functional expression of {beta}-defensin 2 in airway epithelial cells and associates with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2010;182:163-9.
20. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J* 2005;26:319-38.
21. Altshuler DM, Gibbs RA, Peltonen L, et al. Integrating common and rare genetic variation in diverse human populations. *Nature* 2010;467:52-8.
22. Trulock EP, Christie JD, Edwards LB, et al. Registry of the International Society for Heart and Lung Transplantation: twenty-fourth official adult lung and heart-lung transplantation report-2007. *J Heart Lung Transplant* 2007;26:782-95.
23. Le Marchand L, Derby KS, Murphy SE, et al. Smokers with the CHRNA lung cancer-associated variants are exposed to higher levels of nicotine equivalents and a carcinogenic tobacco-specific nitrosamine. *Cancer Res* 2008;68:9137-40.
24. Thorgeirsson TE, Gudbjartsson DF, Surakka I, et al. Sequence variants at CHRNB3-CHRNA6 and CYP2A6 affect smoking behavior. *Nat Genet* 2010;42:448-53.
25. Wang H, Yu M, Ochani M, et al. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. *Nature* 2003;421:384-8.
26. Fu XW, Lindstrom J, Spindel ER. Nicotine activates and up-regulates nicotinic acetylcholine receptors in bronchial epithelial cells. *Am J Respir Cell Mol Biol* 2009;41:93-9.
27. Wang J, Spitz MR, Amos CI, et al. Mediating effects of smoking and chronic obstructive pulmonary disease on the relation between the CHRNA5-A3 genetic locus and lung cancer risk. *Cancer* 2010;116:3458-62.
28. Saccone SF, Hinrichs AL, Saccone NL, et al. Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. *Hum Mol Genet* 2007;16:36-49.
29. Liu JZ, Tozzi F, Waterworth DM, et al. Meta-analysis and imputation refines the association of 15q25 with smoking quantity. *Nat Genet* 2010;42:436-40.
30. Bierut LJ. Nicotine dependence and genetic variation in the nicotinic receptors. *Drug Alcohol Depend* 2009;104 Suppl 1:S64-9.
31. DeMeo DL, Mariani T, Bhattacharya S, et al. Integration of genomic and genetic approaches implicates IREB2 as a COPD susceptibility gene. *Am J Hum Genet* 2009;85:493-502.
32. Pillai SG, Kong X, Edwards LD, et al. Loci Identified by Genome-wide Association Studies Influence Different Disease-related Phenotypes in COPD. *Am J Respir Crit Care Med* 2010;15:1498-505.

Supplement Chapter 7

Variants in the 15q24/25 locus associate with lung function decline in active smokers

Supplementary note 1:

COPACETIC study population:

The **COPACETIC** cohort included 2,246 participants from the NELSON trial recruited by the University Medical Centers in Groningen and Utrecht. Of these 2,246 participants a random sample of 1,226 participants was selected for a genome-wide association study. The inclusion criteria of the NELSON trial are discussed in detail elsewhere [1]. Briefly, the NELSON trial included current and former heavy smokers (pack-years <20) fit enough to undergo surgery. Those with moderate or bad self-reported health status were excluded. Only males were included based on the high risk to develop lung cancer or COPD as fewer women in the Dutch population have accumulated a long-term exposure to cigarettes compared to men. Baseline details on smoking habits were gathered through questionnaires which included questions about age at started smoking, duration of smoking, number of pack-years smoked, smoking status at enrolment (current or former smoker) and if applicable duration of cessation.

Additional information regarding the COPACETIC genome-wide association study:

COPACETIC is an international and multicenter study that aims to elucidate the genetic basis of individual variance in the susceptibility to COPD and emphysema. In the discovery phase, 1078 participants with emphysema, 1036 participants with obstruction and 1677 non-obstructive, non-emphysema participants were included. These participants were recruited from the general population of 7 districts in the Netherlands: Groningen, Drenthe, Utrecht, Eemland, Midden-Nederland, Kennemerland and Amstelland-de Meerlanden [1]. In particular, no COPACETIC subjects have been recruited in Belgium. All participants have been analyzed using Illumina's genome-wide SNP arrays (Human610-Quad BeadChip) and a stringent quality control assessment on the data was performed. Since genome-wide findings in discovery cohorts are prone to high false-positive discovery rates, interesting SNPs are currently being replicated in >10,000 replication samples from COPACETIC. The replication phase is only expected to finish mid 2012. Some of the methods and results of this study have previously been reported in the form of abstracts (12, 13).

Importantly, the 1,226 COPACETIC participants in the current study represent only a minority (32%) of the COPACETIC GWAS participants and even less (10%) of the combined COPACETIC discovery/replication cohort. In particular, these 1,226 subjects were selected

because of the availability of lung function decline data, which are not available and will not be reported in the GWAS. The second study population of the current study, the LEUVEN cohort (which is described in detail below), is not part of the COPACETIC GWAS.

LEUVEN study population:

The **LEUVEN** cohort included 366 Belgian participants of the NELSON trial that were recruited from the general population of 14 municipalities around LEUVEN (19). These 366 population-based subjects were not previously diagnosed with COPD. In addition, 517 heavy smokers with symptoms compatible with COPD were included at the LEUVEN respiratory outpatient clinic. Of these 517 subjects, 123 were listed for lung transplantation because of disabling end-stage COPD [2]. Inclusion criteria for these 883 LEUVEN participants were a smoking history of at least 15 pack-years, a minimal age of 50 years and the availability of a complete pulmonary function test. Subjects with a suspicion or diagnosis of asthma were excluded, as well as those with other respiratory diseases affecting pulmonary function. General listing criteria for lung transplantation were end-stage lung disease with serious repercussions on daily life activities, a life expectancy <18 months, <65 years of age and a smoke-free period of ≥6 months. Patients with COPD or emphysema were classified as having end-stage disease if they had a FEV_1 <1 litre or an FEV_1 % predicted <25, an arterial oxygen tension <60 mmHg and/or an arterial carbon dioxide tension >55 mmHg, failure of the right ventricle caused primarily by the respiratory disorder (cor pulmonale) or progressive deterioration of lung function despite optimal treatment (inclusive respiratory revalidation and oxygen therapy). Arterial blood gas values and echocardiographical measurements were only available for the minority of the total population (45% and 35%, respectively, and mainly consisting of the patients listed for transplantation) and were therefore not studied as separate phenotypes.

Genotyping:

In COPACETIC, the rs1051730 and rs8034191 genotypes were extracted from Human610-Quad BeadChip data (Illumina Inc., San Diego, CA, USA). Quality control for these data included the removal of *i)* SNPs located in copy number variations, *ii)* SNPs out of Hardy-Weinberg equilibrium, *iii)* SNPs with a minor allele frequency <5%, and *iv)* SNPs with >5% of genotype data missing. Ethnic outliers were removed using principle component analysis as well as related individuals. From 620,901 SNPs genotyped, 522,902 SNPs passed the first round quality control, of which rs1051730 and rs8034191.

Genotyping in the LEUVEN cohort for the rs1051730 and rs8034191 SNPs was performed in a blinded manner using iPLEX technology on a MassARRAY Compact Analyser (Sequenom Inc., San Diego, CA, USA), as reported previously [3]. Quality control was performed by genotyping 13 samples in duplicate, with a duplicate concordance of 100% between the samples.

Supplementary note 2:

Power calculations:

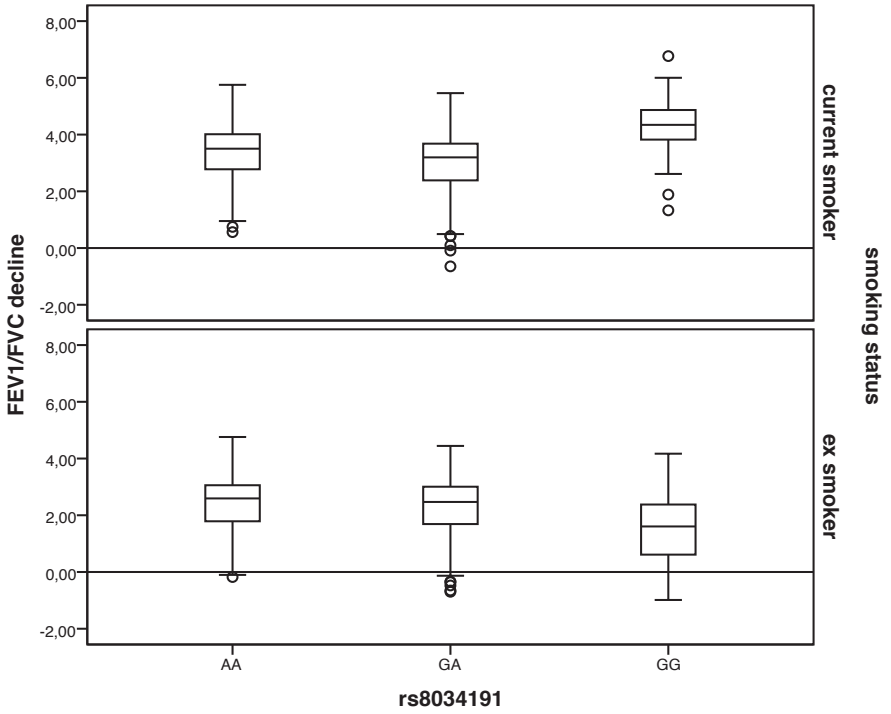
We calculated the observed power to discover a significant association of genotypes, smoking status (current versus former) and the genotype*smoking status interaction term with lung function decline over a three-year follow-up period, as shown in the table below. The observed power for each test was calculated by use of the Generalized Linear Models method in SPSS (SPSS software version 18.0, Chicago, Illinois, USA).

	Observed power
1. Decline in FEV1/FVC	
genotype	
rs1051730	24%
rs8034191	15.2%
smoking status	~100%
genotype * smoking status interaction	
rs1051730	81.9%
rs8034191	88.0%
2. Decline in FEV1	
Genotype	
rs1051730	5.8%
rs8034191	7.8%
smoking status	~91%
genotype * smoking status interaction	
rs1051730	33.4%
rs8034191	45%
3. Decline in MEF50	
Genotype	
rs1051730	15.3%
rs8034191	10.5%
smoking status	95%
genotype * smoking status interaction	
rs1051730	59.3%
rs8034191	62.8%

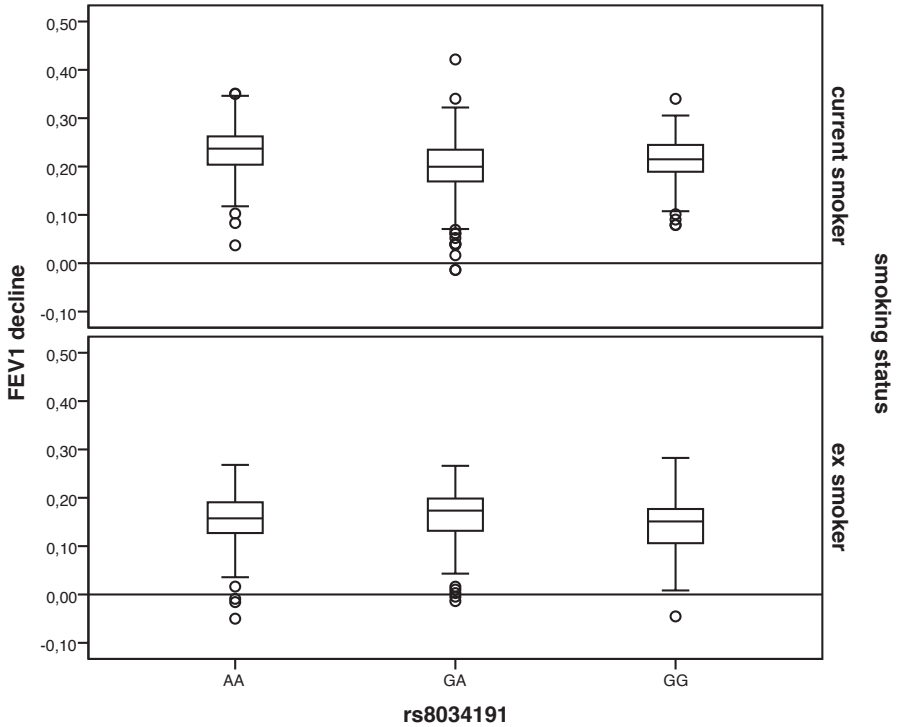
Regarding the observed power for the association between decline in FEV1/FVC and the genotype*smoking status interaction, the high established power (81.9% and 88.0%, for rs1051730 and rs8034191, respectively) indicates that there is a high probability that the absence of an association between genotype*former smoking status and lung function decline is a true negative finding. The same rationale applies to the observations for decline in MEF_{50}.

In addition, to further demonstrate that the smaller sample size of the group of former smokers (n=479 compared with 753 current smokers) did not influence our findings, we stratified this group of ex-smokers according to rs1051730 genotypes and used the non-inferiority or equivalent approach, as described previously [4], to calculate a one-sided 95% confidence interval (CI) of the ratio of two means. We determined the two-sided 90% CI's (which corresponds to a one one-sided 95% CI) for the differences between the ln-transformed FEV1/FVC values in the AA-, AG- and GG- genotypes of rs1051730. The width of those intervals is directly related to the power of the analysis. The largest difference in this stratified analysis appeared to be between AA- and AG-genotypes with a ratio of exp(0.010447)=101.105%. In other words, FEV1/FVC for AA-carriers at follow-up was 1.0111 times the value of AG-carriers. The 90% CI of that ratio ranges from exp(-0.006822) to exp(0.027715) or from 0.9932 to 1.0281. This means that, in the population of former smokers, the FEV1/FVC values will, with 90% certainty, be within the range of 99.320% to 102.810% of the FEV1/FVC value of AA-carriers at follow-up. The width of this interval is 101.105 – 99.320 = 1.785%. This means that the difference in FEV1/FVC between AA- and AG-carriers for rs1051730 will, with 95% certainty, not exceed 1.785% of the FEV1/FVC value at follow-up. Analogously, the FEV1/FVC difference between the GG and AG groups of rs8034191 will not exceed 0.8% of the actual mean FEV1/FVC in the GG group.

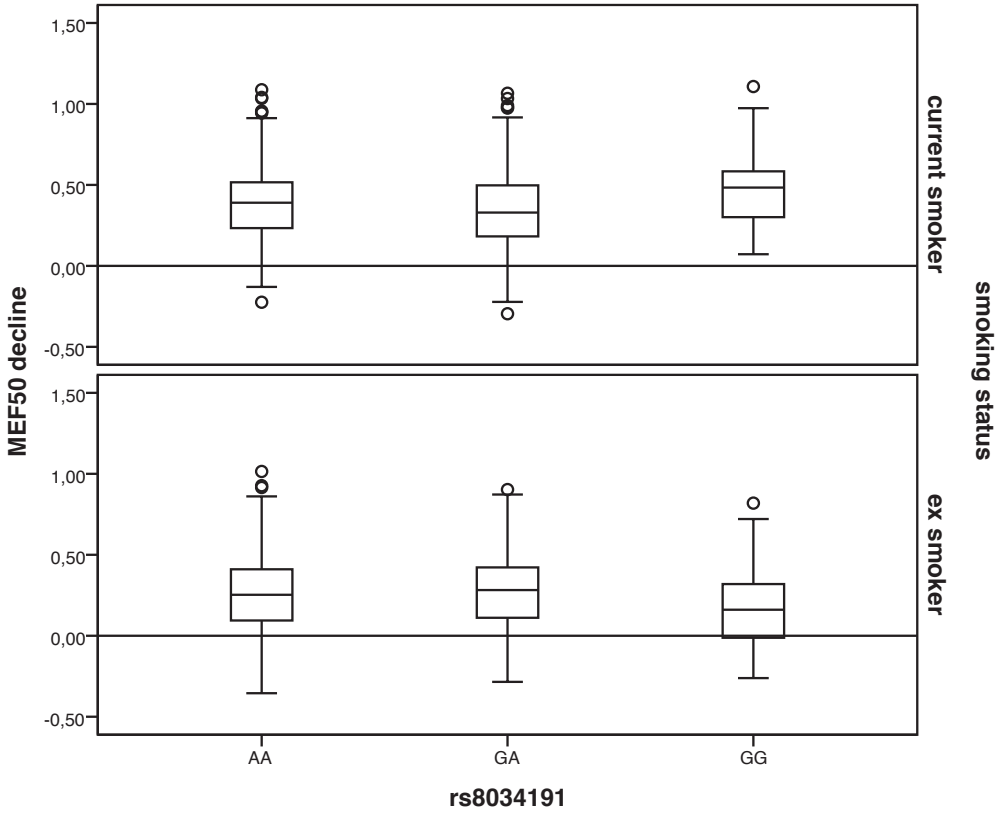
Supplementary figure 1. Decline in FEV_1/FVC over 3-year follow-up stratified by smoking status rs8034191 genotypes. Box plot of FEV_1/FVC decline over a 3-year period split by the genotype. The horizontal bar in the box shows a median. FEV_1: forced expiratory volume in one second; FVC: forced vital capacity.



Supplementary figure 2. Decline in FEV_1 over 3-year follow-up stratified by smoking status and rs8034191 genotypes. Box plot of FEV_1 decline over a 3-year period split by the genotype. The horizontal bar in the box shows a median. FEV_1: forced expiratory volume in one second.



Supplementary figure 3: Decline in MEF_{50} over 3-year follow-up stratified by smoking status and rs1051730 genotypes. Box plot of MEF_{50} decline expiratory flow when 50% of the FVC has been exhaled.



Reference List

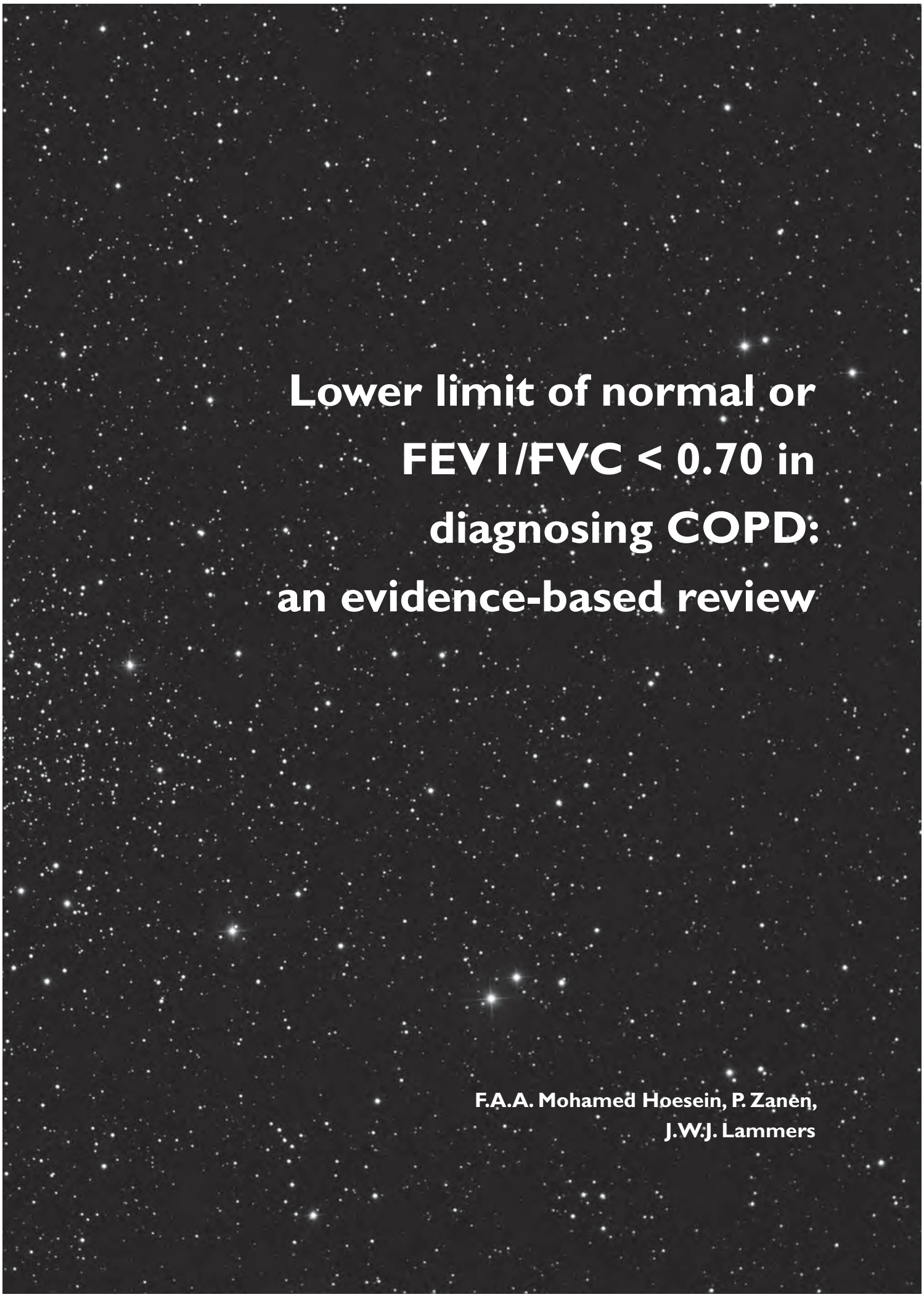
1. van Iersel CA, de Koning HJ, Draisma G, et al.R isk-based selection from the general population in a screening trial: selection criteria, recruitment and power for the Dutch-Belgian randomised lung cancer multi-slice CT screening trial (NELSON). *Int J Cancer* 2007; 120: 868-874.
2. Janssens W, Bouillon R, Claes B, et al. Vitamin D deficiency is highly prevalent in COPD and correlates with variants in the vitamin D-binding gene. *Thorax*; 65: 215-220.
3. Lambrechts D, Buysschaert I, Zanen P, et al. The 15q24/25 susceptibility variant for lung cancer and chronic obstructive pulmonary disease is associated with emphysema. *Am J Respir Crit Care Med* 2010; 181: 486-493.
4. Schuirmann DJ. A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. *J Pharmacokinet Biopharm* 1987; 15: 657-680.

Part

2

Spirometric definition of COPD


Chapter
8



Lower limit of normal or FEV1/FVC < 0.70 in diagnosing COPD: an evidence-based review

F.A.A. Mohamed Hoesein, P. Zanen,
J.W.J. Lammers

Abstract

Purpose To review the currently available literature comparing the FEV_1/FVC LLN with a fixed value of FEV_1/FVC <0.70 in diagnosing airflow obstruction in subjects aged >40 years.

Methods A structured MEDLINE, EMBASE and Cochrane search of English-language literature was conducted. Studies comparing prevalence rates according to the LLN and a fixed value were included. Attention was paid to the choice of the reference test or gold standard used.

Results Eighteen studies met the inclusion criteria. Sixteen studies compared the rates of subjects diagnosed with airflow obstruction by either definition of airflow obstruction without using a non-independent reference standard (level 4 studies). Using a fixed value of FEV_1/FVC, an overall higher number of subjects were diagnosed with airflow obstruction that increased with age. Two studies included a follow-up phase comparing risks of either hospitalization or occurrence of respiratory symptoms and mortality (level 2b studies). Adjusted risks of hospitalization (HR 2.6) or mortality (HR 1.3) were significantly larger in subjects with an FEV_1/FVC below 0.70 but above the LLN (in-between group) compared to subjects with normal lung function.

Conclusion The prevalence of spirometry-based COPD is greater when using the fixed value of FEV_1/FVC in comparison to using the LLN. Based on one longitudinal study the in-between group appears to have a higher risk of hospitalization and mortality; therefore it seems that using the LLN of FEV_1/FVC underestimates COPD. In absence of a gold standard of COPD longitudinal research will be necessary to determine which criterion is better and more clinically relevant.

Introduction

Chronic obstructive pulmonary disease (COPD) will constitute the third leading cause of death by 2020 and it is currently the only chronic disease with increasing mortality rates.[1] COPD is preventable but irreversible and stabilizing the progression of the disease in an early phase appears to be the best therapy for decreasing morbidity and mortality and reducing health costs.[2] Consensus on the proper diagnostic criteria for COPD is essential.

It is widely accepted that the presence of airflow obstruction is key in diagnosing COPD.[2-3] Airflow obstruction is present when the forced expiratory flow in 1 second (FEV_1)/ forced vital capacity (FVC) - ratio is reduced.[3] Another important sign of COPD is a decline in FEV_1 which is consequently used as a measure of the severity of airflow obstruction.[2]

There is still a controversy regarding the appropriate cut-off values for FEV_1/FVC.[4-6] In 2001, the Global Initiative for Chronic Obstructive Lung Disease (GOLD) committee was the first to publish a consensus statement propagating the use of a fixed FEV_1/FVC <0.70 value and fixed FEV_1 values to classify severity.[7] One of the objectives in introducing fixed values was to standardize and increase the awareness of diagnosing COPD, i.e. to simplify the diagnosis. However, more recently the GOLD committee recognized that using a fixed value of <0.70 may lead to potential overdiagnosis in the elderly.[2]

In 2004, the ERS and the ATS issued a combined statement advocating the use of the lower limit of normal (LLN) instead of a fixed criterion.[8] The LLN is statistically defined by the lower fifth percentile of a reference population and can be calculated by subtracting 1.64 times the standard deviation from the mean, i.e. the expected value. The LLN is age-corrected. Using a fixed percentage instead of the LLN has several drawbacks as mentioned by Pellegrino et al.4 One of the main arguments for discarding the fixed FEV_1/FVC criterion is that it can lead to a COPD diagnosis in non-smoking elderly .[9]

A large number of studies have compared the two COPD definitions in an attempt to decide which is more appropriate. This review aims to clarify the strengths and weaknesses of studies comparing the LLN with a fixed criterion. We focused on the study design and the choice of reference tests that were employed in these studies.

Methods

Search Strategy

A structured search was conducted in Medline, EMBASE and the Cochrane library containing studies from January 1966 till June 2010. The search strings that were used contained synonyms and related terms for the "lower limit of normal". The Boolean operator "OR" was used to combine the search terms; the complete search string is noted in Table 1. Moreover, all references from the bibliographies of the included articles were reviewed.

Table 1: Search strategy used in MEDLINE, EMBASE and Cochrane library June 2010.

Search terms	Results
"lower limit of normal" OR "lower limits of normal" OR LLN OR "fifth percentile" OR "five percentile" OR "5th percentile"	1,954

Article Inclusion

After removing duplicates, F.M.H. and P.Z. independently screened all articles' titles and abstracts by applying inclusion and exclusion criteria (see flowchart, Figure 1). Articles were included if *(a)* they pertained to studies comparing the LLN with a fixed criterion to diagnose spirometry-based COPD, *(b)* spirometry was performed in an adult population containing subjects ≥ 40 years and *(c)* they were written in English. If the abstract alone did not provide sufficient information to conclude that the article compared the LLN with a fixed value of FEV_1/FVC, the full-text was retrieved. Differences with respect to article inclusion were resolved by consensus between the two reviewers. Full-text versions of the included articles were retrieved if available and were read independently by both reviewers.

Study Appraisal

All included articles were appraised independently by F.M.H. and P.Z. on criteria concerning validity: (*a*) design, (*b*) study population, (*c*) presence or choice of a gold standard or reference test and (*d*) subject inclusion. Special attention was paid to the choice of the reference test / gold standard used. It was noted whether (*1*) only prevalence was reported according to either definition while no reference standard was used, (*2*) either one of the definitions was used as a reference test or (*3*) another kind of reference standard, like follow-up or a range of tests, was used. Levels of evidence

were classified according to Oxford Centre for Evidence-based Medicine (CEBM) levels of evidence, see Table 2.[10] Disagreement in study appraisal was resolved by consensus.

Table 2: Levels of evidence according to the Oxford Centre for Evidence-based Medicine Levels of Evidence (CEBM) [10]

Level	
1a	Systematic reviews with homogeneity or studies with clinical prediction rule from different clinical centres
1b	Validating cohort studies with good reference standards or studies with clinican prediction rule within one centre
1c	Studies reporting high sensitivity/ specificity
2a	Systematic reviews with homogeneity of Level >2 studies
2b	Systematic reviews with homogeneity or studies with clinical prediction rule from different clinical centres
3a	Systematic reviews with homogeneity of Level >3 studies
3b	Non-consecutive studies or without consistently applied reference standards
4	Case-control studies, poor or non-independent reference standard
5	Expert opinion

Data Extraction

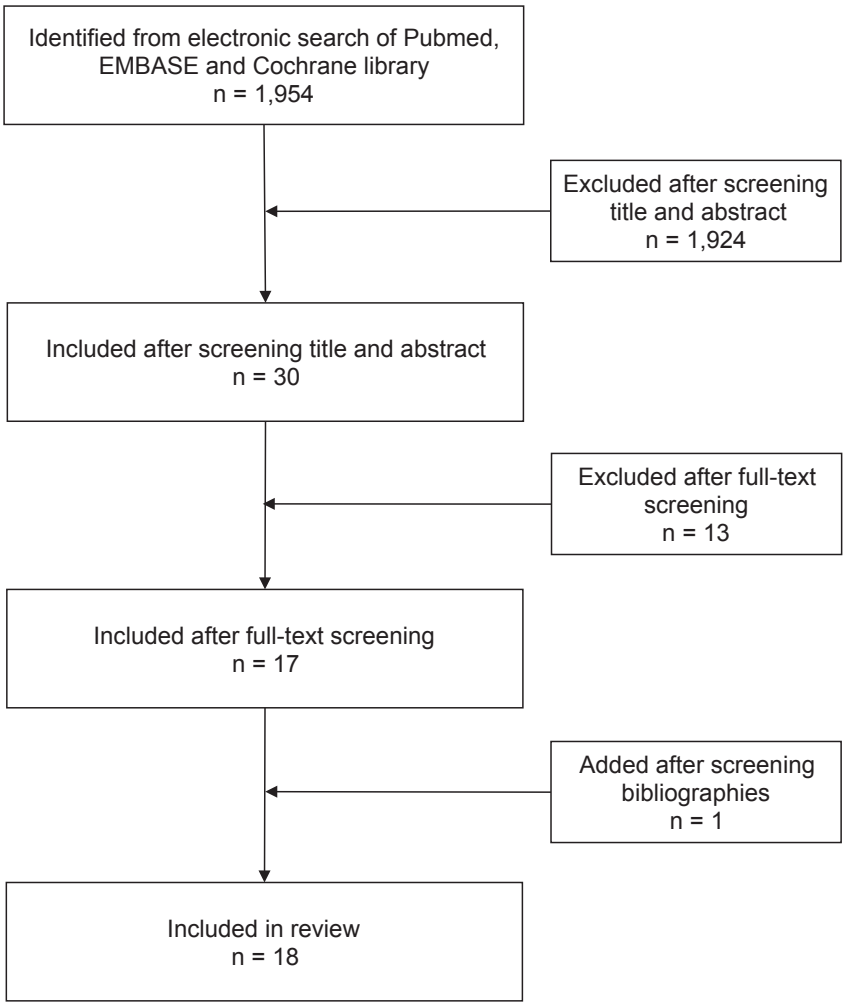
The reviewers independently extracted the following data: *(a)* number of subjects included, *(b)* percentage of males, *(c)* mean ages (standard deviation), *(d)* percentage of current smokers and *(e)* prevalence of COPD according to LLN and fixed FEV_1/FVC criteria If it was not provided, the percentage of smokers and prevalence rates were calculated by using the data provided in the text and tables of the article. Differences in the extracted data were resolved by consensus between the two reviewers.

Results

General

The search of the three databases yielded 1,954 individual articles. After applying the inclusion criteria on the titles and abstracts, 30 articles were selected and the full-texts were retrieved. Two articles were not available as full text.[11-12] Eleven articles were excluded after full-text screening because they did not meet the inclusion criteria. Screening of the references resulted in one additional publication.[13] In total, 18 articles were included as is mentioned in the flowchart search strategy (Figure 1).

Figure 1: Flowchart search strategy. Search performed June 2010.



In Table 3, the authors, year of publication, number of included patients, percentage of males, study population, reference equation used for calculating predicted lung function and LLN, age groups, smoking status, and use of post-bronchodilator values were noted separately for each included study.

Six studies performed post-bronchodilator spirometry.[14-19] The majority of the studies compared the fixed value of FEV_1/FVC <0.70 against the LLN and thus used the LLN as their gold standard or reference test, see Table 4. Only Mannino et al. used occurrence of COPD-based hospitalization and mortality during follow-up as a reference, while Vaz Fragoso used mortality and occurrence of respiratory symptoms as a reference.[20-21] The remaining studies provided prevalence and discordance rates according to either definition, see Table 4. The CEBM levels of evidence are provided in Table 4.

Table 3: Percentages of males, age groups included, smoking status of included subjects and use of post-bronchodilator values. NHANESIII = Third National Health and Nutrition Examination Survey, BOLD= Burden of Long Disease Initiative, CHS= Cardiovascular Health Study, HSE9596= Health Survey for England 1995-1996

	Study	Year	Subjects No.	Male [%]	Population	Mean age (SD) [yr]	Age range [yr]	Post-broncho-dilator	Smo-kers[%]	Never smo-kers [%]
Referred/ hospitalized subjects	*Aggarwal et al.22]*	2006	18,112	56.1	India	M 48.2 (16.2) / F 44.8 (14.8)	≥15	no	NA	NA
	Dejsomritrutai et al.23]	2002	1,754	54.1	Thailand	NA	12 > - 91		NA	NA
	Margolis et al.13]	1997	166	95.1	USA	58.9 (13)	≥24	no	43	11
	Roberts et al.24]	2006	1,503	47	USA	NA	≥20	no	NA	NA
	Schermer et al.14]	2008	14,056	47	Netherlands	M 54.2 (15.2) / F 51.9 (15.1)	≥ 21	yes	NA	31
Subjects from general population	*Celli et al.25]*	2003	9,838	47.8	NHANES III	48.3 (13.6)	≥30	no	30.3	42.8
	Hansen et al.26]	2007	9,508	43.1	NHANES III	NA	20 - 80	no	36.8	62.8
	Hnizdo et al.27]	2006	13,842	NA	NHANES III	NA	20 - 80	no	NA	NA
	Hwang et al.15]	2009	2728	46.3	Korea	41 (median)	≥ 18	yes	28.9	NA
	Ko et al.16]	2008	1,008	26.9	China	74.2 (6.4)	≥ 60	yes	4.5	74.9
	Lau et al.28]	2007	525	100	Hong Kong	NA	≥20 - 80	no	68.3	0
	Mannino et al.20]	2007	4,965	43.0	CHS	NA	≥ 65	no	11.6	45.9
	Perez-Padilla et al.19]	2007	5,183	40.0	PLATINO	56.2	≥ 40	yes	NA	NA
	Roche et al.29]	2008	4,764	48.1	France	59.9 (10.1)	≥ 45	no	18.1	48.2
	Shirtcliffe et al.17]	2004	749	54.2	New Zealand	54.9 (12.8)	≥30	yes	10.1	46.2
	Swanney et al.30]	2008	40,646	NA	NHANESIII/ HSE9596 / Dutch	NA	≥17	no	NA	NA
	Vaz Fragoso et al.21]	2010	2,480	49.5	NHANESIII	71.7 (4.5)	65 - 80	no	15.0	44.7
	Vollmer et al.18]	2009	10,001	47.7	BOLD	NA	≥ 40	yes	57.1	42.9

Table 4: Prevalence of airflow obstruction according to definition used, reference standard used, levels of evidence and conclusion of the authors.

	Study	Prevalence GOLD [%]	Prevalence LLN [%]	Reference Standard	Level of Evidence*	Authors conclusion / recommendation
Referred/ hospitalized subjects	*Aggarwal et al.22]*	overall: 23.6	overall: 28.2	LLN	4	Use LLN instead of fixed criterion based on statistical considerations.
	Dejsomritrutai et al.23]	overall: 51.5	overall: 37.9	LLN	4	Fixed criterion result in poor agreement with LLN. Use FEV_1/FVC 90% of predicted.
	Margolis et al.13]	overall: 24.5	overall: 24	None	4	Discrepancies in diagnosis between LLN and fixed criterion exist.
	Roberts et al.24]	overall: 40	overall: 37	LLN	4	At extreme age using fixed criterion leads to higher prevalence of airflow obstruction when compared to LLN
	Schermer et al.14]	51 - ≥ 81: 27.5 - 45	51 - ≥ 81: 20 - 25	LLN	4	Use LLN because fixed criterion leads to overestimation.
	Celli et al.25]	overall: 25.6 - 41.7	overall: 19.1 - 22.7	LLN, self-reported COPD	4	Discrepancies in diagnosis between LLN and fixed criterion exist. Opinion leaders should agree upon definition.
	Hansen et al.26]	Sk: M 41.2-62.4; F 50.1-18.8 NvSk: M 9.8-26.3; F 6.9-33.1	Sk: M 32.2-42.9 ; F 25.4-31.3 NvSk: M 4.6-6.0; F 6.2-12.1	LLN	4	Discard fixed criterion because it leads to overestimation of airflow obstruction in elderly.
	Hnizdo et al.27]	50 – 80: 30.5	50 – 80: 19.2	LLN, physician-diagnosed	4	Using fixed criterion overestimates airflow obstruction in subjects age >50.
	Hwang et al.15]	≥45: 13.5; ≥75:30.7	≥45: 10.7; ≥75: 16.4	LLN	4	Using LLN might reduce risk of over diagnosis in elderly.
	Ko et al.16]	Overall:25.9 Sk: M 40.4; F 25.3 NvSk: M 21.0; F 23.1	Overall: 12.4 Sk: M 9.6; F 20.7 NvSk: M 5.7; F 13.1	LLN, symptoms	4	Longitudinal studies necessary to assess appropriate definition ormation
	Lau et al.28]	≥60-80: 45.4	≥60-80: 27.6	LLN	4	Use LLN calculated from local reference equation.
	Mannino et al.20]	overall: 42.1	overall: 19.3	Follow-up : Mortality/ COPD-related hospitalization	3b	LLN may miss subjects likely to have complications

Subjects from general population

Perez-Padilla et al.19]	14.0	10.8	none	4	No preference stated.
Roche et al.29]	Sk: 12.53; NvSk: 6.62	Sk: 9.13; NvSk: 4.85	None	4	No preference stated. Need for homogenizing definition of airflow obstruction.
Shirtcliffe et al.17]	≥ 40: 14.2	≥ 40: 9.0	LLN, self-reported COPD	4	Discrepancies in diagnosis between LLN and fixed criterion exist. Longitudinal studies necessary to determine correct definition.
Swanney et al.30]	Sk: M 41.2-62.4; F 50.1-18.8 NvSk: M 9.8-26.3; F 6.9-33.1	Sk: M 32.2-42.9; F 25.4-31.3 NvSk: M 4.6-6.0; F 6.2-12.1	LLN	4	Use LLN instead of fixed criterion. Perform spirometry only in subjects with complaints and prior tobacco exposure to avoid over diagnosis.
Vaz Fragoso et al.21]	overall: 33.5	overall: 7.2	Follow-up : Mortality/ presence respiratory symptoms	3b	Concerns about using fixed criterion. No preference stated
Vollmer et al.18]	Sk: NA ; NvSk:16-27	Sk: NA ; NvSk 6-8	LLN	4	Use LLN because fixed criterion leads to misclassification

Clinically based studies

Five studies were clinically based, i.e. including only referred or hospitalized subjects, and all had a cross-sectional design.[13-14, 22-24] All five studies included subjects over a wide age range, but unfortunately prevalence rates according to either definition were not consistently divided by age groups. Only the study by Schermer et al. provided separate rates for subjects aged 51 to 60; 61 to 70 and ≥71 years: it was the only clinically based study to include post-bronchodilator spirometry values.14 Prevalence rates according to a fixed FEV_1/FVC of <0.70 were higher in these age groups than the prevalence rates according to the LLN, which ranged from 27.5% to 45% and from 20% to 25%, respectively. Discordant prevalence rates occurred in 4% up to 20% of the cases in this study.

Aggarwal et al. found an overall lower prevalence rate when applying FEV_1/FVC<0.70 instead of the LLN, 23.6% and 28.2% respectively.[22] This stands in contrast to the other studies, but it should be noted that they included younger subjects. The studies by Margolis et al., Dejsomritrutai et al. and Roberts et al. found overall higher prevalence rates when FEV_1/FVC <0.70 was used rather than the LLN (Table 4).[13, 23-24] Discordant results in these three studies occurred in 6%, 7.5% and 14.7% of the cases respectively. All five articles mentioned in their discussion the observation of growing prevalence differences between the two definitions based on increasing age, although exact numbers were not provided.

Population-based studies

Thirteen population-based studies were included.[15-21, 25-30] Six studies used the NHANES III study population.[21, 25-27, 30-31] Three studies were performed in an Asian population.[15-16, 28] The studies by Mannino et al., Roche et al., Vollmer et al. and Vaz Fragoso included only subjects ≥40 years.[18, 20-21, 29] Five studies performed post-bronchodilator spirometry.[16-19, 28] In all other population-based studies only pre-bronchodilator values were used. Prevalence rates were higher in all studies after applying a FEV_1/FVC <0.70 and the differences grew with increasing age (Table 4). Prevalence rates according to FEV_1/FVC increased more with age than did the prevalence rates according to LLN.

The study by Mannino et al. had a longitudinal design with mortality and COPD-related hospitalization as the primary outcomes.[20] 4,965 subjects (age >65 years) from the Cardiovascular Health Study (CHS) were included and were followed for nine years. The subjects were classified according to the FEV_1/FVC <0.70 or LLN. 1,134 subjects had an FEV1/FVC <0.70, but >LLN (in-between group). Subjects in the in-between group had a

higher adjusted mortality risk (HR 1.3, CI95% 1.1-1.5) and an increased COPD-related hospitalization (HR 2.6, CI95% 2.0-3.0) in comparison to subjects with normal lung function. The outcome suggests that using the FEV_1/FVC <0.70 identifies at-risk patients who would have been classified as normal according to LLN.

Vaz Fragoso et al. assessed data of 2,480 subjects, aged 65-80, who were followed for 12 years and compared the risks of all-cause mortality and on respiratory symptoms between subjects with a FEV_1/FVC <0.70 and <LLN (<5th percentile).[21] 831 subjects had an FEV_1/FVC <0.70, and 179 of these also had an FEV_1/FVC <LLN. The adjusted hazard ratio of all-cause mortality in <LLN subjects was 2.01$^{(95\%CI\ 1.60-\ 2.54)}$ and 1.24$^{(95\%CI\ 1.04-1.47)}$ for those who had <0.70 compared to those with normal lung function. The authors found no significant effects of either classification on the risk of respiratory symptoms being present. This outcome suggest that those with <0.70 have a lower risk of all-cause mortality compared to those with <LLN, yet the risk is still higher than those with normal lung function.

Comparing healthy never-smokers and current smokers

Vollmer et al., Hansen et al., Roche et al. and Swanney et al. also compared healthy never-smokers and found that COPD prevalence rates were higher when using a fixed value of FEV_1/FVC<0.70.[18, 26, 29, 30] These differences increased especially with increasing age. The study by Vollmer et al. used post-bronchodilator values, but unfortunately only numbers for the non-smokers and not for the current smokers were reported.[18]

Discussion

This evidence-based review presents an overview of studies comparing the FEV_1/FVC <LLN with FEV_1/FVC <0.70 in diagnosing spirometry-based COPD. The majority of studies reviewed had a cross-sectional design and all concluded that using the <0.70 approach resulted in a greater prevalence of COPD, which was often interpreted as 'overdiagnosing COPD'. As mentioned by Vollmer et al, using the LLN as a threshold would probably miss subjects with mild airflow obstruction, but would correctly diagnose subjects with more profound and advanced airflow obstruction.[18] Taking this into consideration we have to ponder whether we can afford to miss those subjects who have only mild airflow obstruction since treatment options already may be present at an early stage.

Based on Table 4 it can be concluded that 13 out of 18 researchers made the a priori

choice to adopt the LLN as their reference test. Unfortunately, this approach is flawed because the outcome of the comparison becomes predictable. Instead, the correct approach should be to select the diagnostic test that outperforms the other when both are tested against a gold standard. Unfortunately that golden standard test for COPD is lacking in all cases. The GOLD committee states that their cut-off points for COPD have not been clinically validated.

Ideally, the gold standard should be the perfect diagnostic test with a sensitivity and specificity of 100%. By adopting LLN as a reference or standard, one implicitly declares that this test is the gold standard and any that other test (e.g. the <0.70 approach) can deliver a maximal sensitivity / specificity of 100%, i.e. deliver equivalent results as the gold standard in terms of diagnosing or ruling out COPD. When the LLN is a "less than perfect" gold standard, and thus sometimes misdiagnoses or misses COPD as a result, one implicitly expects the new test to make the same mistakes in its attempt to deliver a maximal sensitivity / specificity of 100%. Even when the new test is "perfect" , a comparison with a "less than perfect" test will deliver a sensitivity / specificity <100% and thus be inclined to regard the new test as being inferior. This is known as an 'imperfect gold standard bias' and is an important concept to bear in mind when interpreting the outcomes of such studies.[32] Reversing the choices by adopting the FEV_1/FVC <0.70 as the reference would predictably lead to a sensitivity / specificity <100% of the LLN. The only valid conclusion one may draw when comparing diagnostic tests without a gold standard, is that they may produce different results. A conclusion regarding superiority or inferiority is not possible.

Some of the included studies did not report sensitivity and specificity values but provided agreement stated as kappa. Kappa is used to depict the extent of agreement between tests and its value ranges from 0 (no agreement) to 1 (perfect agreement). Again, in the absence of a gold standard, one should be cautious when interpreting kappa because in case of good agreement, one can only conclude that two tests are equivalent, not that either one is correct. As a consequence, due to the lack of a gold standard, the level of evidence according to the CEBM is 4.

A longitudinal study is probably the best option for obtaining a correct COPD diagnosis: time will tell whether or not a smoker's lung function declines. The decline in the lung function can be compared with the lung function of a never-smoker and the decline in lung function becomes the gold standard. Unfortunately, only two longitudinal studies were performed comparing the risks of hospitalization, the occurrence of respiratory symptoms and the mortality of subjects diagnosed with an FEV_1/FVC <0.70 or <LLN.[20-21]

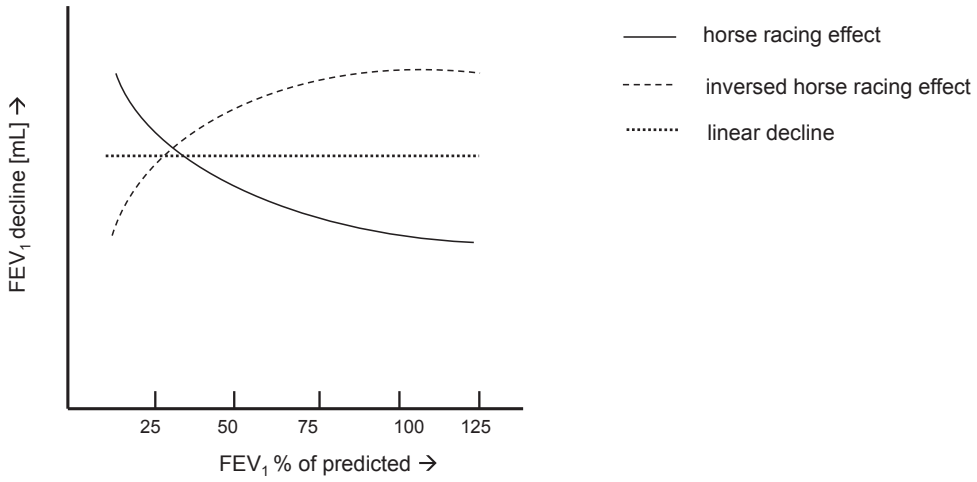
The study by Mannino et al. showed that subjects with an FEV_1/FVC <0.70 but above the LLN had increased hospitalization and mortality rates, with hazard ratios of 2.6 and 1.3, respectively. At the same time, the study by Vaz Fragoso showed that subjects with an FEV_1/FVC <LLN have higher risks of mortality compared to those with <70%, with hazard ratios of 2.0 and 1.2 respectively. Unfortunately the latter study did not examine the mortality risks of the in-between group.

The disadvantage of cross-sectional studies is that they miss subjects with an accelerated decline in lung function, which may still have >0.70 or >LLN: it is inevitable that both measures will fall short in being the perfect diagnostic tools in cross-sectional studies. The point in time at which either of the two boundaries will be crossed partly depends on the initial height of lung function: subjects with a high initial lung function require more time to be labeled as COPD-subjects even though their lung function can decline as rapidly as in others. They remain ' healthy smokers' for a longer time. When rapidly declining subjects with an FEV_1/FVC>0.70 are still labeled as 'non-diseased', a large number of COPD-subjects will be missed.

The pattern of lung function decline is therefore an issue in determining which of the two diagnostic tests is the least imperfect under cross-sectional conditions. In their well-known study Fletcher and Peto found that FEV_1-decline was initially small but became stronger as lung function decreased.[33] This phenomenon is referred to as the "horse-racing effect".[34] By contrast, in the placebo-arms of the UPLIFT and TORCH studies, two randomized controlled studies (RCT's), lung function decline was more pronounced initially and slowed down as lung function lowered.[35-36] Both patterns of decline are schematically depicted in Figure 2.

If the 'Fletcher and Peto' pattern is valid, subjects will hover above both thresholds for longer periods of time and in cross-sectional studies neither the FEV_1/FVC <0.70 or <LLN will be able to discriminate sufficiently between susceptible and non-susceptible smokers. On the other hand, when the 'UPLIFT and TORCH' pattern is valid, susceptible smokers will show rapid FEV_1/FVC declines initially and therefore cross the <LLN threshold in an early phase of their disease and the <70% threshold in a later phase. The risk of missing susceptible smokers in a cross-sectional study will thus be lower using the FEV_1/FVC <0.70 value, whereas for the FEV_1/FVC <LLN more time will pass, allowing greater lung function deterioration.

Figure 2: Patterns of lung function decline: FEV_1 decline against FEV_1 percentage of predicted. The horse racing effect: FEV_1 decline increases with decreasing FEV_1 percentage of predicted.



Another approach would be to distinguish those subjects with respiratory symptoms from those without. It has been shown that mild COPD subjects (GOLD stage I) without respiratory symptoms show no significantly faster FEV_1-decline compared to those with normal lung function, i.e. FEV_1/FVC >70%.[37] These results suggest that the presence of respiratory symptoms should be part of the definition of COPD. Our review focused on literature including older subjects, although one study conducted in younger subjects is worth mentioning. The study by De Marco et al. included subjects aged 20-44 years and compared FEV_1-decline and hospitalization rates according to definition of airflow obstruction (<70% or <LLN).[38] The outcome was that subjects with respiratory symptoms had more significant FEV_1-decline than subjects without respiratory complaints, regardless of which spirometric criteria were used.

An important and often cited argument for not using the FEV_1/FVC <0.70 threshold is the observation of high prevalence rates of COPD in elderly healthy never-smokers. This argument is correct in our view, but at the same time is valued too high. COPD screening in subjects without prior tobacco addiction is less sensible.[39] No physician will / may label subjects without a prior smoking history and respiratory complaints as COPD based only on the outcomes of an FEV_1/FVC value.

The availability of population-specific reference equations is essential for the utilization of the LLN worldwide. However, in many parts of the world, these population-specific (post-bronchodilator) reference equations are not (yet) established. Instead, reference equations based on, for instance, the US-population like the NHANESIII are used. This may result in biased outcomes when applying in non-US populations.

This present review has several limitations which need some attention. First, the majority of included studies only performed pre-bronchodilator spirometry. Differences in prevalence of airflow obstruction, as defined by FEV1/FVC <0.7 or <LLN, can be pre-/ post-bronchodilator spirometry study dependent. However, at the moment it is not possible to estimate to what degree due to the lack of data. The few studies that performed both pre- and post-bronchodilator measurements did not report the pre-bronchodilator data. Second, studies with inconclusive results may not have been published and thus missed in our search. Third two articles were not available in full text.[11-12] Fourth, an English-language bias may be present because only the English-language literature was searched. Seeing as these limitations are similar to those encountered by the studies included in this review, we believe that this will not affect the validity of our conclusions.

Conclusion

A major shortcoming of the cited literature is that FEV_1/FVC <0.70 and <LLN were not compared to a gold standard. However, defining such a standard is difficult. Nine out of the 18 included articles defined only the FEV_1/FVC <LLN as their reference test and they influenced the outcome in favor of the FEV_1/FVC <LLN. Therefore, based on the current available literature it cannot be determined whether it is preferable to use the LLN rather than to a fixed percentage of FEV_1/FVC. Nevertheless, the outcome of the only included longitudinal study in which subjects with an FEV1/FVC <70% but >LLN are compared to those with normal lung function suggest that LLN may miss subjects at risk. Further longitudinal research will be necessary to determine which criterion is better and more clinically relevant. Until then, neither of the two approaches can be claimed to be superior over the other.

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Reference List

1. Murray, C. J. and A. D. Lopez. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. *Lancet* 1997;349:1498-1504.
2. Rabe, K. F., S. Hurd, A. Anzueto, P. J. Barnes, S. A. Buist, P. Calverley, Y. Fukuchi, C. Jenkins, R. Rodriguez-Roisin, W. C. van, and J. Zielinski. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med* 2007;176:532-555.
3. Hogg, J. C. Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. *Lancet* 2004;364:709-721.
4. Pellegrino, R., V. Brusasco, G. Viegi, R. O. Crapo, F. Burgos, R. Casaburi, A. Coates, C. P. van der Grinten, P. Gustafsson, J. Hankinson, R. Jensen, D. C. Johnson, N. Macintyre, R. McKay, M. R. Miller, D. Navajas, O. F. Pedersen, and J. Wanger. Definition of COPD: based on evidence or opinion? *Eur Respir J* 2008;31:681-682.
5. Miller, M. R., O. F. Pedersen, R. Pellegrino, and V. Brusasco. Debating the definition of airflow obstruction: time to move on? *Eur Respir J* 2009;34:527-528.
6. Mannino, D. M. Defining chronic obstructive pulmonary disease... and the elephant in the room. *Eur.Respir J* 2007;30:189-190.
7. Pauwels, R. A., A. S. Buist, P. M. Calverley, C. R. Jenkins, and S. S. Hurd. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary. *Am J Respir Crit Care Med* 2004;163:1256-1276.
8. Celli, B. R. and W. MacNee. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. *Eur Respir J* 2004;23:932-946.
9. Janssens, J. P., J. C. Pache, and L. P. Nicod. Physiological changes in respiratory function associated with ageing. *Eur Respir J* 1999;13:197-205.
10. Oxford Centre for Evidence-based Medicine (CEBM). Levels of Evidence; http://www.cebm.net (accessed September 2009). 2009.
11. Ra, S. W., J. S. Oh, S. B. Hong, T. S. Shim, C. M. Lim, Y. S. Koh, S. D. Lee, W. S. Kim, D. S. Kim, W. D. Kim, and Y. M. Oh. Effect of the changing the lower limits of normal and the interpretative strategies for lung function tests. *Tuberc Respir Dis.* 2006;61:129-136.
12. Ruppel, G. L. Spirometric determination of chronic obstructive pulmonary disease. *J Organ Dysfunct.* 2007;3:221-223.
13. Margolis, M. L., F. J. Montoya, and W. R. Palma, Jr. Pulmonary function tests: comparison of 95th percentile-based and conventional criteria of normality. *South Med J* 1997;90:1187-1191.
14. Schermer, T. R. J., I. J. M. Smeele, B. P. A. Thoonen, A. E. M. Lucas, J. G. Grootens, T. J. Van Boxem, Y. F. Heijdrae, and C. Van Weel. Current clinical guideline definitions of airflow obstruction and COPD overdiagnosis in primary care. *Eur Respir J* 2008;32:945-952.
15. Hwang, Y. I., C. H. Kim, H. R. Kang, T. Shin, S. M. Park, S. H. Jang, Y. B. Park, C. H. Kim, D. G. Kim, M. G. Lee, I. G. Hyun, and K. S. Jung. Comparison of the prevalence of chronic obstructive pulmonary disease diagnosed by lower limit of normal and fixed ratio criteria. *J Korean Med Sci.* 2009;24:621-626.

16. Ko, F. W. S., J. Woo, W. Tam, C. K. W. Lai, J. Ngai, T. Kwok, and D. S. C. Hui. Prevalence and risk factors of airflow obstruction in an elderly Chinese population. *Eur Respir J* 2008;32:1472-1478.

17. Shirtcliffe, P., M. Weatherall, S. Marsh, J. Travers, A. Hansell, A. McNaughton, S. Aldington, H. Muellerova, and R. Beasley. COPD prevalence in a random population survey: a matter of definition. *Eur Respir J* 2007;30:232-239.

18. Vollmer, W. M., T. Gislason, P. Burney, P. L. Enright, A. Gulsvik, A. Kocabas, and A. S. Buist. Comparison of spirometry criteria for the diagnosis of COPD: results from the BOLD study. *Eur Respir J* 2009;34:588-597.

19. Perez-Padilla, R., P. C. Hallal, J. C. Vazquez-Garcia, A. Muino, M. Maquez, M. V. Lopez, M. M. de Oca, C. Talamo, G. Valdivia, J. Pertuze, J. Jardim, and A. M. Menezes. Impact of bronchodilator use on the prevalence of COPD in population-based samples. *COPD* 2007;4:113-120.

20. Mannino, D. M., A. S. Buist, and W. M. Vollmer. Chronic obstructive pulmonary disease in the older adult: What defines abnormal lung function? *Thorax* 2007;62:237-241.

21. Vaz Fragoso, C. A., J. Concato, G. McAvay, P. H. Van Ness, C. L. Rochester, H. K. Yaggi, and T. M. Gill. Chronic obstructive pulmonary disease in older persons: A comparison of two spirometric definitions. *Respir Med* 2010. doi:10.1016/j.rmed.2009.10.030

22. Aggarwal, A. N., D. Gupta, D. Behera, and S. K. Jindal. Comparison of fixed percentage method and lower confidence limits for defining limits of normality for interpretation of spirometry. *Respir Care* 2006;51:737-743.

23. Dejsomritrutai, W., P. Wongsurakiat, N. Chierakul, S. Charoenratanakul, A. Nana, and K. N. Maranetra. Comparison between specified percentage and fifth percentile criteria for spirometry interpretation in Thai patients. *Respirology* 2002;7:123-127.

24. Roberts, S. D., M. O. Farber, K. S. Knox, G. S. Phillips, N. Y. Bhatt, J. G. Mastronarde, and K. L. Wood. 2006. FEV1/FVC ratio of 70% misclassifies patients with obstruction at the extremes of age. *Chest* 130:200-206.

25. Celli, B. R., R. J. Halbert, S. Isonaka, and B. Schau. Population impact of different definitions of airway obstruction. *Eur.Respir J* 2003;22:268-273.

26. Hansen, J. E., X. G. Sun, and K. Wasserman. Spirometric criteria for airway obstruction: Use percentage of FEV1/FVC ratio below the fifth percentile, not < 70%. *Chest* 2007;131:349-355.

27. Hnizdo, E., H. W. Glindmeyer, E. L. Petsonk, P. Enright, and A. S. Buist. Case definitions for chronic obstructive pulmonary disease. *COPD* 2006;3:95-100.

28. Lau, A. C., M. S. Ip, C. K. Lai, K. L. Choo, K. S. Tang, L. Y. Yam, and M. Chan-Yeung. Variability of the prevalence of undiagnosed airflow obstruction in smokers using different diagnostic criteria. *Chest* 2008;133:42-48.

29. Roche, N., F. Dalmay, T. Perez, C. Kuntz, A. Vergnenegre, F. Neukirch, J. P. Giordanella, and G. Huchon. FEV1/FVC and FEV1 for the assessment of chronic airflow obstruction in prevalence studies: do prediction equations need revision? *Respir Med* 2008;102:1568-1574.

30. Swanney, M. P., G. Ruppel, P. L. Enright, O. F. Pedersen, R. O. Crapo, M. R. Miller, R. L. Jensen, E. Falaschetti, J. P. Schouten, J. L. Hankinson, J. Stocks, and P. H. Quanjer. Using the lower limit of normal for the FEV1/FVC ratio reduces the misclassification of airway obstruction. *Thorax* 2008;63:1046-1051.

31. Vaz Fragoso, C. A., J. Concato, G. McAvay, P. H. Van Ness, C. L. Rochester, H. K. Yaggi, and T. M. Gill. The ratio of FEV1 to FVC as a basis for establishing chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2010;181:446-451.

32. Valenstein, P. N. Evaluating diagnostic tests with imperfect standards. *Am.J Clin.Pathol.* 1990;93:252-258.

33. Fletcher, C. and R. Peto. The natural history of chronic airflow obstruction. *Br Med J* 1977;1:1645-1648.

34. Burrows, B., R. J. Knudson, A. E. Camilli, S. K. Lyle, and M. D. Lebowitz. The "horse-racing effect" and predicting decline in forced expiratory volume in one second from screening spirometry. *Am Rev Respir Dis* 1987;135:788-793.

35. Tashkin, D. P., B. Celli, S. Senn, D. Burkhart, S. Kesten, S. Menjoge, and M. Decramer. A 4-year trial of tiotropium in chronic obstructive pulmonary disease. *N Engl J Med* 2008;359:1543-1554.

36. Celli, B. R., N. E. Thomas, J. A. Anderson, G. T. Ferguson, C. R. Jenkins, P. W. Jones, J. Vestbo, K. Knobil, J. C. Yates, and P. M. Calverley. Effect of pharmacotherapy on rate of decline of lung function in chronic obstructive pulmonary disease: results from the TORCH study. *Am J Respir Crit Care Med* 2008;178:332-338.

37. Bridevaux, P. O., M. W. Gerbase, N. M. Probst-Hensch, C. Schindler, J. M. Gaspoz, and T. Rochat. Long-term decline in lung function, utilisation of care and quality of life in modified GOLD stage 1 COPD. *Thorax* 2008;63:768-774.

38. De Marco, R., S. Accordini, J. M. Anto, T. Gislason, J. Heinrich, C. Janson, D. Jarvis, N. Kunzli, B. Leynaert, A. Marcon, J. Sunyer, C. Svanes, M. Wjst, and P. Burney. Long-term outcomes in mild/moderate chronic obstructive pulmonary disease in the European community respiratory health survey. *Am J Respir Cri Care Med 2009;*180:956-963.

39. Qaseem, A., V. Snow, P. Shekelle, K. Sherif, T. J. Wilt, S. Weinberger, and D. K. Owens. Diagnosis and management of stable chronic obstructive pulmonary disease: a clinical practice guideline from the American College of Physicians. *Ann Inter Med* 2007;147:633-638


Chapter
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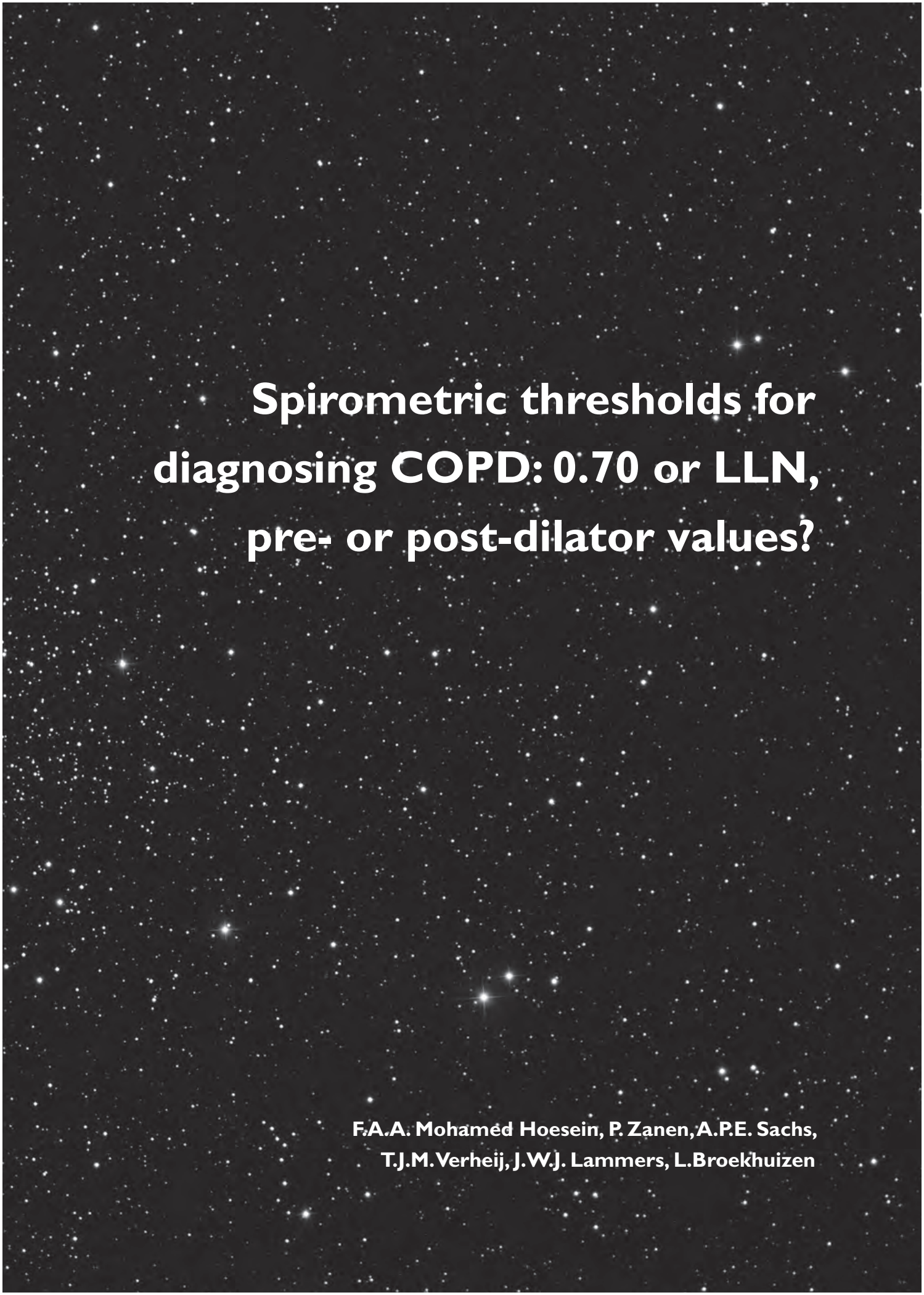

Spirometric thresholds for diagnosing COPD: 0.70 or LLN, pre- or post-dilator values?

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Abstract

Purpose In absence of a gold standard for chronic obstructive pulmonary disease (COPD) it remains difficult to compare the true diagnostic characteristics of the forced expiratory volume in 1 second to the forced vital capacity (FEV_1/FVC) <0.70 and < lower limit of normal (LLN). COPD is a clinical diagnosis, based on symptoms signs and lung function results combined, and an expert panel assessment would be an adequate reference standard.. We compared the diagnostic properties of FEV_1/FVC <LLN and <0.70 against this panel diagnosis

Methods 342 participants, aged >50, consulting for persistent cough, but without physician diagnosed COPD, were prospectively enrolled. All underwent extensive history taking, physical examination, spirometry and diffusion testing. An expert panel, including a board certified respiratory physician, assessed all diagnostic information to determine the presence or absence of COPD and served as reference standard.

Results 104 participants were diagnosed with COPD by the panel. The reproducibility of the panel diagnosis was high (kappa of 0.94). Sensitivity estimates of <0.70 were significantly higher than that of <LLN (0.73 and 0.47, respectively, $p<0.001$), specificity rates were comparable (0.95 and 0.99, respectively, $p<0.001$). There was no significant difference in diagnostic property when using pre- or post-bronchodilator FEV_1/FVC ($p=0.615$).

Conclusion In a symptomatic primary care population, the FEV_1/FVC <0.70 was more accurate to detect COPD.

Introduction

A diagnosis of COPD is usually confirmed by spirometric airflow obstruction defined as a lowered ratio of the forced expiratory volume in one second to the forced vital capacity (FEV_1/FVC ratio). A debate exists on the appropriate threshold value: the lower limit of normal (LLN) which represents the lowest 5th percentile according to age and gender, or the fixed 0.70 value. [1 2] The ATS and ERS guidelines advocate using the LLN [3] whereas the Global Initiative for Chronic Obstructive Lung Disease (GOLD) the 0.70 value. [4]

Many studies compared the two thresholds [5] but unfortunately, did not compare them with an independent gold standard for COPD, but rather selected one of the proposed thresholds as the reference test. The reference test unavoidably is superior in such analyses, rendering the outcome of these comparative studies predictable: nominating e.g. the <0.70 threshold as a reference leads unavoidably to an outcome of an inferior LLN and vice versa. [5]

A preferable design would be to compare both thresholds with an independent reference standard for COPD. [6] COPD is a clinical diagnosis and is based on symptoms, signs and lung function results combined. A panel diagnosis, taking into account other relevant clinical factors / parameters, is the best way forward. [7] To the best of our knowledge, no studies have been published comparing the LLN and <0.70 approaches against a panel diagnosis of COPD.

The objective of the current study was to compare the two mentioned thresholds of FEV_1/FVC against a panel diagnosis of COPD as the reference standard. Secondly, we investigated whether the diagnostic characteristics were influenced by using pre- and post-bronchodilator values.

Methods

Design and study population

A diagnostic study was performed between 2006 and 2009 in the Netherlands, the FRESCO study (From Respiratory Symptoms to COPD). The study protocol is described in detail elsewhere.[8] Inclusion criteria for patients were: an age over 50 years and consulting their general practitioner (GP) for cough lasting ≥14 days. Exclusion criteria were physician diagnosed COPD, suspected pneumonia, and terminal illness. All participants gave written informed consent and the ethics committee of the University Medical Center Utrecht approved the study.

Diagnostic work up

On the day a patient presented with cough, a standardized history was taken (including smoking habits and respiratory complaints) and a full physical examination was performed. The use of medication and comorbidities were registered from the GP's medical file.

Subsequently, all patients underwent further extensive diagnostic work-up, including full lung function testing (spirometry, body plethysmography and diffusing capacity of the lung for carbon monoxide by the single breath method) 90 days after the initial visit to their GP to ensure stable phase measurements. Lung function results were obtained before and after bronchodilation with 400 microgram of salbutamol, and expressed as percentage of predicted for age, gender and height. [9] Lower limits of normal (LLN) were calculated by using the reference equations from the European Coal and Steel Community (ECSC). [9] Finally, clinical follow up information, like new diagnoses or hospital admissions, was provided by the participants' GP.

Panel diagnosis of COPD

We used an outcome panel of two physicians, one GP with expertise in COPD and a board certified respiratory physician to decide by consensus and using (inter)national guidelines whether COPD or asthma was present or not. [10] [4] The decisions were determined during a meeting in which all available patient information was presented. All lung function test results were available to the panel.

For a diagnosis of COPD, recurrent complaints of cough, sputum or breathlessness were obligatory, as well as a post bronchodilator obstruction. A history of smoking was supportive but not obligatory for COPD. Obstruction was defined as a lowered FEV_1/FVC ratio and a concave dip in the second part of the curve. However, no strict threshold of FEV_1/FVC was applied for the presence of obstruction, rather this was assessed per individual. As an illustration, a 52 year old woman with recurrent complaints of cough and dyspnoea who had smoked 25 pack years, with a post bronchodilator FEV_1/FVC ratio of 0.71 and an FEV_1 of 80 % of predicted, was diagnosed with COPD because both a lowered diffusion capacity (transfer coefficient for carbon monoxide (Kco) = 70 % of predicted) and an increased residual volume (160% of predicted) supporting this diagnosis. When no consensus was reached, a third physician (BDLB) could be consulted.

Reproducibility of the expert panel

The reproducibility of the panel was estimated by repeating the consensus diagnosis

procedure after more than a year, of a random sample of 41 patients, without information on the original diagnosis, resulting in Cohen's kappa of 0.94 (good). The panel included an independent respiratory physician not involved in the initial panel diagnosis.

Statistical analysis

Mean and standard deviation (SD) were calculated for normally distributed continuous variables and proportions or frequencies for categorical variables.

Differences between groups were evaluated using (un)paired T-tests or analysis of variance where appropriate; for categorical variables the ^{2} or the McNemar test was used (the latter for within-subject comparisons). 95% confidence intervals (CI95%) were calculated where appropriate. [11]

The post-dilator FEV_1/FVC was dichotomized based on a] the GOLD 0.70 threshold and b] the appropriate European Coal and Steel Community (ECSC) lower limit of normal (LLN). [9] Using the panel diagnosis of COPD as a reference, the sensitivity and specificity for these categorical variables were estimated. The differences between 'LLN' and 'GOLD' COPD sensitivity and specificity were evaluated using the McNemar test for paired proportions. [12] CI95% of the differences was calculated. [11] Diagnostic accuracy was defined by the sum of the number of true positives and true negatives divided by the total number of subjects.

Again using the panel diagnosis of absence / presence of COPD as reference, the area under the receiver operating characteristic curve (ROC area) of the continuous pre/post-dilator FEV1/FVC values was estimated. Differences with CI95% between the two resulting ROC areas were evaluated with the method of Metz, [13] which adjusts for the fact that variables are strongly correlated. CI95% of the differences was calculated.

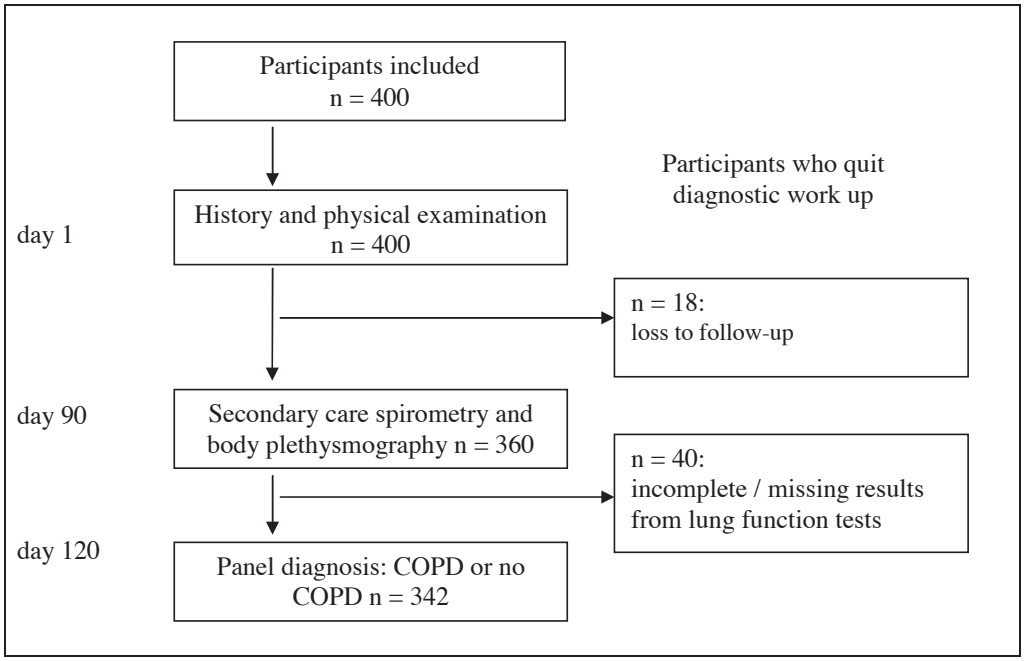
P-values <0.05 were considered statistically significant. All statistical analyses were performed using SPSS 18 for Windows (SPSS, Chicago, Illinois, USA).

Results

Baseline demographics

Of the 400 participants initially included, 58 were excluded (40 due to missing / incomplete results from lung function tests and 18 based on other reasons (death, poor health, moving, etc.), see Figure 1. Of the remaining 342 participants, 53.5% were females and the mean (SD) age was 63.0 (8.3) years. Detailed demographics and spirometry, stratified by COPD-status is provided in Table 1.

Figure 1: Flowchart of inclusion criteria and number of participants included.



Diagnosis of COPD: FEV_1/FVC <0.70 versus <LLN

COPD was present in 104 participants (30.5%). Those diagnosed with COPD were more often male of higher age or current smoker (Table 1).

Table 1: Participants demographics and results from the pulmonary function tests stratified the panel diagnosis. * median (interquartile range) BMI = body mass index; TLC = total lung capacity; RV = residual volume; DLco = transfer factor for carbon monoxide; Kco = transfer coefficient for carbon monoxide. MRC = Medical Research Counsil

	Total (N=342)	No COPD (N=238)	COPD (N=104)	p-value for difference
Age [years]	63.0 (8.3)	61.9 (7.5)	65.5 (9.5)	<0.001
Females (%)	53.50%	143 (60.1%)	40 (38.5%)	<0.001
BMI [kg*m-2]	28.3 (5.4)	28.8 (5.5)	27.1 (5.0)	0.008
Height [meters]	1.70 (0.09)	1.70 (0.09)	1.71 (0.09)	0.13
Packyears	23.2 (5.4)	12.2 (22.7)	26.2 (21.5)	<0.001
Never smokers (%)	94 (26.9)	86 (36.1)	8 (7.7)	<0.001
Current smokers(%)	72 (21.1)	29 (40.3)	43 (59.7)	0.022
Ex-smokers (%)	176 (50.6)	123 (69.9)	53 (30.1)	<0.003
FEV_1 [L]	2.65 (0.78)	2.85 (0.77)	2.20 (0.61)	<0.001
FEV_1 [L] post-bronchodilator	2.76 (0.78)	2.94 (0.78)	2.35 (0.62)	<0.001
FEV_1 reversibility [%] *	3.3 (0.7 – 6.7)	3.1 (0.3 – 6.1)	4.8 (2.1 – 8.5)	0.001
FEV_1% predicted	96.5 (19.7)	104.7 (15.5)	79.2 (16.0)	<0.001
FEV_1% predicted post-bronchodilator	100.4 (18.9)	107.4 (15.5)	84.5 (16.1)	<0.001
FEV_1/FVC %	72.7 (9.9)	77.3 (6.1)	62.2 (9.0)	<0.001
FEV_1/FVC % post-bronchodilator	74.8 (10.1)	79.5 (6.0)	64.0 (9.4)	<0.001
TLC [L]	6.41 (1.35)	6.16 (1.27)	6.94 (1.37)	<0.001
RV [L]	2.53 (0.71)	2.29 (0.54)	3.02 (0.79)	<0.001
RV/TLC %	39.6 (7.8)	37.7 (7.1)	43.6 (7.6)	<0.001
DLco [mmol/min/kPa/l]	7.36 (2.00)	7.69 (1.94)	6.63 (1.92)	<0.001
Kco [mmol/min/kPa/l]	1.34 (0.26)	1.41 (0.23)	1.17 (0.25)	<0.001
Kco % predicted	94.9 (18.8)	98.4 (17.1)	87.1 (20.0)	<0.001
MRC dyspnoea score	3.0 (1.5)	3.0 (1.5)	3.1 (1.6)	0.599
Complaints of phlegm [%]	76.1	72.6	84.0	<0.001
Complaints of wheeze [%]	49.1	41.2	63.1	<0.001

The sensitivity of FEV_1/FVC <0.70 for diagnosing COPD was significantly higher than that of FEV_1/FVC <LLN 0.73 and 0.47, respectively (p<0.001; CI95% 0.18 – 0.34). The specificity of FEV_1/FVC <LLN was slightly, but significantly higher compared to FEV_1/FVC <0.70: 0.99 and 0.95, respectively (p<0.001; CI95% 0.01 – 0.06). The positive and negative predictive values of <0.70 and <LLN are provided in Table 2.

Table 2: Positive and negative predictive values of FEV_1/FVC <0.70 and <LLN for COPD, (post-bronchodilator values).

	FEV_1/FVC <0.70	FEV_1/FVC <LLN
Positive predictive value	86.2 %	94.1 %
Negative predictive value	89.0 %	81.0 %

A larger number of participants had COPD according to FEV_1/FVC <0.70 compared to <LLN, 88 (25.7%) and 52 (15.2%), respectively. The LLN-approach 'missed' 55 participants with COPD, the GOLD approach 28, when compared to the expert panel diagnosis. The false-positives and false-negative rates according to either the LLN and <0.70 are presented in Table 3. The diagnostic accuracy of the <0.70 was higher than that of <LLN, 91.2% and 83.0%, respectively.

Table 3: Number (percentage) of correct and wrong diagnoses of COPD according to FEV_1/FVC <0.70 and <LLN (post-bronchodilator values).

	FEV_1/FVC <0.70	**FEV_1/FVC <LLN**
True-positives (%)	76 (22.2)	49 (14.3)
False-positives (%)	12 (3.5)	3 (0.9)
True-negatives (%)	226 (66.1)	235 (68.7)
False-negatives (%)	28 (8.2)	55 (16.1)

Diffusion tests and bodyplethysmography

The Kco% predicted in participants with FEV_1/FVC <0.70 was significantly lower than those with FEV_1/FVC >0.70, 87.6% (19.5) and 97.6% (17.8), p<0.001, respectively. Comparable results were found using the LLN as threshold, 86.4% (20.4) and 96.4% (18.1), p=0.005, respectively.

The RV/TLC% ratio, which represents the degree of air trapping, was significantly higher in participants with FEV_1/FVC <0.70 than those with FEV_1/FVC >0.70, 43.2% (7.2) and 38.2% (8.0), p<0.001, respectively. Similar results were seen in participants with FEV_1/FVC <LLN and with >LLN, 44.9% (7.4) and 38.6% (7.4), p<0.001, respectively.

Pre- versus post-bronchodilator values

The difference between the ROC area of the pre-and post-bronchodilator FEV_1/FVC values was not significant, 0.929 and 0.933, respectively (δ=0.004, p=0.615; CI95% -0.02 – 0.01) (Figure 2).

Figure 2: Receiver operating curve (ROC) for pre- and post-bronchodilator FEV_1/FVC values in diagnosing COPD.

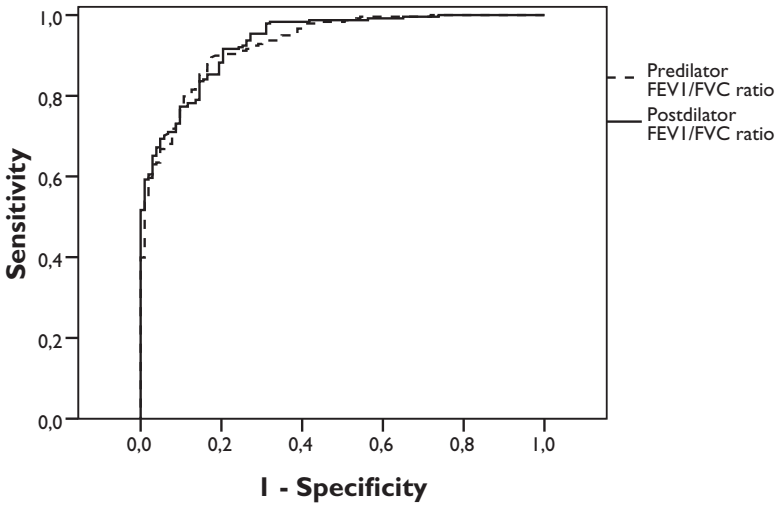


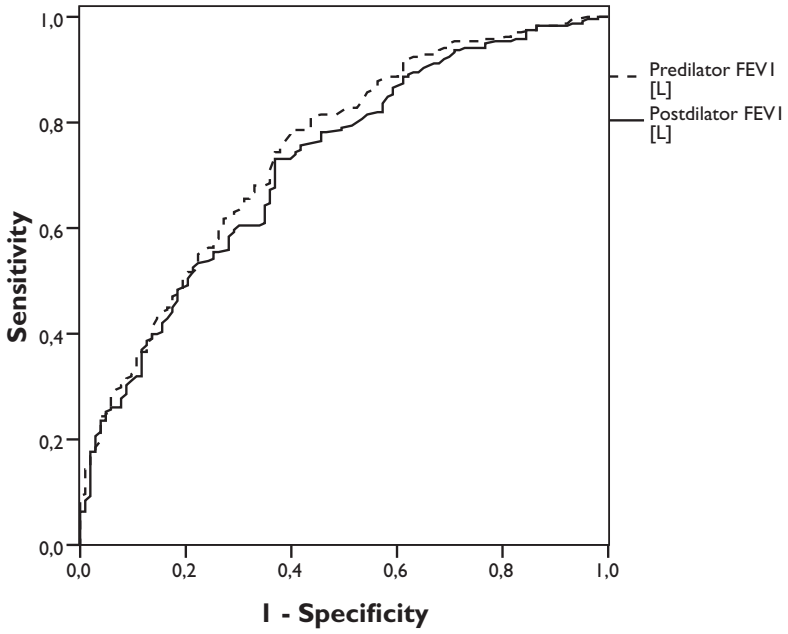
Table 4: Number of participants changing from below the thresholds to above the thresholds or vice versa after the administration of a bronchodilator according to FEV_1/FVC <0.70 and <LLN.

		Post-bronchodilator	
		FEV_1/FVC >0.70	FEV_1/FVC <0.70
Pre-bronchodilator	FEV_1/FVC >0.70	274	**5**
	FEV_1/FVC <0.70	**17**	46

		Post-bronchodilator	
		FEV_1/FVC >LLN	FEV_1/FVC <LLN
Pre-bronchodilator	FEV_1/FVC >LLN	218	**5**
	FEV_1/FVC <LLN	**37**	82

For the FEV_1/FVC <0.70 12.3% of the participants had a change in their COPD status, while this was 6.5% for the FEV_1/FVC <LLN (Table 4). Also for the FEV_1 as percent of predicted, there were no significant differences (δ=0.013; p 0.336; CI95% -0.026 – 0.009) in the ROC area between pre- and post-bronchodilator values, 0.862 and 0.849, respectively (Figure 3).

Figure 3: Receiver operating curve (ROC) for pre- and post-bronchodilator FEV_1 [L] values in diagnosing COPD.



Participants with COPD had significantly greater degree of reversibility, i.e. increase in FEV_1 as percentage of predicted according to age gender and height (Table 1). The discrimination (ROC area) of this reversibility for diagnosing COPD was poor (0.622).

Discussion

Main results

In the current study we compared FEV_1/FVC <0.70 with <LLN for diagnosing COPD, using a panel diagnosis of COPD as reference, in subjects presenting with persistent cough. We showed that the FEV_1/FVC <0.70 sensitivity was higher compared to the LLN, while specificity estimates were comparable. These results demonstrate that the LLN will miss a larger proportion of subjects with COPD. Secondly, we showed that using pre- or post-bronchodilator values for the FEV_1/FVC or FEV_1 did not increase discrimination between presence or absence of COPD.

Comparing with other studies

A large number of studies showed that COPD prevalence rates were higher when applying the FEV_1/FVC <0.70 compared to <LLN and interpreted this as "overdiagnosis"

of COPD resulting from using a fixed value of <0.70. Our results show the same pattern, however, we stress that the results of any head to head comparison of two thresholds or tests is predictable and without a proper reference test, or gold standard, it is impossible to label either of them as superior. Furthermore, most previous studies used open populations including asymptomatic subjects in which the risk of overdiagnosis is greater than in our study population. [5]

To our knowledge no previous studies reported the comparison of FEV_1/FVC <0.70 and <LLN to a panel diagnosis of COPD. The arguments in the past that the using <0.70 as threshold leads to 'overdiagnosis' is not substantiated by our data: the specificity of the two thresholds do not differ much and the negative predictive value of the <0.70 was higher than of the <LLN. In addition, the results from the diffusion tests, reflecting the functional capacity of the lungs, did not substantially differ between participants with an FEV_1/FVC <0.70 and <LLN

One previous study compared both thresholds against prognostic outcomes and found that participants labelled healthy according to the <LLN approach, but diseased according to the <0.70 had a higher mortality risk and suffered from more COPD-related hospital admissions compared to subjects with a normal lung function. [14] Another, cross-sectional, study reported a worse quality of life in subjects with comparable lung function results (<0.70 but >LLN). [15] These studies suggest that the LLN may miss affected subjects.

Implications for practice

When choosing between two tests, the ideal one shows the highest specificity and sensitivity. The FEV_1/FVC <0.70 showed a higher sensitivity and the LLN labels fewer subjects with COPD. Whether we can 'afford' to miss these subjects is debatable: there is evidence that the lung function declines faster in the early stage of the disease. [16] To prevent loss of lung function as much as possible, requires to use the threshold with the greatest sensitivity, which in our results was the FEV_1/FVC <0.70.[17] The resulting therapeutic treatment, i.e. stop smoking interventions, are widely accepted, cost-effective and available.

In addition, even the more sensitive <0.70 threshold missed a considerable number of subjects with COPD. This illustrates that COPD is a clinical diagnosis in which physicians consider many more characteristics than only the FEV_1/FVC ratio. Subjects with still normal FEV_1/FVC ratios, either according to 0.70 or LLN, may still be labelled as having COPD for instance based on abnormal diffusion tests and / or bodyplethysmography results.

Our results suggest that there is no need to obtain also post-bronchodilator values for the diagnosis of COPD in a symptomatic population with persistent cough. A number of participants was reclassified from below to above the thresholds or vice versa, but this did not result in significant changes in the diagnostic characteristics of pre- and post-bronchodilator FEV_1/FVC values. This is in contrast with the recommendation of the GOLD committee to use post-bronchodilator values of FEV_1/FVC. Our finding is of interest because only performing pre-bronchodilator spirometry will shorten the time needed to perform spirometry. Especially in a primary care setting this could enhance the use of spirometry and early detection of COPD. However, it is uncertain whether these results are generalisable to other populations than our study patients.

The poor discrimination (ROC area) of the of the FEV_1-reversibility as sole diagnostic in diagnosing COPD in our study underscores the recent amendment by the GOLD committee that the degree of reversibility is not longer a prerequisite in the diagnosis of COPD.

Strong and weak points of the study

One of the strengths of this study is the inclusion of a group of participants in whom the diagnostic dilemma is most urgent: middle aged and elderly subjects without physician diagnosed COPD who seek medical help because of persistent respiratory complaints (cough). Other studies mainly analysed open population subjects, including also never-smokers, in whom the need for diagnosing or exclusion of COPD is less clear, pre-test probability of COPD is low and risk of overdiagnosis is larger.[5] However, our results may not be applicable to the general population or to asymptomatic subjects as prevalence of COPD and symptoms and signs probably differ from our study population.

A second strength is that we validated the panel diagnosis of COPD by repeating the complete diagnostic process in a random sample of subjects. The validity of the 'reference standard' used in the current study, i.e. the panel diagnosis, is of key importance when interpreting the outcomes of this study. The panel diagnosis provided the 'best' available diagnosis of COPD, which was a combined assessment of all symptoms, signs, spirometry tests outcomes (including the shape of the flow-volume curve) and diffusion tests outcomes. The reproducibility was high (kappa=0.94). Moreover, the reproducibility of the panel diagnosis was tested by an independent physician not being involved in the first panel diagnosis (inter-observer agreement).

We acknowledge that the panel diagnosis still may not be the 'perfect' diagnosis. This possible 'imperfect standard bias' however probably did not bias the ranking of the

thresholds in terms of sensitivity / specificity. Because FEV_1/FVC <0.70 and <LLN were both compared against this panel diagnosis both suffered to the same extent from its possible diagnostic inaccuracy. In other words, although the panel diagnosis can not be 100% correct, the ranking of the diagnostic capability of FEV_1/FVC <0.70 and <LLN remains valid. [12]

Conclusions

In our study population the fixed criterion showed better sensitivity rates when compared to the LLN, while specificity rates were comparable. Secondly, there were no differences in diagnostic accuracy between pre- and post-bronchodilator values.

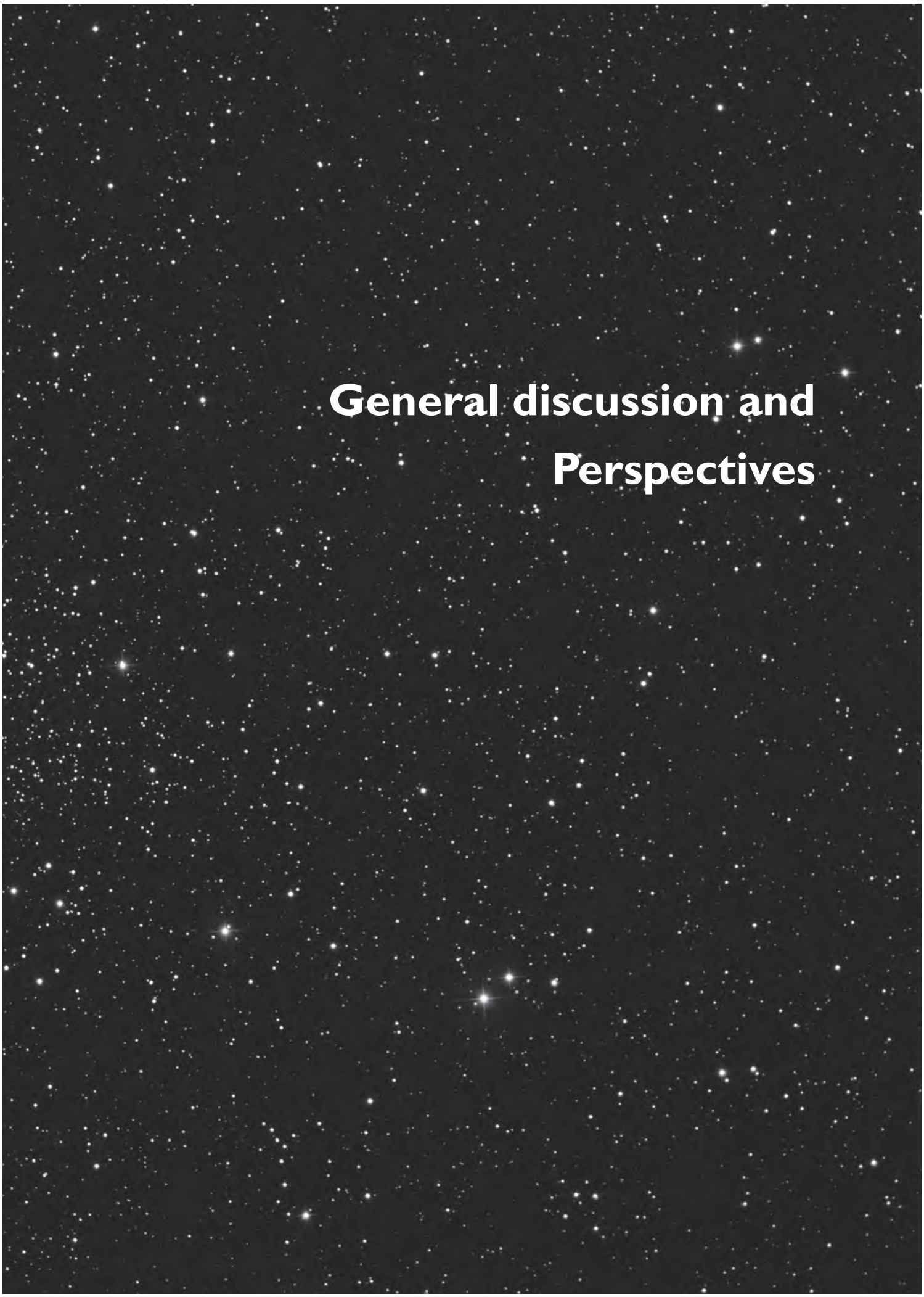
Reference List

1. Enright, P. and V. Brusasco. 2010. Counterpoint: should we abandon FEV/FVC < 0.70 to detect airway obstruction? Yes. *Chest* 138:1040-1042.
2. Celli, B. R. and R. J. Halbert. 2010. Point: should we abandon FEV/FVC <0.70 to detect airway obstruction? No. *Chest* 138:1037-1040.
3. Brusasco,V., R. Crapo, and G.Viegi. 2005. Coming together: the ATS/ERS consensus on clinical pulmonary function testing. *Eur.Respir.J.* 26:1-2.
4. Rabe, K. F., S. Hurd, A. Anzueto, P. J. Barnes, S. A. Buist, P. Calverley, Y. Fukuchi, C. Jenkins, R. Rodriguez-Roisin, W. C. van, and J. Zielinski. 2007. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am.J Respir Crit Care Med* 176:532-555.
5. Mohamed Hoesein, F. A., P. Zanen, and J. W. Lammers. 2011. Lower limit of normal or FEV(1)/FVC <0.70 in diagnosing COPD: An evidence-based review. *Respir.Med.*
6. Reitsma, J. B., A. W. Rutjes, K. S. Khan, A. Coomarasamy, and P. M. Bossuyt. 2009. A review of solutions for diagnostic accuracy studies with an imperfect or missing reference standard. *J.Clin.Epidemiol.* 62:797-806.
7. Weller, S. C. and N. C. Mann. 1997. Assessing rater performance without a "gold standard" using consensus theory. *Med.Decis.Making* 17:71-79.
8. Broekhuizen, B. D., A. P. Sachs, A. W. Hoes, K. G. Moons, J. W. van den Berg, W. H. Dalinghaus, E. Lammers, and T. J. Verheij. 2010. Undetected chronic obstructive pulmonary disease and asthma in people over 50 years with persistent cough. *Br.J Gen Pract.* 60:489-494.
9. Quanjer, P. H., G. J. Tammeling, J. E. Cotes, O. F. Pedersen, R. Peslin, and J. C. Yernault. 1993. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur.Respir.J.Suppl* 16:5-40.
10. Levy, M. L., P. H. Quanjer, R. Booker, B. G. Cooper, S. Holmes, and I. Small. 2009. Diagnostic spirometry in primary care: Proposed standards for general practice compliant with American Thoracic Society and European Respiratory Society recommendations. *Prim.Care Respir J* 18:130-147.
11. 2000. In Douglas G Altman, David Machin, Trevor N Bryant, and and Martin J Gardner, editors Statistics with confidence,2nd ed. BMJ Books, London.
12. 2002. In Xiao-Hua Zhou, Nancy A.Obuchowski, and Donna K.McClish, editors Statistical Methods in Diagnostic Medicine,first ed. John Wiley & Sons Inc, New York.
13. Metz, C. E., B. A. Herman, and C. A. Roe. 1998. Statistical comparison of two ROC-curve estimates obtained from partially-paired datasets. *Med.Decis.Making* 18:110-121.
14. Mannino, D. M., B. A. Sonia, and W. M. Vollmer. 2007. Chronic obstructive pulmonary disease in the older adult: what defines abnormal lung function? *Thorax* 62:237-241.
15. Garcia-Rio, F., J. B. Soriano, M. Miravitlles, L. Munoz, E. Duran-Tauleria, G. Sanchez, V. Sobradillo, and J. Ancochea. 2010. Subjects "over-diagnosed" as COPD by the 0.7 fixed ratio have a poor health-related quality of life. *Chest.*

16. Decramer, M. and C. B. Cooper. 2010. Treatment of COPD: the sooner the better? *Thorax* 65:837-841.

17. van Schayck, C. P. and N. H. Chavannes. 2003. Detection of asthma and chronic obstructive pulmonary disease in primary care. *Eur.Respir.J.Suppl* 39:16s-22s.


Chapter
10



General discussion and Perspectives

Introduction and outline

Chronic obstructive pulmonary disease (COPD) is one of the main causes of mortality worldwide and estimated to be the third leading cause of death by 2020. COPD is the only chronic disease with increasing mortality rates. Cigarette smoking is the major risk factor for COPD. Identifying subjects at high risk for COPD is of utmost important to prevent COPD or its progression. Especially since COPD is considered as an incurable disease prevention is of paramount importance to stop this (future) burden.

In **part 1** determinants of lung function decline and progression of emphysema were examined in participants of a lung cancer screening trial. The inclusion criteria led to a cohort of relatively healthy, but heavy, smokers. The majority of included subjects (still) had normal lung function values, i.e. no airflow obstruction. Otherwise, due to their high smoking exposure, of on average 40 packyears, the group is at a high risk to 1) develop COPD, 2) show a rapid decrease in lung function, 3) develop emphysema and 4) show a rapid progression of the latter. In **part 2** of this thesis it was investigated which threshold of FEV_1/FVC is most appropriate for diagnosing airflow obstruction.

In this chapter the major findings, methodological considerations and implications for clinical practice will be discussed. Finally, the conclusions of this thesis and future perspectives will be presented.

Discussion

Part 1: Lung function decline and emphysema progression

Chapters **3-7** in this thesis described studies examining the association of some clinical (chapters **2, 5, 6**), radiological (chapters **3** and **4**) and genetic determinants (chapter **7**) with lung function decline and progression of emphysema in heavy smokers.

A strong point of the studies is that the longitudinal design and therefore one could examine associations with progression of disease. COPD is a heterogeneous and complex disease with components like chronic bronchitis and emphysema. Although it is currently mostly diagnosed when airflow obstruction is present, i.e. the FEV_1/FVC is below a predefined threshold, signs of the disease are probably already present at an earlier time point. COPD is considered as a progressive disease which can be expressed as a stronger lung function decline than as in healthy individuals. Fletcher and Peto were the first to describe a progressive lung function decline in COPD patients and also found that smokers had a more progressive decline than non-smokers. Progression of disease in COPD is traditionally expressed as lung function decline. Structural changes

in lung parenchyma, like emphysema, are currently easily measured by lung densitometry on CT scans. Because the progressive nature of COPD is fundamental to the disease, it is of interest to asses these phenomena as markers for early detection of COPD in heavy smokers.

Clinical determinants

An important and intriguing finding of chapter **2** is that the decline in lung function was the steepest in participants with higher lung function values. This is in contrast with the classical findings by Fletcher and Peto who described that lung function decline increased with decreasing lung function, the so-called horse-racing effect. However, the outcomes from the TORCH and UPLIFT trials, including large numbers of moderate COPD subjects, showed that lung function decline was steepest in participants with higher lung function values. Our findings thus expand these finding to subjects with normal lung function and mild COPD (stage I). Regarding emphysema progression we found that it was lowest in the group with high lung function values and strongest in participants with lower lung function values.

One could argue that the occurrence of regression towards the mean might have influenced our results. This is, however, unlikely because the correlation between the baseline and follow-up lung function tests was high. In case of high correlations between the baseline and follow-up measurement the possibility of occurrence of regression towards the mean is low. Secondly, we used the preferred analysis technique, analysis of covariance (ANCOVA), to analyze the lung function decline and inserted the baseline value as covariate. This is the recommended method for minimizing the occurrence of regression towards the mean.

Our findings may have important implications because they emphasize that in a very early stage, when smoking subjects still have normal lung values, lung function decline is the largest. Intervention programs possibly will have the highest yield when subjects still have a normal lung function because they may prevent disease progression. Especially since COPD is regarded as an irreversible disease this may be of importance.

The only intervention known to halt lung function decline in COPD is smoking cessation. Several studies have investigated this and found that smoking cessation normalized lung function decline over time to that of never-smokers. However, no studies investigated the effect of smoking cessation on emphysema progression. In chapter **5** we examined the effects of duration of smoking cessation on lung function decline and progression

of emphysema in heavy smokers with and without airflow obstruction. We showed that smoking cessation for >4 years stabilized lung function decline and emphysema progression in both obstructed as non-obstructed participants. Our results show that smoking cessation is beneficial even in participants with a high smoking exposure. Although the effect on emphysema progression was significant, it was small. Unfortunately, there is limited knowledge on the natural course of emphysema progression making it difficult to compare the effect we found. Nonetheless, it may be concluded that smoking cessation has differential effects on lung function and emphysema otherwise. The results from this study may be used to convince smokers to cease smoking even before airflow obstruction is present.

The Kco reflects the properties of the alveolar-capillary membrane which is damaged in the presence of emphysema and as such it is a marker for disease in COPD. In chapter **6** we showed that the diffusion capacity of the lungs for carbon monoxide (Kco) was associated with lung function decline and progression of CT-quantified emphysema. These associations were independent of the baseline level of FEV_1/FVC indicating that there is already evidence of structural changes before lung function becomes abnormal. Using CT-scans for assessing emphysema progression has several disadvantages like radiation exposure and higher costs. Diffusion testing on the other hand is safe, has no contra-indications and is less expensive than CT-scanning. In current practice the Kco is not determined routinely; however the results of our study show that the Kco may act as an independent predictor of progression of disease.

Radiological determinants

Emphysema is frequently found as a secondary finding on CT-scans of heavy smokers participating in a lung cancer screening trial, but is also detected on CT-scans in smokers when made for other indications. Although several studies found an association between CT-quantified emphysema and lung function parameters, [1] [2;3] it remained unclear what the (clinical) consequences of this CT-quantified emphysema are. Especially in relatively healthy smokers the clinical value of emphysema was uncertain. To our knowledge, no studies examined the association of CT-quantified emphysema and lung function in a longitudinal study. In chapter **3** we reported that a higher extent of CT-quantified emphysema, either expressed as Perc15 or <-950 HU, was associated with a stronger decline in lung function and with an increased risk of the development of COPD during a 3-year follow-up. Our results show that CT-quantified emphysema may be used to select subjects at risk to develop COPD and/or with a more rapid lung function decline.

In addition we showed in chapter **4** that, besides the overall extent of CT-quantified emphysema, the distribution pattern also was associated with lung function decline. Participants with upper lobe predominant emphysema had a lower FEV_1/FVC after follow-up than those with lower lobe predominant emphysema, independent of the total extent of emphysema. A strong point of this study was that the distribution pattern was based on the anatomical lung lobes enabling a more precise characterization. Most former studies used merely lung volumes derived from CT to qualify the emphysema distribution. [4 5;6]

From chapters **3** and **4** it may be concluded that measuring emphysema on CT can be used for stratification of subjects at higher risk of lung function decline or COPD. Significant structural changes of lung parenchyma thus already occur before airflow obstructions is present. Soft-ware packages are widely available and automatic emphysema measurements may be completed in minutes. However, our results may only apply to heavy smokers and it should be formally investigated whether emphysema measurements at CT are also applicable in subjects with lower smoking exposures. In addition, it should be noted that standardization of emphysema measurements across different CT-scanners may be problematic. It is known that differences exist between vendors and therefore comparison of emphysema measurements of subjects scanned on different CT-scanners may not be possible without a correction factor. This may limit implementation in daily clinical practice.

Genetic determinants

In chapter **7** we have shown that polymorphisms of the *nAChR* gene were associated with lung function decline. Smoking participants with AA genotypes of rs1051730 and participants with GG genotypes of rs8034191 showed a significantly larger FEV_1/FVC decline than former smokers with the same genotypes.

Several studies showed a cross-sectional association between lung function and *nAChR* polymorphisms, but so far, we are the first, to our knowledge, to show this association with lung function decline. The previous reported cross-sectional associations imply that *nAChR* polymorphisms are associated with lower lung function already early in life. Our findings are important because they show that *nAChR* polymorphism are associated with lung function decline. COPD is associated with an accelerated lung function decline and by showing that *nAChR* polymorphisms are also associated with lung function decline the importance of these polymorphisms is emphasized.

It has been hypothesized that the effects of *nAChR* polymorphisms are mediated through

an association with nicotine dependency and smoking habits. However, we failed to find this association and showed that the effects on lung function decline were independent of nicotine dependency and smoking habits.

The association was only present in smokers and not in former smokers with a comparable smoking exposure, which may point out that the *nAChR* needs to be triggered (continuously) by nicotine to elucidate its effects. However, one should be cautious with implying causal effects because strictly given we only have found a significant association. Biological studies need to be performed to answer this question.

Part 2: Spirometric diagnosis of COPD: time to move on?

A heated debate is held on the correct threshold of FEV_1/FVC when diagnosing COPD; the fixed value of 70% or the lower limit of normal (LLN). The LLN is the lowest 5th percentile lung function of a healthy reference population. Proponents and opponents of the 70% seem to be in an impasse and no consensus is reached. One of the reasons to choose for a fixed ratio of FEV_1/FVC is for the sake of generalization and simplicity. The LLN requires population specific reference equations and these may not be present for all populations. In addition, individual's LLN may differ according to the reference equation applied. A fixed ratio is easy to remember for physicians worldwide. Otherwise, because with increasing age the FEV_1 decreases more than the FVC, the FEV_1/FVC maybe lowered leading to a FEV_1/FVC < 70%. The LLN of normal takes this aging process in account.

In chapter **8** we described a literature review of studies comparing diagnostic criteria of spirometric COPD: FEV_1/FVC below the lower limit of normal (LLN) or below a fixed percentage of 70%. Several studies retrieved attempted to find out which cut off value is superior. The majority of studies had a cross-sectional design and compared the prevalence rates according to either the LLN or the fixed criterion. Prevalence rates according to the fixed criterion were higher than those according to the LLN. Therefore, in subjects aged>45 years, it was generally concluded that the fixed criterion over diagnosed COPD. The major flaw of the cross sectional studies is that they compared the fixed criterion with the LLN, by taking the LLN as gold standard. By definition, no test can perform better than the test, i.e. gold standard, against it is compared, the outcome of such studies is highly predictable. The gold standard always 'wins' and the alternative test always turn out to be inferior. Another approach was taken by Mannino et al; they grouped subjects by their baseline FEV_1/FVC ratio: 1) FEV_1/FVC >70% and >LLN; 2) FEV_1/FVC <70%, but >LLN; and 3) FEV_1/FVC <70% and <LLN. Group 2 subjects had

significantly higher risks on mortality and respiratory-related hospitalizations compared to group 1 (hazard ratios 2.6 and 1.3, respectively). The results from this longitudinal study indicate that subjects with an increased risk on mortality will be missed by the LLN. Since COPD is associated with a more rapid decline in lung function, longitudinal studies assessing the rate of lung function decline might be a solution to overcome the drawbacks of the cross-sectional studies comparing the LLN and fixed criterion. If it would be shown that subjects with a FEV_1/FVC <70%, but with >LLN have a comparable lung function decline to that of those with <LLN this would indicate that the <70% should not be discarded. Similar studies with longitudinal outcomes like morbidity and mortality have been conducted showing that subjects with FEV1/FVC <70%, but >LLN are comparable to subjects with <LLN.

In an attempt to investigate which threshold to prefer, we performed a diagnostic study to evaluate the diagnostic values of the FEV_1/FVC <LLN and <70%. As described in chapter **8** the main problem is lack of a true gold standard for COPD. In chapter **9** we therefore used a panel diagnosis of COPD, taking in account not only the FEV_1/FVC, but also other relevant clinical factors, as reference standard. This may be considered as a good reference standard for COPD because COPD itself is a clinical diagnosis in which physicians take far more in account than the FEV_1/FVC levels only. The FEV_1/FVC <LLN and <70% were compared against that panel diagnosis of COPD and sensitivity and specificity was calculated. Specificity numbers were comparable (0.95 and 0.99, respectively, $p<0.001$), but large differences in the sensitivity existed (0.73 and 0.47, respectively, $p<0.001$). The <70% criterion had a higher sensitivity than the LLN, and thus the LLN labeled fewer subjects as having COPD. The outcomes of this study suggest that the <70% is preferred above the <LLN to diagnose COPD in subjects presenting with persistent cough to their general practitioner. When we would apply the LLN instead of the 70% in general practice we indeed would diagnose less COPD. It is however questionable whether we can afford to miss these subjects as more evidence becomes available indicating that an increased lung function decline occurs already in the early stage of the disease. If our goal is to diagnose COPD in an early stage and we do not choose to miss a great number of diseased subjects, we need to adopt the threshold with the greatest sensitivity. Especially given the larger number of false-negatives according to <LLN when compared to <70% we need to be cautious as adopting the <LLN may prevent adequate counseling and treatment.

The sensitivity rate of the 70% threshold was good, 0.73, but not perfect. From this and also pointed out by the GOLD committee, it can be learned that COPD is a clinical

diagnosis in which physicians take more factors in account than the FEV_1/FVC level only. In clinical practice the FEV_1/FVC result is just a part of the information used. Incorporating all the present information, including the FEV_1/FVC results leads to a COPD diagnosis. A clinical diagnosis of COPD should not be based on one single FEV_1/FVC value only. Therefore, we need to look beyond the discussion about the correct threshold of FEV1/FVC. It is time to move on. Our ultimate goal is to prevent and reduce morbidity and mortality caused by COPD. For that reason we should classify subjects based on their risk of disease development and progression.

A second finding of chapter **9** was in this specific population no significant differences existed in diagnostic properties of using either pre- or post-bronchodilator values for diagnosing COPD. This finding is in contrast to the recommendation of the GOLD committee to perform broncho-dilator spirometry. It should however be noted that in other populations, for instance in the general healthy population this may not be the same. It would be of interest to perform similar studies in other more general populations.

Methodological considerations

There are some general methodological considerations of the studies presented in chapters **2-7**. These can be divided mainly in issues concerning the design of the study and the method of CT emphysema measurements.

Study design:

Only males were included. This was due to the inclusion criteria of the NELSON study which primarily focused on inclusion of males as females in the Netherlands are less heavily exposed to cigarette smoking. Since differences in lung function decline and severity of (CT-quantified) emphysema have been reported between males and females our findings may not be extrapolated directly to females.

The majority of studies subjects had no airflow obstruction or only mild to moderate COPD. Due to the inclusion criteria of the NELSON study, aimed on recruiting heavy, but relatively healthy smokers, no subjects with more severe COPD were included. One could argue that this is a limitation of the studies presented in this thesis. On the other hand, we were able to study determinants of lung function decline and emphysema progression in those most eligible for interventional measures, like smoking cessation.

The follow-up time was relatively short (median 3 years). It is known that lung function values

may show a high intra-individual variability and that therefore long follow-up periods are necessary to detect real decline. However, because we included a large number of participants, we were able to detect clinically significant decline in lung function (FEV_1 and FEV_1/FVC) over time. The same could be true of emphysema measurements. Unfortunately, little is known about the natural course of CT-quantified emphysema in smokers and COPD patients. It has been shown that COPD exacerbations are associated with increased progression of CT-quantified emphysema. [8] However, reports of large longitudinal CT studies in COPD subjects are not yet available, but hopefully will be in the near future. [9]

Broncho reversibility was not assessed. The GOLD committee states that COPD is present if the post-bronchodilator FEV_1/FVC is below 70%. As we only performed pre-bronchodilator spirometry a percentage of participants could have been erroneously classified as having COPD. However, because we analyzed lung function values continuously we do not expect that this has biased our results greatly. Secondly, because both baseline as follow-up spirometries were performed without bronchodilatation, it is not expected that a subsequent decline will differ largely.

CT acquisition and emphysema measurements:

Emphysema at CT was quantified by using the Perc15. Emphysema is characterized by destruction of alveolar walls and widening of the alveoli distal to the terminal bronchioles. CT-scanning of the lungs enables us to assess the severity and extent emphysema by measuring the low-attenuation areas in the lungs. Several measures have been proposed to quantify CT-detected emphysema like the percentage of low-attenuation voxels <–910 HU and below <–950 HU and the 15th percentile point of the low-attenuation voxels distribution (Perc15). In all the studies described in this thesis we used the Perc15 as emphysema severity measure because the Perc15 may be considered the most robust measure of emphysema in follow-up studies. [10 11] Secondly, the Perc15 was already normally distributed in our study population unlike the <-950% measure. Furthermore, the <-950% measure and Perc15 measure are highly correlated and as expected the results of our studies did not differ when using the <-950% as emphysema measure instead of the Perc15.

The CT-scans were not spiromatically gated. Ideally, the extent of low-attenuation areas at follow-up should be assessed at the same inspirational level at baseline. Larger lung volumes 'darken' the CT-image of the lungs as they contain more air, which has a HU of -1000. As a result real progression of emphysema may be overestimated. Logically,

this also works the other way around and smaller lung volumes at follow-up CT-scans underestimate the progression of emphysema. A solution would be to spiromatically gate the CT-scans, however this showed not to be feasible in large multi-centered clinical trials, like the NELSON trial. [12] However, mean total lung CT volumes for the baseline scans and the follow-up scans were not significantly different (6684 mL and 6703 mL, p=0.835) indicating that our measurements are valid.

One of the limitations of the review described in chapter **8** is that relevant studies might have been missed in the literature search. We performed searches in both MEDLINE and in EMBASE. In addition we performed a hand search of the references of the included studies. This has limited the occurrence of studies being missed. Despite this potential of missing data, the issue of nominating one of the thresholds as gold standard and thereby influencing the outcome is a general problem, a few articles missed will not alter that.

In chapter **9** we used a panel diagnosis as reference standard to compare the diagnostic accuracy of the FEV_1/FVC <70% and < LLN. Ideally, the reference test is the test with the highest sensitivity and specificity, i.e. the gold standard test, and any other reference standard is an imperfect gold standard. Because there is no gold standard test for COPD a panel diagnosis, taking in account information from history taking, physical examination and lung function testing, may be the best test available. We recognize that the panel diagnosis may be considered as an imperfect gold standard. However, because we compare both thresholds against this panel diagnosis both suffered to the same extent from its possible diagnostic inaccuracy and therefore comparison of the thresholds will still be valid.

Clinical implications

In current practice the evaluation of subjects at risk for or with recognized COPD is based on spirometry. In this thesis we investigated the role of several different determinants to predict lung function decline and emphysema progression. The question arises which of these determinants could and should be implemented in daily clinical routine.

It is known that significant amounts of emphysema can be present in subjects with normal spirometry. Spirometry alone therefore is not sensitive enough to detect emphysema. Because emphysema is associated with a lower diffusion capacity it may be used to detect emphysema. We showed that a lower Kco was associated with lung function decline and emphysema progression. Diffusion capacity tests therefore may

be used for a better evaluation and characterization of heavy smokers. CT scans also provide information about the distribution of emphysema and, are as shown in chapter 4, also associated with lung function decline. However, diffusion testing has the advantage of being less expensive and harmless. Lung diffusion testing is easily available in almost every hospital. In conclusion, lung diffusion testing may be useful to better characterize disease progression in heavy smokers and provides additional information beyond spirometry.

Although it has been more 20 years that CT emphysema measurements have been described for the first time they are not yet embedded in daily clinical routine. There are several reasons for this. First, there is disagreement on the quantification method of emphysema either the 15th percentile method or the -950HU method. To overcome this issue studies should compare both against the gold standard for emphysema; pathology specimens, however these studies are not feasible easily. Second, it is known that large differences exist between different CT machines, vendors and algorithms. Therefore, emphysema measurements obtained on different CT machines may not be compared straightforwardly, limiting clinical use. Last, differences in lung volume during acquisition may influence emphysema measurements. Spiromatically gating of the CT scans could overcome this problem, but is not considered feasible in daily practice. Hence, encouraging every subject who undergoes CT scanning to inspire at their maximum level may be adequate enough. [7] Taken together all these factors limit the embedding in clinical practice.

Summarizing the above, in the evaluation of heavy smokers for the presence of COPD spirometry testing should be the first step. Ideally, also lung diffusion testing should be performed because spirometry fails to discriminate between chronic bronchitis and emphysema. If the FEV_1/FVC ratio is normal results from the lung diffusion tests may provide additional information about future lung function decline and progression of CT-quantified emphysema. In case of normal spirometry and diffusion testing results a low-dose CT scan could be performed. The CT-scan could provide information about the presence and distribution of structural abnormalities, i.e. emphysema, and the risk on future lung function decline and development of COPD.

Smoking cessation is the most important intervention for preventing disease progression in COPD. Even more emphasis and attention should be paid to motivate smokers to quit. Results from this thesis showed that smoking cessation is beneficial for lung function decline and emphysema progression. This information could be used in information campaigns and patient education programs. As COPD is a progressive and irreversible disease smoking cessation as early as possible is imperative.

One of the main goals of implementation of genetics in clinical care is to make risk

predictions on who has a higher chance to develop COPD or to have a larger lung function decline. This will make a individualized and targeted approach possible with the goal to eventually prevent COPD. Therefore, lot of research efforts has and is been put in unraveling the genetics of COPD and lung function. Unfortunately, results have not been consistent enough to be implemented in clinical care yet. Results from candidate-gene studies of the 15q24/25 locus have been associated with several smoking-related disorders like peripheral arterial disease, lung cancer and COPD. Recent GWAS also found that variants of the 15q24/25 locus were associated with COPD. These results are promising because GWAS are hypothesis-free and therefore the found associations may confirm the previous candidate-gene studies. Unfortunately, the effect sizes of the found associations are small and thus unlikely to be used for risk prediction in clinical practice yet. It is recognized that COPD is a complex disease with many possible gene-environment interactions. Therefore future research should also take the interaction for instance between smoking status and genes in account.

Also the heterogeneity of COPD hampers the finding of associating genes. Although spirometry, i.e. an FEV_1/FVC below a predefined threshold, has been used to define COPD it does not discriminate well between emphysema and airway wall changes.

As shown in chapters 3 and 4 smokers without airflow obstruction yet already have significant CT-quantified emphysema. From chapter 6 it can be learned that smokers with lower diffusion tests results show a stronger progression of emphysema. Spirometry thus does not differentiate well between the different COPD phenotypes and therefore future genetic studies should study genetic associations with other COPD phenotypes based only on spirometry. Quantitative CT may be used for phenotyping of emphysema and is currently being explored.

In conclusion, our findings of chapter 7 may help to further elucidate the role of variants in the 15q24/25 locus in COPD, but is not ready for application in clinical care. Hopefully, results from ongoing GWAS will shed some light on the genetics of COPD and eventually make risk stratification possible.

The current GOLD guideline recommend the fixed value of FEV_1/FVC for diagnosing COPD. The findings of chapters 8 and 9 support the GOLD in their recommendation. From the current literature it cannot be concluded which threshold is superior. What we do now is that applying the lower limit of normal will miss more subjects with COPD in a symptomatic population. Therefore in daily clinical practice the more strict 70% threshold may be preferred. Especially, since COPD is under diagnosed in primary care we need to ponder whether we can afford to miss COPD subjects.

Conclusions

The work presented in this thesis has improved our insight on the question which threshold of FEV_1/FVC is most appropriate in diagnosing COPD. Secondly, it improved our insights in important determinants of lung function decline and emphysema progression in heavy smokers.

Lung function decline and progression of emphysema

Greater extents of CT-quantified emphysema are associated with a more rapid lung function decline in heavy smokers, independent whether they have airflow obstruction. Furthermore, in non-obstructive participants (FEV_1/FVC>70%) a greater extent of CT-quantified emphysema is a risk factor to become obstructive over time. Besides the total extent of CT-quantified emphysema the distribution of this emphysema is also associated with lung function decline. Participants with upper lobe predominant emphysema have a more rapid lung function decline.

In heavy smokers, lung function decline is steepest in subjects with a still normal lung function value compared to subjects with already abnormal lung function. In contrast, the emphysema progression as assessed at CT is steepest in subjects with abnormal lung function and least in subjects with normal lung function values. Smoking cessation for >4 years in heavy smokers stabilizes lung function decline and emphysema progression. Together, these observations indicate the importance of an early recognition and intervention in COPD.

Diffusion testing, i.e. the Kco, may be used to selected heavy smokers who will have a more rapid lung function decline and progression of emphysema.

In conclusion, we showed that it is possible to select participants with an increased lung function decline and emphysema progression based on specific characteristics. The results may be used to select heavy smokers at risk of a higher lung function decline and emphysema progression. These subjects may be eligible for intensive smoking cessation counseling and eventually prevent further disease progression.

Spirometric definition of COPD

Based on the findings from the evidence based review is may be concluded that the current literature does not provide a definite answer on which threshold of FEV_1/FVC is to prefer due to methodological shortcomings of studies included. From the results

of chapter 3, comparing the two threshold against a panel diagnosis of COPD, it may be concluded that the FEV_1/FVC <70% has better diagnostic accuracy than the <LLN in symptomatic subjects of >50 years for diagnosing COPD. Therefore in such a population the <70% may be preferred. Secondly, from chapter 3 it may be concluded that there were no differences in diagnostic properties between pre- and post-bronchodilator values for diagnosing COPD.

Perspectives

A better understanding of the longitudinal course of COPD will help to improve our knowledge on the different phenotypes of the disease. These findings may also help to find those subjects eligible for more aggressive smoking cessation programs to prevent (more severe) COPD. However, inevitably, the importance of smoking cessation needs to be highlighted in every smoker given the (many) undesirable and detrimental effects on human health.

Besides the extent of low-attenuation areas at CT also other radiological findings may be associated with lung function decline. Markers for airway disease like increased airway wall thickness, decreased airway diameter and presence / extent of air trapping have been associated with COPD in cross-sectional studies. By taking these other factors in account in future longitudinal studies the prediction of lung function decline by CT-quantified factors in heavy smokers may be improved. Future longitudinal studies should investigate whether low-attenuation areas, and other CT-derived parameters, are associated with an increased mortality.

In our longitudinal studies we only looked at the association with parameters of disease, i.e. lung function values and/ or CT-quantified emphysema, but not with parameters of survival. It would be of interest to examine for instance the effect of smoking cessation length or emphysema on morbidity and mortality rates.

Increasing emphasizes is put on the presence of extra-pulmonary manifestations of COPD and co-morbidities, most importantly being osteoporosis, cardiovascular disease and muscle wasting. [13] [14] [15] Unfortunately, the relationship between these extra-pulmonary manifestations and co-morbidities and COPD is not yet fully understood. Systemic inflammation is thought to play an important role. Signs of osteoporosis and cardiovascular disease can be found on chest CT-scans of heavy smokers. Future studies could examine whether these co-morbidities are associated with disease progression in COPD.

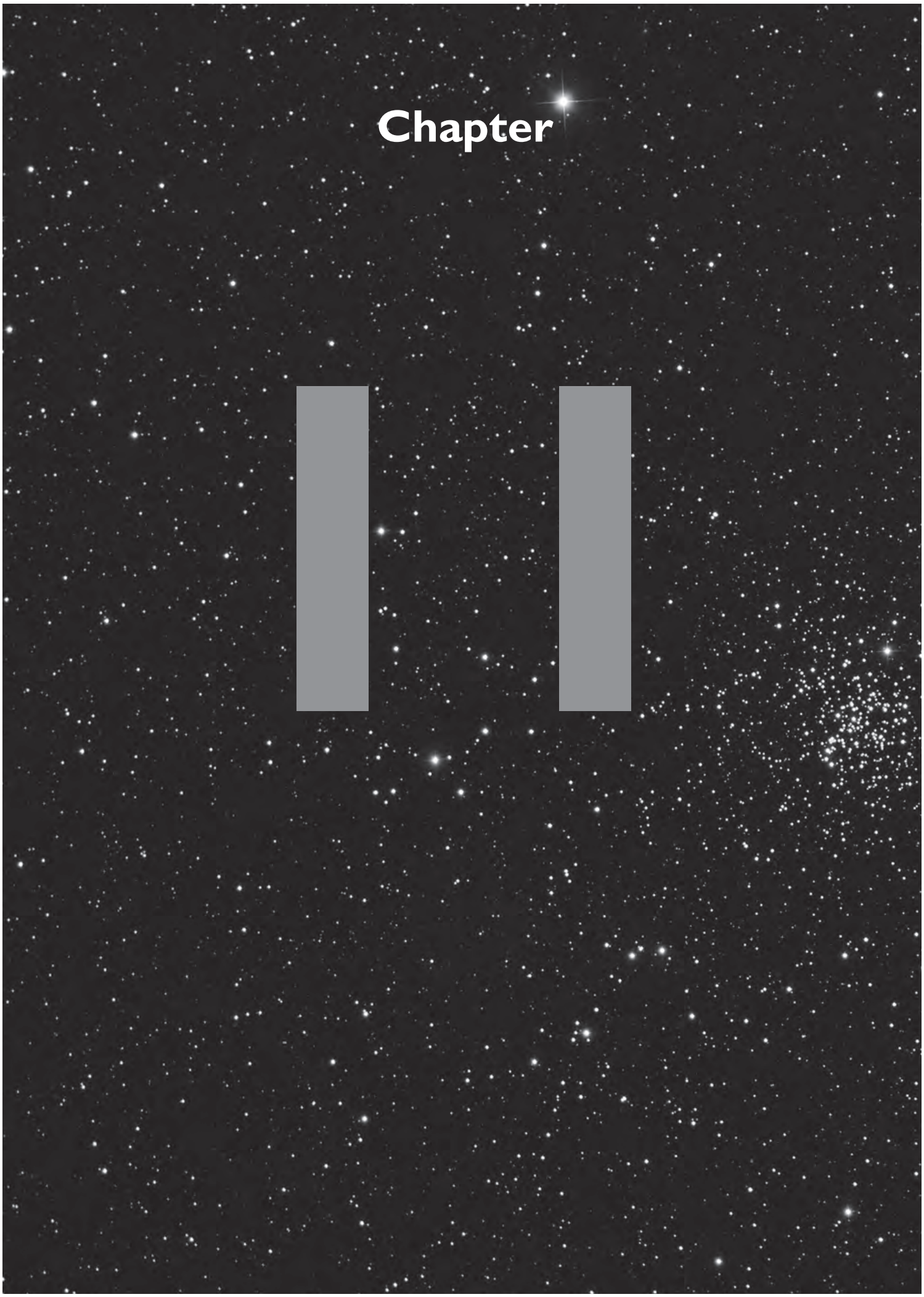
Hypothesis-free approaches, like genome-wide association studies (GWAS), may reveal new genetic factors associated with lung function decline. Several cross-sectional GWAS have been performed on COPD and lung function levels, however no GWAS on lung function decline have been reported yet. The results from longitudinal GWAS currently being performed may reveal new genetic associations not yet being discovered in the cross-sectional GWAS. The ultimate goal of finding the genetic factors involved in COPD is to provide a better understanding of the pathophysiological processes of COPD leading to the development of specific drugs targeting these specific processes. Furthermore, and maybe even more importantly, genetic factors may lead to the development of prediction rules for the development of COPD.

The appropriate cut-off value for air flow obstruction is still debated, however the debate should take another dimension. The current available literature comparing these two criteria has important methodological draw backs. In absent of a 'true' gold standard for COPD and to overcome the drawbacks of cross-sectional studies, it will be inevitable to perform longitudinal and prognostic studies. Until then, one should be precautious on discarding the fixed criterion, because subjects missed by the LLN, but who would have been diagnosed by the fixed criterion, have a higher risk on mortality. [16] Future studies should investigate the prognostic values of the two thresholds to decide which is to prefer. COPD remains a clinical diagnosis in which the FEV_1/FVC value is one of the factors taken in account. Other factors and test outcomes may be added to the FEV_1/FVC results to come to a diagnosis or risk prediction. For instance, subjects with a near normal FEV_1/FVC, but with significant extents of CT-quantified emphysema are at a higher risk on developing COPD than subjects with less CT-quantified emphysema.

In conclusion, COPD is a complex and heterogeneous disease. Hopefully, a more precise characterization of heavy smokers with a more rapid lung function decline and a more pronounced progression of CT-quantified emphysema may increase insight in the disease and reveal specific groups eligible for a more specific and aggressive treatment regime.

Reference List

1. van der Lee, I, Gietema HA, Zanen P et al. Nitric oxide diffusing capacity versus spirometry in the early diagnosis of emphysema in smokers. Respir Med 2009; 103(12):1892-1897.
2. Gould GA, Redpath AT, Ryan M et al. Lung CT density correlates with measurements of airflow limitation and the diffusing capacity. Eur Respir J 1991; 4(2):141-146.
3. Gould GA, Redpath AT, Ryan M et al. Lung CT density correlates with measurements of airflow limitation and the diffusing capacity. Eur Respir J 1991; 4(2):141-146.
4. Mair G, Miller JJ, McAllister D et al. Computed tomographic emphysema distribution: relationship to clinical features in a cohort of smokers. Eur Respir J 2009; 33(3):536-542.
5. de Torres JP, Bastarrika G, Zagaceta J et al. Emphysema presence, severity and distribution has little impact on the clinical presentation of a cohort of patients with mild to moderate COPD. Chest 2010.
6. Saitoh T, Koba H, Shijubo N et al. Lobar distribution of emphysema in computed tomographic densitometric analysis. Invest Radiol 2000; 35(4):235-243.
7. Madani A, Van MA, Gevenois PA. Pulmonary emphysema: effect of lung volume on objective quantification at thin-section CT. Radiology 2010; 257(1):260-268.
8. Tanabe N, Muro S, Hirai T et al. Impact of exacerbations on emphysema progression in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2011; 183(12):1653-1659.
9. Regan EA, Hokanson JE, Murphy JR et al. Genetic epidemiology of COPD (COPDGene) study design. COPD 2010; 7(1):32-43.
10. Coxson HO. Quantitative chest tomography in COPD research: chairman's summary. Proc Am Thorac Soc 2008; 5(9):874-877.
11. Parr DG, Sevenoaks M, Deng C et al. Detection of emphysema progression in alpha 1-antitrypsin deficiency using CT densitometry; methodological advances. Respir Res 2008; 9:21.
12. Newell JD, Jr., Hogg JC, Snider GL. Report of a workshop: quantitative computed tomography scanning in longitudinal studies of emphysema. Eur Respir J 2004; 23(5):769-775.
13. Decramer M, Rennard S, Troosters T et al. COPD as a lung disease with systemic consequences--clinical impact, mechanisms, and potential for early intervention. COPD 2008; 5(4):235-256.
14. Wouters EF, Creutzberg EC, Schols AM. Systemic effects in COPD. Chest 2002; 121(5 Suppl):127S-130S.
15. Agusti AG, Noguera A, Sauleda J et al. Systemic effects of chronic obstructive pulmonary disease. Eur Respir J 2003; 21(2):347-360.
16. Mannino DM, Buist AS, Vollmer WM. Chronic obstructive pulmonary disease in the older adult: What defines abnormal lung function? Thorax 2007; 62(3):237-241.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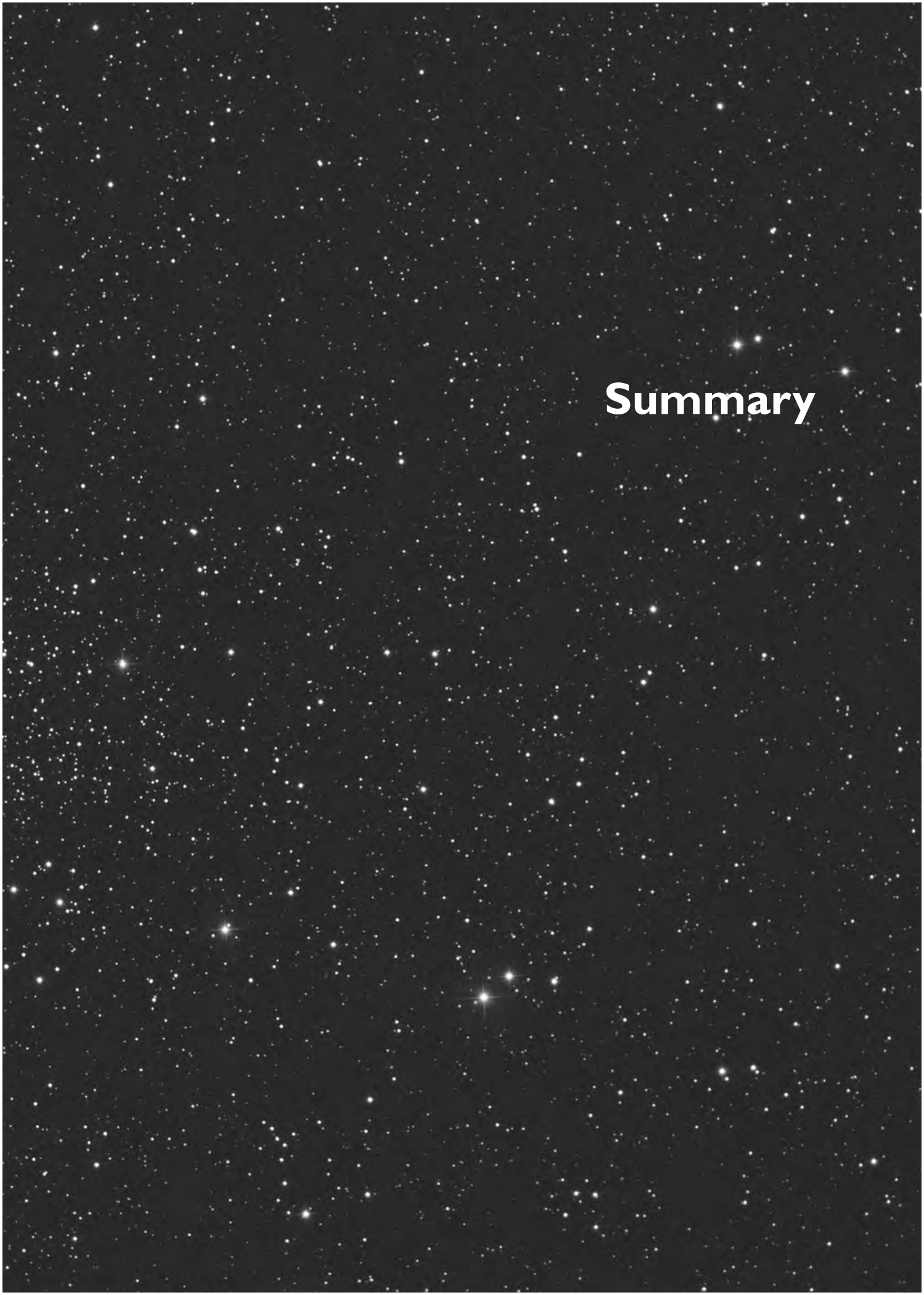


Summary

Summary

In **part 1** determinants of lung function decline and progression of emphysema were examined in heavy smokers. In **part 2** of this thesis it was investigated which threshold of FEV_1/FVC is most appropriate for diagnosing airflow obstruction.

Part 1: Lung function decline and emphysema progression

An important and intriguing finding of chapter **2** is that the decline in lung function was the steepest in participants with higher lung function values. Regarding emphysema progression we found that it was least in the group with high lung function values and strongest in participants with lower lung function values. Our findings may have important implications because they emphasize that in a very early stage, when smoking subjects still have normal lung values, lung function decline is the largest. Intervention programs possibly will have the highest yield when subjects still have a normal lung function because they may prevent disease progression. Especially since COPD is regarded as an irreversible disease this may be of importance.

Although several studies found an association between CT-quantified emphysema and lung function parameters,[1 2;3] it remained unclear what the (clinical) consequences of this CT-quantified emphysema are. Especially in relatively healthy smokers the clinical value of emphysema was uncertain. In chapter **3** we reported that a higher extent of CT-quantified emphysema, either expressed as Perc15 or <-950 HU, was associated with a stronger decline in lung function and with an increased risk of the development of COPD during a 3-year follow-up. Our results show that CT-quantified emphysema may be used to select subjects at risk for COPD and those with a more rapid lung function decline.

In addition we showed in chapter **4** that, besides the overall extent of CT-quantified emphysema, the distribution pattern also was associated with lung function decline. Participants with upper lobe predominant emphysema had a lower FEV_1/FVC after follow-up than those with lower lobe predominant emphysema. This association was independent of the total extent of emphysema. A strong point of this study was that the distribution pattern was based on the anatomical lung lobes enabling a more precise characterization. Most former studies used merely lung volumes derived from CT to qualify the emphysema distribution. [4 5;6]

Smoking cessation is the only intervention known to halt progression of disease in COPD subjects. In chapter **5** we examined the effects of duration of smoking cessation on lung function decline and progression of emphysema in heavy smokers with and without airflow obstruction. We showed that smoking cessation for >4 years stabilized lung function decline and emphysema progression in both obstructed as non-obstructed participants. Our results show that smoking cessation is beneficial even in participants with a high smoking exposure. Although the effect on emphysema progression was significant, it was small and therefore questionable to be clinically relevant. Nonetheless, it may be concluded that smoking cessation has differential effects on lung function and emphysema otherwise.

In chapter **6** we showed that the diffusion capacity of the lungs for carbon monoxide (Kco) was associated with lung function decline and progression of CT-quantified emphysema. The Kco reflects the properties of the alveolar-capillary membrane which is damaged in the presence of emphysema. As we showed that the Kco may be used to predict the progression of CT-quantified emphysema in smokers it may act as a substitute for CT-scanning to assess emphysema progression.

In chapter **7** we showed that variants in the 15q24/25 locus (rs1051730 and rs8034191) are associated with lung function decline in active smokers. Current smokers homozygous for the rs1051730 A-allele or rs8034191 G-allele had significantly greater FEV_1/FVC decline than homozygous carriers of wild-type alleles (3.3% and 4.3%, p=0.026 and p=0.009, respectively). Remarkably, no such association was found in current smokers. These findings were replicated in an independent cohort of healthy smokers and subjects with varying COPD severity (GOLDI –IV) from Leuven. In the LEUVEN cohort, rs1051730 AA-carriers and rs8034191 GG-carriers had a two-fold increased risk to suffer from COPD GOLD IV (p=0.025 and p=0.016, respectively). The same risk alleles conferred, respectively, a five- and four-fold increased risk to be referred for lung transplantation because of end-stage COPD (p=0.004 and p=0.010).

Part 2: Spirometric diagnosis of COPD

In chapter **8** we described the performance of a literature search of studies comparing diagnostic criteria of spirometric COPD: FEV_1/FVC below the lower limit of normal (LLN) or below a fixed percentage of 70%. Several studies were retrieved that did an attempt to find out which cut off value is superior. The majority of studies had a cross-

sectional design and compared the prevalence rates according to either the LLN or the fixed criterion. Prevalence rates according to the fixed criterion were higher than those according to the LLN. Therefore, in subjects aged>45 years, it was generally concluded that the fixed criterion over diagnosed COPD. The major flaw of the cross sectional studies is that they compared the fixed criterion with the LLN, by taking the LLN as gold standard. By definition, no test can perform better than the test, i.e. gold standard, against it is compared. Mannino et al grouped subjects by their baseline FEV_1/FVC ratio: 1) FEV_1/FVC >70% and >LLN; 2) FEV_1/FVC <70%, but >LLN; and 3) FEV_1/FVC <70% and <LLN. Group 2 subjects had significantly higher risks on mortality and respiratory-related hospitalizations compared to group 1 (hazard ratios 2.6 and 1.3, respectively). The results from this longitudinal study indicate that subjects with increased risk on mortality will be missed by the LLN.

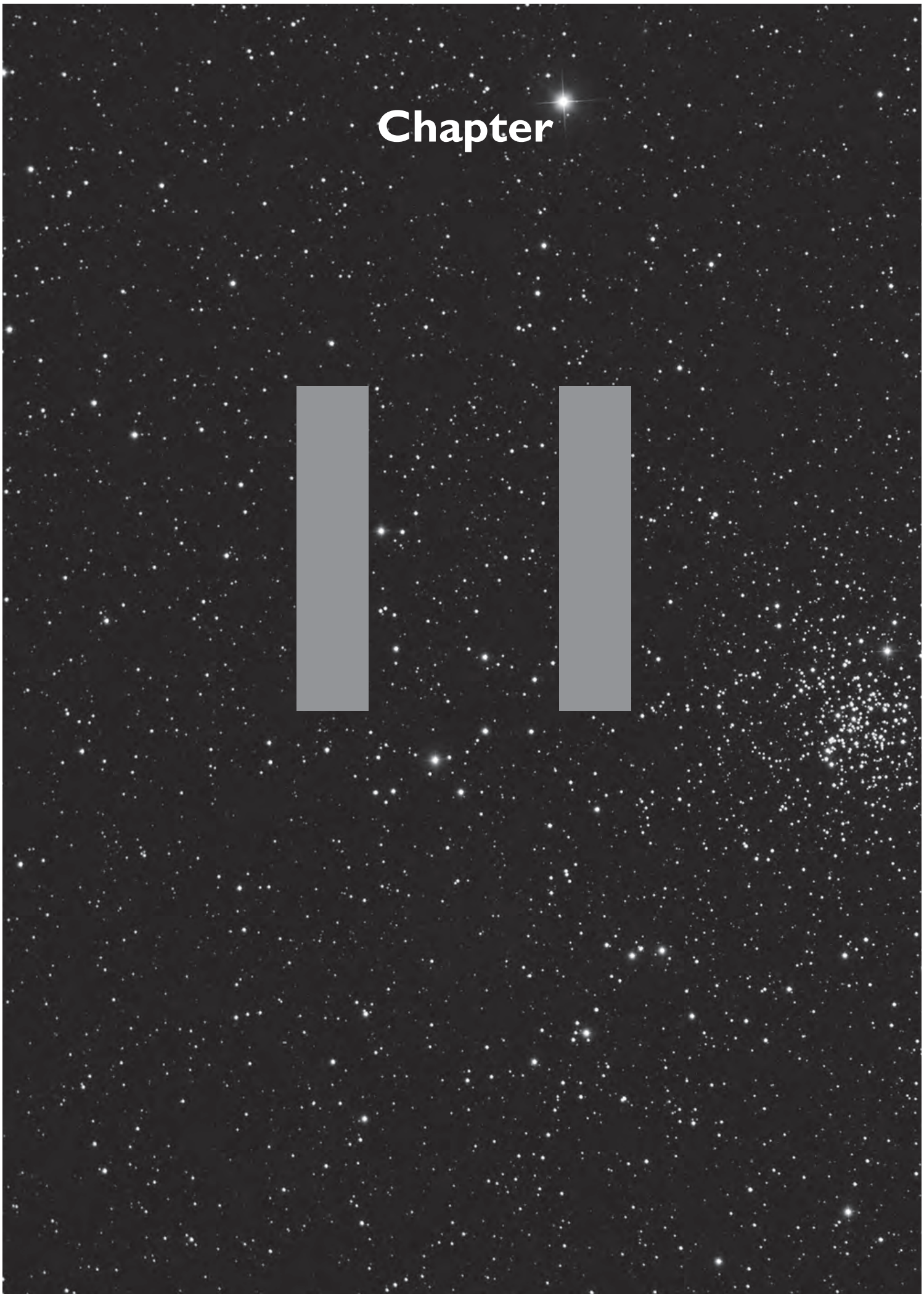
In an attempt to investigate which threshold is to prefer we performed a diagnostic study to evaluate the diagnostic values of the FEV_1/FVC <LLN and <70%. As described in chapter **8** the main problem is lack of a true gold standard for COPD. In chapter **9** we used a panel diagnosis of COPD, taking in account not only the FEV_1/FVC, but also other relevant clinical factors, as reference standard. The FEV_1/FVC < LLN and <70% were compared against a panel diagnosis of COPD and sensitivity and specificity numbers were calculated. Specificity numbers were comparable, but large differences in the sensitivity existed. The outcomes of this study suggest that the <70% is preferred above the <LLN to diagnose COPD in subjects presenting with persistent cough to their general practitioner. Secondly, we showed that in this specific population no significant differences existed in diagnostic properties of using either pre- or post-bronchodilator values for diagnosing COPD.

Finally, in chapter **10** the major findings of this these were discussed and some future perspectives were presented.

In conclusion, the work presented in this thesis has improved our insights in important determinants of lung function decline and emphysema progression in heavy smokers. Secondly, it improved our insight on the question which threshold of FEV_1/FVC is most appropriate in diagnosing COPD.

Reference List

1. van, der Lee., I, H. A. Gietema, P. Zanen, R. J. van Klaveren, M. Prokop, J. W. Lammers, and J. M. van den Bosch. 2009. Nitric oxide diffusing capacity versus spirometry in the early diagnosis of emphysema in smokers. *Respir.Med.* 103:1892-1897.
2. Gould, G. A., A. T. Redpath, M. Ryan, P. M. Warren, J. J. Best, D. C. Flenley, and W. MacNee. 1991. Lung CT density correlates with measurements of airflow limitation and the diffusing capacity. *Eur.Respir.J.* 4:141-146.
3. Gould, G. A., A. T. Redpath, M. Ryan, P. M. Warren, J. J. Best, D. C. Flenley, and W. MacNee. 1991. Lung CT density correlates with measurements of airflow limitation and the diffusing capacity. *Eur.Respir.J.* 4:141-146.
4. Mair, G., J. J. Miller, D. McAllister, J. Maclay, M. Connell, J. T. Murchison, and W. MacNee. 2009. Computed tomographic emphysema distribution: relationship to clinical features in a cohort of smokers. *Eur.Respir.J.* 33:536-542.
5. de Torres, J. P., G. Bastarrika, J. Zagaceta, R. S. Mendiguren, A. B. Alcaide, L. M. Seijo, U. Montes, A. Campo, and J. J. Zulueta. 2010. Emphysema presence, severity and distribution has little impact on the clinical presentation of a cohort of patients with mild to moderate COPD. *Chest*.
6. Saitoh, T., H. Koba, N. Shijubo, H. Tanaka, and F. Sugaya. 2000. Lobar distribution of emphysema in computed tomographic densitometric analysis. *Invest Radiol.* 35:235-243.
7. Newell, J. D., Jr., J. C. Hogg, and G. L. Snider. 2004. Report of a workshop: quantitative computed tomography scanning in longitudinal studies of emphysema. *Eur.Respir.J.* 23:769-775.
8. Tanabe, N., S. Muro, T. Hirai, T. Oguma, K. Terada, S. Marumo, D. Kinose, E. Ogawa, Y. Hoshino, and M. Mishima. 2011. Impact of exacerbations on emphysema progression in chronic obstructive pulmonary disease. *Am.J.Respir.Crit Care Med.* 183:1653-1659.
9. Regan, E. A., J. E. Hokanson, J. R. Murphy, B. Make, D. A. Lynch, T. H. Beaty, D. Curran-Everett, E. K. Silverman, and J. D. Crapo. 2010. Genetic epidemiology of COPD (COPDGene) study design. *COPD.* 7:32-43.
10. Mannino, D. M., A. S. Buist, and W. M. Vollmer. 2007. Chronic obstructive pulmonary disease in the older adult: What defines abnormal lung function? *Thorax* 62:237-241.
11. Decramer, M., S. Rennard, T. Troosters, D. W. Mapel, N. Giardino, D. Mannino, E. Wouters, S. Sethi, and C. B. Cooper. 2008. COPD as a lung disease with systemic consequences--clinical impact, mechanisms, and potential for early intervention. *COPD.* 5:235-256.
12. Wouters, E. F., E. C. Creutzberg, and A. M. Schols. 2002. Systemic effects in COPD. *Chest* 121:127S-130S.
13. Agusti, A. G., A. Noguera, J. Sauleda, E. Sala, J. Pons, and X. Busquets. 2003. Systemic effects of chronic obstructive pulmonary disease. *Eur.Respir J* 21:347-360.


Chapter
II

Nederlandse samenvatting

Achtergrond

"Chronische obstructieve longziekte", beter bekend als "COPD", is een chronische aandoening van de longen die zich klinisch onder andere kenmerkt door een chronische hoest, het opgeven van slijm en kortademigheidsklachten bij toenemende inspanning. Bij longfunctieonderzoek wordt COPD gekenmerkt door een irreversibele luchtwegvernauwing. De belangrijkste risicofactor voor het ontstaan van COPD is roken en het wordt geschat dat ongeveer 20% van alle rokers gedurende zijn of haar leven COPD ontwikkelt. De prevalentie van COPD stijgt nog steeds, zowel wereldwijd als in Nederland, en COPD zal in de nabije toekomst wereldwijd doodsoorzaak nummer 4 worden.

Pathologisch gezien bestaat COPD uit een chronische inflammatie van de kleine luchtwegen en uit destructie van het longparenchym (emfyseem). Beide entiteiten kunnen leiden tot luchtwegobstructie. De mate waarin inflammatie van de kleine luchtwegen en emfyseem bijdragen tot COPD verschilt per individu. Ten gevolge van de ontstekingsreactie van de kleine luchtwegen op de geïnhaleerde sigarettenrook ontstaan chronische hoestklachten en slijmproductie. Emfyseem ontstaat door destructie van longblaasjes ook als gevolg van de ontstekingsreactie van de longen. De destructie van longblaasjes, emfyseem, kan microscopisch worden vastgesteld, maar ook op een CT-scan kan emfyseem worden vastgesteld. Als gevolg van emfyseem zullen de longen namelijk meer "lucht" bevatten wat een daling van dichtheid van het longweefsel tot gevolg heeft. Deze dichtheid kan gemeten worden op een CT-scan van de borstkas en dit is een radiologische maat voor emfyseem.

Helaas zijn er, behalve het stoppen met roken, tot op heden geen behandelingsmogelijkheden beschikbaar die deze mortaliteitcijfers doen dalen. Stoppen met roken blijft een van de belangrijkste pijlers in het voorkomen van COPD en het verminderen van symptomen in COPD-patienten. COPD wordt gediagnosticeerd door middel van het vaststellen van luchtwegobstructie bij spirometrie. Echter, de pathologische processen die leiden tot deze luchtwegobstructie zijn waarschijnlijk al eerder aanwezig.

Inhoud van dit proefschrift

Het eerste deel van dit proefschrift gaat over longfunctiedaling en emfyseemtoename in deelnemers van een longkankerscreeningsstudie, de NELSON studie. De deelnemers aan de NELSON hebben naast een CT-scan van de longen, ook een longfunctietest gehad. Deze twee onderzoeken hebben de deelnemers zowel in het begin van de studie als na een mediane follow-up tijd van 3 jaar gehad. Met al deze informatie waren wij in

staat om te onderzoeken welke factoren geassocieerd waren met 1) het ontwikkelen van COPD, 2) een versnelde achteruitgang in longfunctie en 3) een versnelde toename van emfyseem zoals dat gemeten wordt op de CT-scans.
Het tweede deel van dit proefschrift beschrijft de resultaten van twee studies die ingaan op de vraag welke afkapwaarde voor luchtwegobstructie moet worden gebruikt.

In de studies beschreven in dit proefschrift werden de volgende onderwerpen onderzocht:

1. De relatie tussen de ernst van luchtwegobstructie en achteruitgang van longfunctie en toename van emfyseem op de CT-scan (hoofdstuk 2).
2. De relatie tussen automatisch bepaald emfyseem op de CT-scan en het ontwikkelen van COPD en het hebben van een versnelde longfunctie achteruitgang (hoofdstuk 3 en 4).
3. De relatie tussen de duur van stoppen met roken en longfunctie achteruitgang en toename van emfyseem op de CT-scan (hoofdstuk 5).
4. De relatie tussen de diffusiecapaciteit van de longen voor koolmonoxide en longfunctie achteruitgang en toename van emfyseem op de CT-scan (hoofdstuk 6).
5. De relatie tussen variaties in het 15q24/2 gen en longfunctiedaling (hoofdstuk 7).
6. De juiste afkapwaarde in longfunctieonderzoek om COPD vast te stellen (hoofdstuk 8 en 9).

Deel 1: Longfunctiedaling en emfyseemprogressie

Hoofdstuk **2** beschrijft de achteruitgang van longfunctie en progressie van CT-gekwantificeerd emfyseem in de deelnemers van de NELSON studie in Utrecht en Groningen. Uit het onderzoek blijkt dat de mate van longfunctiedaling het grootste is in deelnemers met een nog normale longfunctie. In tegenstelling, de progressie van emfyseem is het grootste in de deelnemers met de slechtste longfunctie. Deze resultaten zijn in lijn met bevindingen uit grote studies naar het effect van luchtwegmedicatie in COPD patiënten waarin ook werd gerapporteerd dat de deelnemers met de laagste klasse van COPD de snelste longfunctiedaling lieten zien. De resultaten van hoofdstuk **2** onderstrepen het belang van een vroegtijdige herkenning van COPD aangezien de grootste longfunctiedaling al in een vroeg stadium optreedt.

In hoofdstuk **3** hebben wij onderzocht of CT-gekwantificeerd emfyseem een risicofactor is voor het ontwikkelen van COPD en of het geassocieerd is met longfunctiedaling. Emfyseem is strikt genomen een pathologische diagnose, maar de aanwezigheid en mate van emfyseem kan ook geschat worden met behulp van CT-scans van de longen. Speciale ontwikkelde software is in staat om uit te rekenen hoeveel emfyseem aanwezig is. Echter, de klinische waarde van dit CT-gekwantificeerde emfyseem in zware rokers was onduidelijk. De resultaten van hoofdstuk **3** laten zien dat CT-gekwantificeerd emfyseem een risicofactor is voor het ontwikkelen van COPD na 3 jaar follow-up. Daarnaast lieten de deelnemers met meer emfyseem een snellere longfunctiedaling zien.

Naast de totale hoeveelheid van het CT-gekwantificeerde emfyseem, blijkt uit eerder beschreven onderzoek dat de verdeling van dat emfyseem, dominant in bovenste deel van de longen (apex) of juist onderste deel (basaal), ook geassocieerd is met een longfunctie achteruitgang. In hoofdstuk **4** beschrijven we het onderzoek hiernaar in de NELSON deelnemers uit Utrecht die een longfunctietest en CT-scan ondergingen aan het begin van de studie en na gemiddelde 3 jaar. Speciaal ontwikkelde software was in staat om de longen te verdelen volgens de anatomische longkwabben: rechter boven-, midden- en onderkwab en linker boven- en onderkwab. Hierdoor waren we in staat om de verdeling van het CT-gekwantificeerde nog nauwkeuriger te bepalen dan in voorgaande studies. Het bleek dat deelnemers die meer emfyseem hadden in de bovenkwabben dan in de onderkwabben een lagere longfunctie hadden na 3 jaar. Hieruit concludeerden wij dat naast de totale hoeveelheid emfyseem (zoals beschreven in hoofdstuk **3**), ook de verdeling van het emfyseem van invloed is op de longfunctiedaling. Samen met de resultaten van hoofdstuk **4** blijkt dat er mogelijk een rol weggelegd is voor de bepaling van emfyseem op CT-scans van zware rokers. Deze informatie kan worden gebruikt voor een nauwkeurige typering van zware rokers en voor selectie van rokers met een snellere longfunctieachteruitgang.

Stoppen met roken is de belangrijkste interventie in het stoppen van een versnelde longfunctieachteruitgang. In hoofdstuk **5** wordt een studie beschreven naar de relatie van de duur van stoppen met roken met de daling van longfunctie en progressie van CT-gekwantificeerd emfyseem na een mediane follow-up van 3 jaar. Uit de resultaten kwam naar voren dat de deelnemers die voor aanvang van de studie 1 jaar of langer gestopt waren een significant lagere longfunctiedaling en emfyseemprogressie hadden in vergelijking met de deelnemers die wel rookte bij aanvang van de studie. Hoe langer men gestopt was, hoe lager de longfunctiedaling en emfyseemprogressie waren. De

resultaten van hoofdstuk 7 laten zien dat stoppen met roken ook in zware rokers een significant effect hebben op de progressie van ziekte.

Hoofdstuk **6** beschrijft de associatie tussen de diffusiecapaciteit van de longen en longfunctiedaling en emfyseem progressie. De diffusiecapaciteit meet de mate waarin de longen in staat zijn om zuurstof op te nemen. In het geval van emfyseem zijn de longblaasjes beschadigd en zullen de longen als gevolg minder zuurstof kunnen opnemen wat uiteindelijk kan resulteren in een lagere diffusiecapaciteit. Het was echter niet goed bekend wat de waarde van de diffusiecapaciteit was in zware rokers zonder luchtwegobstructie op het natuurlijk beloop van longfunctie en emfyseem. Een lagere diffusiecapaciteit, uitgedrukt in de transfer constante voor koolstofmonoxide (Kco), was geassocieerd met een sterkere progressie van emfyseem en een snellere longfunctiedaling. Deze resultaten laten zien dat diffusietesten kunnen worden gebruikt om zware rokers beter te karakteriseren.

In hoofdstuk **7** worden de resultaten beschreven van een onderzoek naar de associatie tussen varianten in het 15q24/25 gen, een gen eerder in verband gebracht met COPD, emfyseem en longkanker, en longfunctiedaling. Het bleek dat deelnemers met de 'verkeerde' variant een versnelde longfunctieachteruitgang hadden.

Deel 2: Spirometrische definitie van COPD

Er wordt momenteel hevig gedebatteerd over de juiste afkapwaarde van de FEV_1/FVC om COPD vast te stellen. In hoofdstuk **8** wordt een literatuurstudie beschreven naar de juiste afkapwaarde om COPD vast te stellen. Na een uitgebreide zoekprocedure in databases die medische literatuur bevatten, werden in totaal 18 studies gevonden. Al deze 18 studies vergeleken een vaste waarde van FEV_1/FVC <70% met een leeftijdsafhankelijke waarde; de laagste normale limiet, oftewel de 'lower limit of normal' (LLN). Van deze 18 studies hadden 16 een dwarsdoorsnede opzet, dat wil zeggen de prevalentie volgens de vaste waarde werd vergeleken met de prevalentie volgens de leeftijdsafhankelijke waarde (LLN). Hierbij werd de LLN als referentiestandaard gebruikt. Deze aanpak is echter onjuist aangezien geen enkele test beter kan presteren dan de referentietest waarmee deze wordt vergeleken. Slechts twee studies hadden een follow-up periode waarin ze onderzochten of de twee verschillende classificaties geassocieerd waren mortaliteit en klachtenpatroon. De meest correcte methode zou zijn om de vaste waarde en de LLN te vergelijken met een onafhankelijke referentietest.

Hoofdstuk **9** beschrijft de resultaten van een studie waarin de diagnostische waardes van de FEV_1/FVC <70% en <LLN werden vergeleken. Omdat er geen gouden standaard voor COPD bestaat werd gekozen om een paneldiagnose als referentie standaard te gebruiken. 342 deelnemers, >50 jaar, die zich presenteerden met persisterende hoestklachten bij hun huisarts, maar zonder bekend te zijn met astma of COPD, werden geincludeerd. Alle deelnemers ondergingen een uitgebreide anamnese, lichamelijk onderzoek en een uitgebreid longfunctieonderzoek inclusief diffusietesten. De uitkomsten hiervan werden gepresenteerd aan een panel bestaande uit een longarts en een huisarts die samen beoordeelden, op basis van alle beschikbare informatie, of er sprake was van COPD. Sensitiviteitscijfers van de <70% waren significant hoger dan die van de <LLN, terwijl de specificiteitcijfers vergelijkbaar waren.

Tot slot, in hoofdstuk **10**, worden de uitkomsten van het onderzoek beschreven in dit proefschrift samengevat en bediscussieerd en implicaties voor toekomstig onderzoek geschetst.

Toekomstig onderzoek

De studies beschreven in dit proefschrift hebben laten zien dat het mogelijk is om op basis van specifieke karakteristieken te voorspellen wie een snellere longfunctiedaling en progressie van emfyseem zal doormaken.

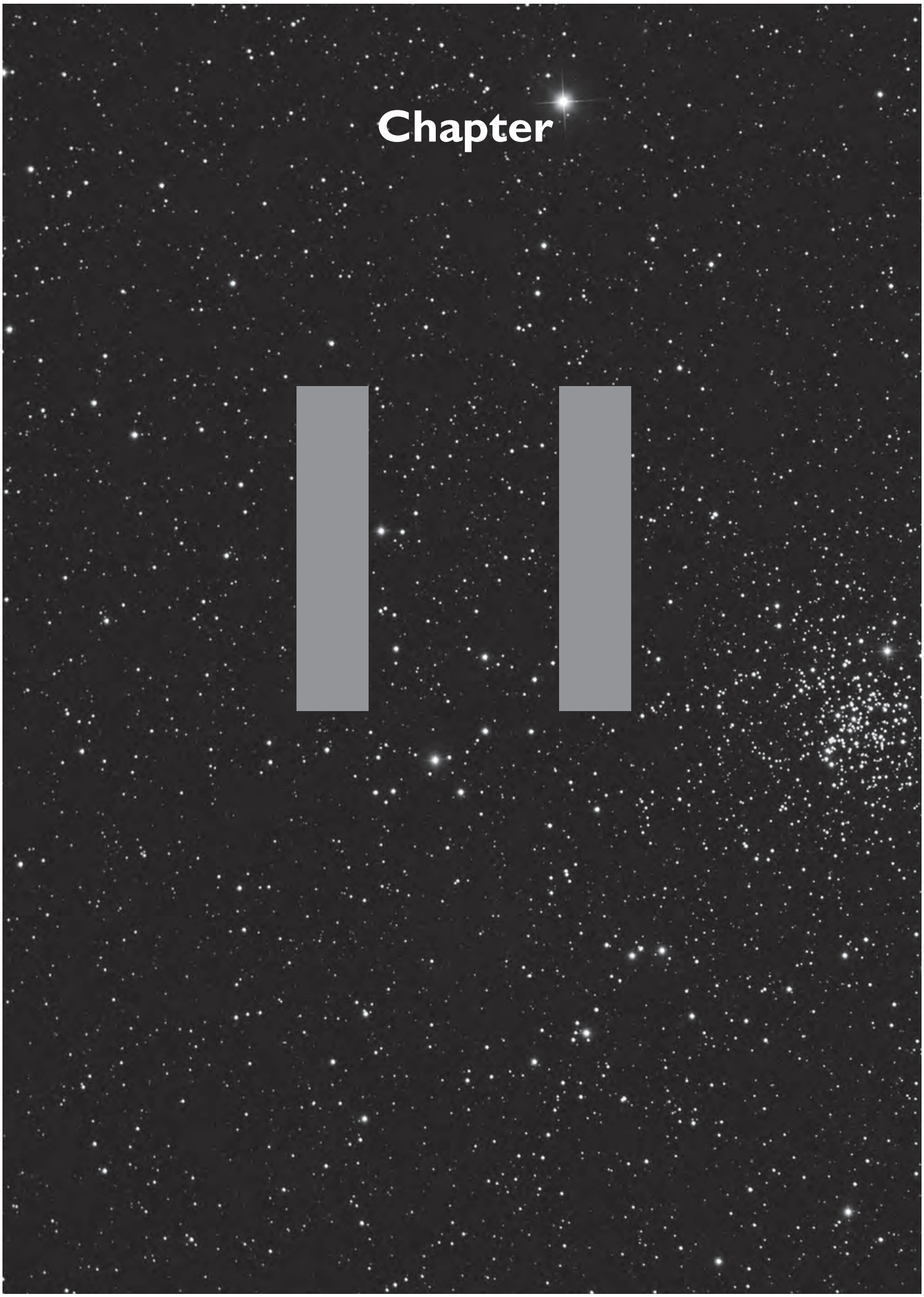
Wij hebben enkel het effect van CT-gedetecteerd emfyseem meegenomen in de analyses, echter het is goed mogelijk dat het toevoegen van luchtwegmetingen en airtrapping de voorspelling zal verbeteren. Toekomstig onderzoek zal dit moeten uitmaken.

COPD is een heterogene ziekte die geassocieerd is met verschillende co-morbiditeiten zoals hart- en vaatziekten en osteoporose. Tekenen van deze twee aandoeningen zijn zichtbaar op de CT-scan en toekomstig onderzoek zou kunnen uitmaken of ze geassocieerd zijn met progressie van COPD.

Het huidige debat over de juiste afkapwaarde voor FEV1/FVC om luchtwegobstructie vast te stellen is niet vruchtbaar. Op basis van de huidige literatuur is het moeilijk om een eenduidig antwoord te geven omdat de grote meerderheid van studies beide afkapwaardes niet hebben vergeleken met een onafhankelijke referentietest. Alhoewel onze studie heeft laten zien dat de sensitiviteit van de vaste waarde hoger is, zal in de toekomst uitgemaakt moeten worden welke afkapwaarde de beste prognostische waarde heeft.

Hypothesevrije methodes zoals de genoomwijde associatie scan (GWAS) kunnen mogelijk nieuwe genetische factors onthullen geassocieerd met COPD die eerder nog niet gevonden waren. Een aantal dwarsdoorsnede GWAS op COPD zijn verricht, maar longitudinale GWAS zullen mogelijk een beter inzicht geven in genetische factoren die geassocieerd zijn met ziekteprogressie.

De discussie over de meest geschikte afkapwaarde voor luchtwegobstructie moet een nieuwe richting op omdat de huidige discussie onvruchtbaar is en zal zijn. Toekomstige studies zullen zich moeten richten op de prognostische waarde van afkapwaarde voor luchtwegobstructie. Daarnaast blijft COPD een klinische diagnose waarbij artsen meer factoren in overweging nemen om tot een diagnose te komen. Deze factoren zouden kunnen worden toegevoegd aan de afkapwaardes om te onderzoeken welke de beste voorspellende waarde heeft.


Chapter
II

Curriculum Vitae

The author was born on January 16th, 1985 in Utrecht, the Netherlands. He completed secondary school (VWO) in 2003 at "College de Klop" in Utrecht and subsequently started with medical school at the University of Utrecht. In 2009 he obtained his medical degree and started as a PhD-student at the department of Respiratory Medicine of the University Medical Center Utrecht under the supervision of prof J.W.J. Lammers and dr. P. Zanen. The results of this research are presented in this thesis. In 2011 he started with his radiology training in the University Medical Center Utrecht under supervision of prof. J.P.J. van Schaik.

De auteur van dit proefschrift is geboren op 16 januari 1985 te Utrecht in Nederland. Na het doorlopen van het VWO op College de Klop in Utrecht begon hij aan de studie geneeskunde aan de Universiteit van Utrecht. In 2009 behaalde hij zijn artsenbul en begon hij als promovendus / arts-onderzoeker op de afdeling Longziekten van het Universitair Medisch Centrum Utrecht onder supervisie van prof. J.W.J. Lammers en dr. P. Zanen. De resultaten hiervan zijn beschreven in dit proefschrift. In 2011 startte hij met de opleiding tot radioloog in het Universitair Medisch Centrum Utrecht onder supervisie van prof. J.P.J. van Schaik.

List of publications

Mohamed Hoesein FA, Zanen P, Sachs AP, Verheij TJ, Lammers JW, Broekhuizen BD. Spirometry to define COPD: 0.70 or LLN, pre- or post-dilator values? COPD: Journal Of Chronic Obstructive Pulmonary Disease 2012, Feb, in-press.

Mohamed Hoesein FA, van Rikxoort E, van Ginneken B, de Jong PA, Prokop M, Lammers JW, Zanen P. CT-quantified emphysema distribution is associated with lung function decline. Eur Respir J. 2012, Feb, online-first *doi:10.1183/09031936.00186311*.

Mohamed Hoesein FA, Gietema HA, Zanen P, Lammers JW. CT-meting van emfyseem bij zware rokers. Ned Tijdschr Geneeskd. 2011;155:A3751

Mohamed Hoesein FA, Zanen P. The epidemiological impasse. Eur Respir J. 2011 Aug;38(2):480

Mohamed Hoesein FA, Zanen P, van Ginneken B, van Klaveren RJ, Lammers JW. Association of the transfer coefficient (Kco) with emphysema progression in male smokers. Eur Respir J. 2011 Nov;38(5):1012-8

Mohamed Hoesein FA, de Hoop B, Zanen P, Gietema H, Kruitwagen CL, van Ginneken B, Isgum I, Mol C, van Klaveren RJ, Dijkstra AE, Groen HJ, Boezen HM, Postma DS, Prokop M, Lammers JW. CT-quantified emphysema in male heavy smokers: association with lung function decline. Thorax. 2011 Sep;66(9):782-7.

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

Mohamed Hoesein FA, Zanen P. New concepts for expressing forced expiratory volume in 1 s: conclusions need nuances. Eur Respir J. 2010 Sep;36(3):693

Mohamed Hoesein FA, Zanen P, Lammers JW. Longitudinal change of prebronchodilator spirometric obstruction. Thorax. 2011 Mar;66(3):267-8

De Boeck BW, Teske AJ, Leenders GE, **Mohamed Hoesein FA**, Loh P, van Driel VJ, Doevendans PA, Prinzen FW, Cramer MJ. Detection and quantification by deformation imaging of the functional impact of septal compared to free wall preexcitation in the Wolff-Parkinson-White syndrome. Am J Cardiol. 2010 Aug 15;106(4):539-546