Epidemiology of Comorbidities in Chronic Obstructive Pulmonary Disease

Lies Lahousse

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Epidemiology of Comorbidities in Chronic Obstructive Pulmonary Disease

Epidemiologie van comorbiditeiten bij chronisch obstructief longlijden

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Chapter 5

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Chapter 6

Lahousse L, Loth DW, Joos GF, Hofman A, Leufkens HG, Brusselle GG, Stricker BH. Statins, systemic inflammation and risk of death in COPD: the Rotterdam Study. *Pulm Pharmacol Ther. 2013 Apr;26(2):212-7.*

Chapter 7

Lahousse L*, Niemeijer MN*, van den Berg ME, Rijnbeek PR, Joos GF, Hofman A, Franco OH, Deckers JW, Eijgelsheim M, Stricker BH, Brusselle GG. Chronic obstructive pulmonary disease and sudden cardiac death: the Rotterdam Study. *Submitted to the Am J Respir Crit Care Med.*

Chapter 8

Lahousse L, Tiemeier H, Ikram MA, Brusselle GG. The lung in relation to brain disorders in the elderly. *In revision for the European Respiratory Journal.*

Chapter 9

Lahousse L, van den Bouwhuijsen Q, Loth DW, Joos GF, Hofman A, Witteman JC, van der Lugt A, Brusselle GG, Stricker BH. Chronic Obstructive Pulmonary Disease and Lipid Core Carotid Artery Plaques in the Elderly: the Rotterdam Study. *Am J Respir Crit Care Med. 2013 Jan 1;187(1):58-64*

Chapter 10

Lahousse L, Loth DW, Vernooij MW, Darweesh SKL, Akoudad S, Joos GF, Hofman A, Stricker BH, Ikram MA, Brusselle GG. Chronic Obstructive Pulmonary Disease and cerebral microbleeds: the Rotterdam Study. *Am J Respir Crit Care Med. 2013;188(7):783-8.*

Chapter 11

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Chapter 12

Lahousse L*, Maes B*, Ziere G, Loth DW, Verlinden VJA, Zillikens MC, Uitterlinden AG, Rivadeneira F, Tiemeier H, Franco OH, Ikram MA, Hofman A, Brusselle GG, Stricker BH. Adverse outcomes of frailty in the elderly: the Rotterdam Study. *European Journal of Epidemiology. 2014 Jun;29(6):419-27.*

Chapter 13

Lahousse L, Ziere G, Verlinden VJA, Zillikens MC, Uitterlinden AG, Rivadeneira F, Tiemeier H, Franco OH, Ikram MA, Hofman A, Brusselle GG, Stricker BH. Chronic Obstructive Pulmonary Disease, comorbidities and frailty: the Rotterdam Study. *Submitted to the Lancet.*

Chapter 14

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PART I: GENERAL INTRODUCTION

1. COPD

1.1 COPD: a common disease in the elderly

Chronic Obstructive Pulmonary Disease (COPD) is defined by the Global initiative for chronic Obstructive Lung Disease (GOLD) as a common preventable and treatable disease, which is characterized by a persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response of the airways and lungs to noxious particles or gases.[1] Since 2010, COPD is globally the third leading cause of death.[2] Each year, approximately 200 000 to 300 000 people die in Europe because of COPD.[3] The morbidity, economic and social impact of COPD is substantial and increasing.[2] Among respiratory diseases, COPD is the leading cause of lost work days and it has an estimated cost of 3 to 4% (or 38.6 billion €) of the total health care budget in the European Union.[3, 4]

Previously, we found that the prevalence of COPD was 11.6% in a Dutch population-based cohort study of subjects aged 55 years and over.[5] A similar prevalence has been observed in the United States.[6] Although COPD is generally known as a disease of older smoking men, a high incidence of COPD was noted in young women and might suggest some shift in the gender distribution of COPD in Western Europe.[7, 8] Moreover, an estimated 25% to 45% of COPD patients worldwide have never smoked, suggesting that long-term exposure to biomass smoke or occupational dusts and air pollution are other major risk factors to develop COPD.[9] Despite the enormous global impact of COPD, there are no current drug therapies that have conclusively demonstrated to prevent disease progression or reduce mortality.[10]

1.2 Pathogenesis

Noxious particles and gases cause abnormal inflammatory immune responses of the lung in patients with COPD. The mechanisms for this amplified inflammation are currently not fully understood but may be genetically determined. Genome-Wide Association Studies (GWAS) for lung function and COPD have identified several genetic loci with genome-wide significance.[1, 11, 12] Both the innate and adaptive immune system are involved in the amplified persistent lung inflammation.[13] Noxious particles cause direct injury of airway epithelial cells, eventually leading to the release of cytokines. These cytokines attract macrophages, neutrophils and dendritic cells to the site of inflammation to set up the innate immune response. Proteolytic enzymes and reactive oxygen species are produced and, if not sufficiently counterbalanced by antiproteases and antioxidant factors, damage will occur, resulting in parenchymal destruction and small-airways fibrosis.[14] On the other hand, immature dendritic cells pick-up self-antigens, released from damaged tissue, and foreign particles, and present them to naïve T-cells in draining lymph nodes. This leads to the recruitment of antigen specific CD-4 and CD-8 T cells and antibody-producing B cells to the lung, driving the response of the adaptive immune system. This adaptive (and sometimes autoimmune) response is partially held responsible for the persistent lung inflammation,

even after smoking cessation.[13] The chronic inflammatory response stimulates remodeling of the small-airway compartment (resulting in small airways fibrosis and increased airway resistance) and emphysematous destruction of parenchyma (resulting in loss of elastic recoil).[15] According to Hogg's hypothesis, terminal bronchiolar loss preceeds the onset of emphysematous destruction.[16] Both widespread narrowing and reduction in numbers of terminal bronchioles contribute to the characteristic physiologic abnormalities and symptoms of COPD, including a progressive decline of forced expiratory volume in 1 second (FEV_1), inadequate lung emptying on expiration, and subsequent static and dynamic hyperinflation.[17, 18]

1.3 Diagnosis, classification and assessment within the Rotterdam Study

According to the GOLD guidelines, a clinical diagnosis of COPD should be considered in any patient experiencing dyspnea, chronic cough and/or chronic sputum production, who has a history of exposure to risk factors for COPD.[1] The presence of a post-bronchodilator ratio of FEV_1 to forced vital capacity (FEV_1/FVC) less than 0.70 is required to make the diagnosis.[1] FEV_1 is the maximum volume of air exhaled in the first second of a forced expiration from a position of full inspiration. FVC is the maximum volume of air exhaled with maximally forced effort from a maximal inspiration, i.e. the vital capacity performed with a maximally forced expiratory effort.[19] Both parameters are measured during the most common pulmonary function test, i.e. spirometry.

All studies of this thesis are embedded within the Rotterdam Study, a prospective population-based cohort study among almost 15 000 participants in the city of Rotterdam, the Netherlands.[20] In short, all inhabitants of the Ommoord district of Rotterdam, aged 55 years of age and older were invited to participate (n=10 215).[21] At baseline, from 1990 through 1993, 7 983 participants (response rate 78%) were included (*Figure* 1). In 2000, an additional 3 011 participants, out of 4 472 invitees, were enrolled (response rate 67%). This extension consisted of all persons living in the study district who had become 55 years old or had moved into the study district. A second similar extension of the cohort was initiated in 2006, in which 3 932 participants (out of 6 057 invitees; response rate 65%), aged 45 years and older were included.[20] Follow-up examinations are conducted periodically, approximately every four to five years. Examinations consist of a home interview and an extensive set of tests at a specially built research facility in the study district. Besides this, participants are continuously monitored for major morbidity and mortality through linkage of records from general practitioners and municipality to the study base.

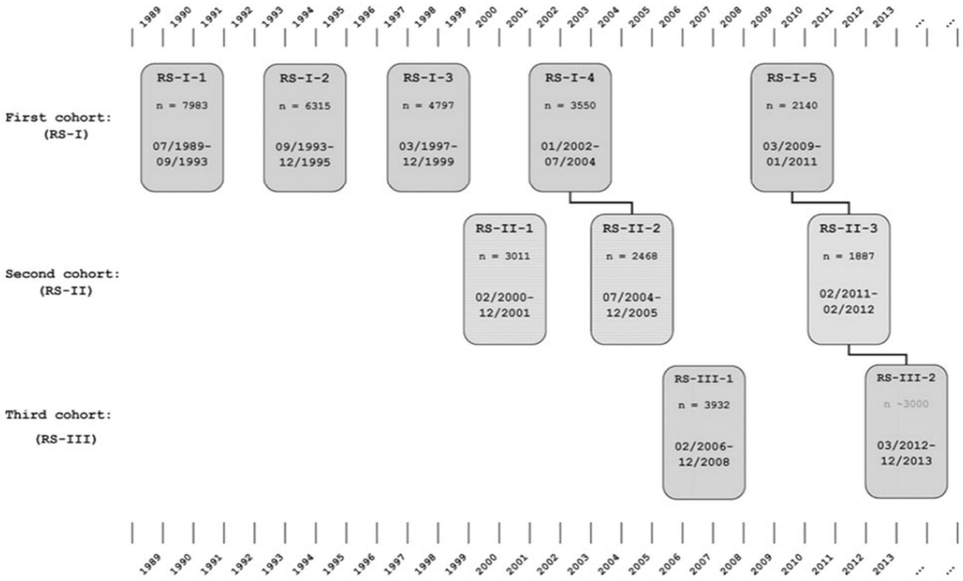


Figure 1: "Diagram of examination cycles of the Rotterdam Study (RS). RS-I-1 refers to the baseline examination of the original cohort (pilot phase 07/1989–12/1989; cohort recruitment 01/1990–09/1993). RS-I-2, RS-I-3, RS-I-4, and RS-I-5 refer to re-examinations of the original cohort members. RS-II-1 refers to the extension of the cohort with persons in the study district that became 55 years since the start of the study or those of 55 years or over that migrated into the study district. RS-II-2 and RS-II-3 refer to re-examinations of the extension cohort. RS-III-1 refers to the baseline examination of all persons aged 45 years and over living in the study district that had not been examined already (i.e., mainly comprising those aged 45–60 years). RS-III-2 refers to the first re-examination of this third cohort which will be completed by June 2014. Examinations RS-I-4 and RS-II-2 were conducted as one project and feature an identical research program. Similarly, examinations RS-I-5, RS-II-3, and RSIII-2 share the same program items." *(Adapted from Hofman et al., EJEP, 2013)*

Within the Rotterdam Study, COPD was diagnosed by an obstructive spirometry ($FEV_1/FVC < 0.70$) performed at the research center visit since 2002. Since 2009, diffusing capacity tests are additionally performed using the Master Screen® PFT Pro (CareFusion, San Diego, CA) by trained paramedical personnel according to the American Thoracic Society(ATS)/European Respiratory Society (ERS) guidelines.[22, 23] To check reproducibility, multiple efforts were required. The values of the best acceptable effort have been considered for spirometry and the mean of two acceptable and reproducible efforts for diffusing capacity. Spirometries and diffusing capacity tests that did not meet ATS/ERS acceptability and reproducibility criteria were classified as "not interpretable". In absence of spirometry, all medical information, including files from specialists and general practitioners, of subjects who used respiratory medication for at least 6 months (Anatomical Therapeutic Chemical (ATC) classification code:

R03) or indicated multiple respiratory symptoms in a questionnaire, was reviewed. Reversibility tests were not performed in this population-based setting; however, asthma patients were consistently excluded from COPD analyses. For cross-sectional analyses at the time of the research center visit, subjects with a spirometry report suggestive of a restrictive syndrome (FEV1/FVC ≥ 0.7 and FVC < 80% predicted) were also excluded. For longitudinal analyses, an incident COPD date was defined as the date of COPD diagnosis in the medical records, the date of a first COPD medication prescription or the date of an obstructive lung function examination, whichever came first. Medication use was obtained through automated linkage with pharmacy filled prescription data.

COPD is a very complex, heterogeneous disease and it is of current debate which characteristics or clusters of characteristics (i.e. phenotypes) define the disease severity best or would lead to the most optimal tailored treatment options. According to the modified GOLD criteria (2007), COPD is classified according to the severity of airflow limitation into mild ($FEV_1 \geq 80\%$), moderate ($50\% \leq FEV_1 < 80\%$), severe ($30\% \leq FEV_1 < 50\%$) and very severe COPD ($FEV_1 < 30\%$).[24] A recent GOLD update (2011) however, highlights that the classification of a patient with COPD should include the evaluation of symptoms and the history of exacerbations next to the severity of airflow limitation, leading to four categories (A, B, C, and D, *see Figure 2*).[1] COPD exacerbations are episodes of worsening of symptoms, accompanied by increased airway and systemic inflammation and physiological changes, leading to substantial morbidity and mortality.[25]

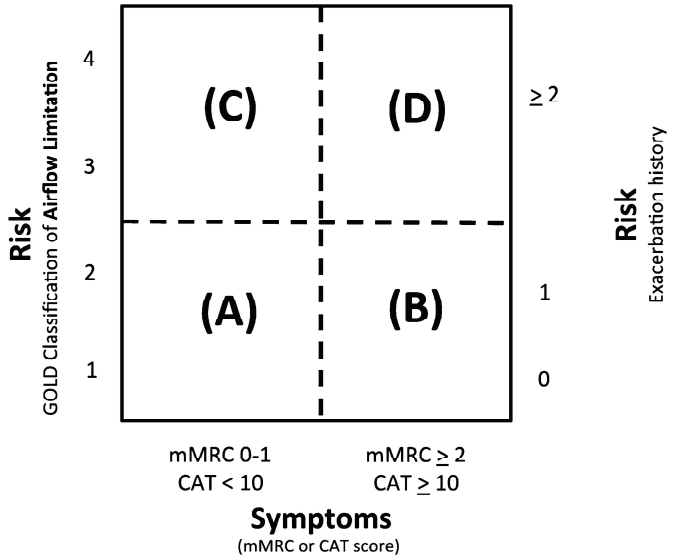


Figure 2: "Combined COPD assessment. When assessing risk, choose the highest risk according to GOLD spirometric grade or exacerbation history"

Reprinted with permission of the American Thoracic Society. Copyright © 2014 American Thoracic Society. Vestbo J, Hurd SS, Agusti AG, et al. 2013 Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med. 187(4):347-65. Official Journal of the American Thoracic Society.

Group A consists of patients with a low risk and less symptoms (i.e. mild or moderate airflow limitation), (n)one exacerbation a year and a modified Medical Research Council Dyspnea Scale (mMRC) grade 0 to 1 or a COPD Assessment Test (CAT) score less than 10. Group B includes patients with a low risk but with more symptoms (mMRC grade ≥ 2 or CAT score ≥ 10). Group C gathers patients with high risk (severe or very severe airflow limitation and/or ≥ 2 exacerbations a year and/or > 1 hospitalized exacerbation a year) but less symptoms. Finally, group D consists of patients with a high risk and more symptoms.

Within the Rotterdam Study, we evaluated the ABCD distribution of a cross-sectional study sample.(*Figure 3*) Our distribution of ABCD (group A: 49.1%, group B: 35.2%, group C: 4.2% and group D: 11.5%) is similar to two recent studies which sampled from the general population in Copenhagen and Norway.[26, 27] Group C consistently represents the smallest group. Because we sampled from the general population where the airflow limitation is frequently still mild, Group A is the most prevalent group, whereas in studies recruiting patients from secondary and tertiary care, group D is the most prevalent group.[28]

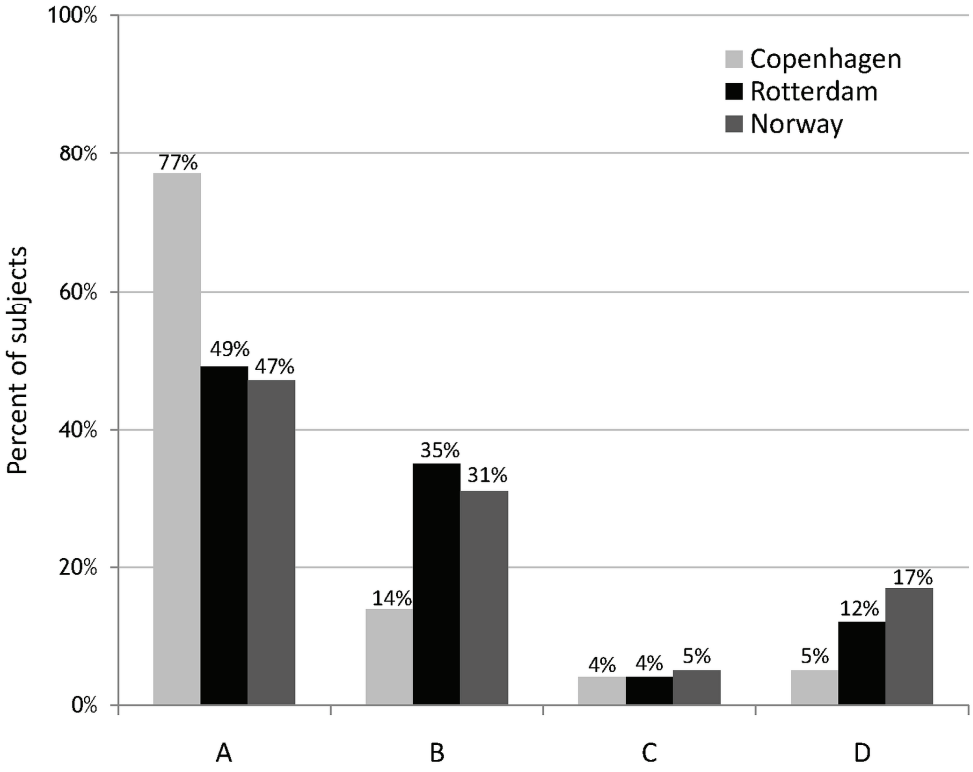


Figure 3: Comparison of the GOLD ABCD distribution of participants with COPD across studies which sampled from the general population.[26, 27]

2. Systemic effects and comorbidities of COPD

2.1 Frequently described comorbidities in COPD

A comorbidity is a disease coexisting with the primary disease of interest, here COPD.[29] It is increasingly recognized that COPD is not only affecting the lungs, as systemic consequences and several comorbid diseases often coexist and are common at any severity of COPD.[30] Known comorbidities are cardiovascular disease, metabolic syndrome, skeletal muscle dysfunction, osteoporosis, depression, and lung cancer.[1] Although the causal mechanisms by which COPD might increase the risk for other concomitant diseases is still under investigation, there is no doubt on the major impact of these comorbidities on the quality of life and survival in patients with COPD. Comorbidities not only significantly contribute to the clinical presentation of patients with COPD, their presence might increase moreover the need for hospitalization during a COPD exacerbation.[24] Furthermore, a substantial proportion of mortality is due to nonrespiratory diseases, especially in COPD patients with mild and moderate disease.[31] Therefore, the identification and treatment of comorbid diseases are key elements in COPD management.[1] In future, more holistic care might be achieved by shifting from comorbidity towards multimorbidity. Herein, the prioritized specific disease approach is left in favor of multidisciplinary collaboration to treat the co-occurrence of multiple diseases within one person.

2.2 Potential underlying mechanisms

The precise pathobiology of the association between COPD and the described comorbidities is still unknown, although several hypotheses including biological and genetic mechanisms are proposed. Next to the fact that airflow limitation and hyperinflation affects cardiac function and gas exchange, other potential underlying mechanisms as systemic inflammation, oxidative stress, physical inactivity, abnormalities of the vascular wall, accelerated aging, and protease/antiprotease imbalances were enumerated.[32] Regarding the systemic inflammation, a spill-over of the abnormal inflammatory lung response to the systemic circulation is supposed to initiate or aggravate the consequential (cardiovascular) diseases.(*Figure 4*) Patients with COPD, especially those with comorbidity or an acute exacerbation, have indeed increased levels of systemic biomarkers of inflammation and procoagulant activity, including circulating cytokines such as interleukin 6 (IL6) and acute phase response proteins as high sensitivity C reactive protein (hsCRP), and fibrinogen.[33] However, since a persistent systemic inflammation was only observed in a proportion of moderate to very severe COPD patients in a recent clinical cohort study, the exact and unique role of systemic inflammation remains subject of debate.[34] The debate is further fueled by the role of common risk factors of COPD and the frequently described comorbidities, including aging, smoking and sedentarism. These factors could also induce a chronic low grade systemic inflammation in susceptible persons leading to a multimorbidity state where COPD represents only the pulmonary abnormalities. Although a recent systematic review concluded that COPD is associated with an increased risk of cardiovascular

disease independent from (known) shared risk factors, further research might reveal yet unknown common risk factors or shed new light on the underlying mechanisms.[35]

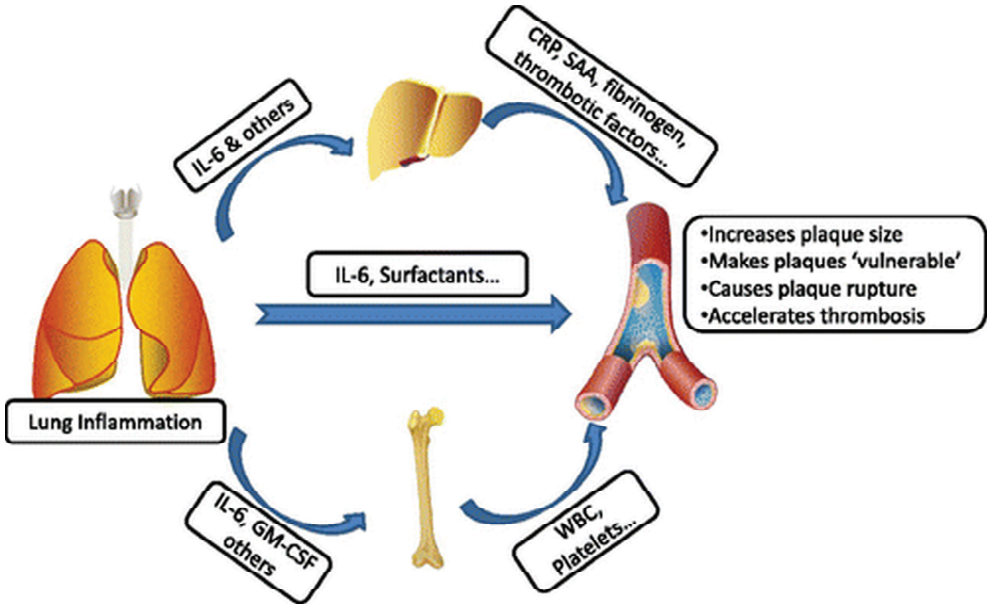


Figure 4: "Proposed mechanism by which lung inflammation causes cardiovascular disease in chronic obstructive pulmonary disease (COPD). COPD is associated with chronic lung inflammation. Some inflammatory mediators such as IL6, GM-CSF, and others escape into the systemic circulation and induce bone marrow to release inflammatory cells such as neutrophils, monocytes, and megakaryocytes that can directly invade atherosclerotic plaques. IL6 and other inflammatory cells released by the lungs can also stimulate hepatocytes to release acute phase reactants such as CRP, SAA, fibrinogen, and procoagulant factors such as factor VIII into the systemic circulation, which together confer a "proinflammatory" phenotype to the plaques. The inflamed lungs can release inflammatory proteins such as IL6 and certain surfactants that can directly promote atherogenesis. Thus, directly or indirectly (through the liver or bone marrow), the inflamed COPD lungs can make plaques more vulnerable by promoting their growth, increasing their lipid content, causing hemorrhage, and accelerating thrombosis (after plaque rupture or erosion). CRP = C-reactive protein; GM-CSF= granulocyte macrophage colony–stimulating factor; SAA = serum amyloid A; WBC = white blood count.

3. Frailty

3.1 Frailty: the identification of vulnerable elderly

One of the major rising determinants of COPD-prevalence and incidence is the aging population. In Europe, the population aged ≥ 65 years is expected to rise from 17% in 2010 to 30% in 2060, and those aged ≥ 80 from 5% to 12% over the same period.[36] The concept of frailty was introduced as being the most problematic expression of population aging.[37] Frailty is defined as a biological syndrome in which a progressive, cumulative decline in the reserve capacity of multiple physiological systems elicits an abnormal vulnerability to common stressors.[38] In short, frailty could be defined as the disability to compensate function loss. 25%-50% of people aged ≥ 85 years are estimated to be frail and these people have a substantially increased risk of adverse health outcomes including disability, falls, hospitalizations, institutionalizations and death.[37-42] The demographic change is a great challenge for social welfare systems, pensions and healthcare funding where healthy aging is becoming increasingly important. Therefore, the identification of vulnerable elderly and their risk factors is crucial to optimize prevention and treatment strategies.

3.2 Pathogenesis

Frailty is a disorder of several interrelated physiological systems and the number of abnormal systems seems more predictive for frailty than an abnormality in any of the individual systems, implying a potential threshold of age-related cumulative decline.[37, 43]

First, the aging brain might be a potential regulator of organismal aging, through hormonal feedback circuits.[37, 44] Especially microglial cells, the macrophages of the brain, and neurons with high metabolic demands, for example in the hippocampus, seem to be affected.[37] Brain injury, local and systemic inflammation activate microglial cells which become hyperresponsive with aging.[37] Moreover, the hippocampus might lose its ability of repressing glucocorticoid release by the hypothalamus due to chronically elevated glucocorticoid concentrations during aging, which exposes the entire organism to elevated stress hormone concentrations.[44] This leads us inevitably to the frail endocrine system wherein circulating hormones like insulin-like growth factor-1 (IGF-1) and dehydroepiandrosterone sulfate (DHEA-S) are decreased, possibly contributing to the observed skeletal muscle weakness.[37] A third organ system related to the development of frailty is the immune system, in which several inflammatory markers, such as white blood cell (WBC) count, CRP, IL6 and tumor necrosis factor-α (TNF-α) are elevated.[37, 45-51] IL6 in particular has been associated with anemia, decreased lean body mass and sarcopenia and is inversely correlated with IGF-1 and DHEA-S in frail elderly.[47, 52, 53] All these observations make clear that the different systems are intrinsically linked and that accumulating deficits during aging might generate a vicious circle leading towards frailty in susceptible persons.

4. Aims and outline of this thesis

The main aim of this thesis is to identify and explore systemic effects and comorbidities of COPD. First, a general aim will be to identify all COPD subjects within the Rotterdam Study by validating the lung function tests and additionally, by carefully exploring all relevant information from the study questionnaires and from the linkage of the study database with the medical files and filled prescriptions of the participants. Furthermore, as mentioned in this introduction, exacerbations are associated with an increased airway and systemic inflammation and are recently introduced in the classification of COPD patients because of their important influence on the morbidity and mortality in patients with COPD. Therefore, another general aim will be the assessment of exacerbations to identify frequent exacerbators and evaluate the influence of COPD exacerbations on the systemic effects and investigated comorbidities.

Regarding the specific aims, because there is a high need for research into the underlying inflammatory mechanisms of COPD, we want to know whether interleukin 6 (IL6) is causally involved in the pathogenesis of COPD or just a bystander. To examine this causal effect, we will use the Mendelian randomization approach in **chapter 5.** The underlying idea is that if common variation in the *IL6* gene (which raises plasma levels of IL6) modifies the susceptibility of an individual to develop COPD, then increased plasma levels of IL6 are causally related to incident COPD. Previously, our Laboratory for Translational Research in Obstructive Pulmonary Diseases demonstrated in a murine model of COPD that - although IL6 levels were significantly increased after cigarette smoke exposure - the cigarette smoke-induced pulmonary and systemic inflammation were *IL6* independent.[54] However, human studies are highly clinical relevant since tocilizumab, a humanized monoclonal IL6 receptor antagonist, is already successfully implemented in the treatment of refractory rheumatoid arthritis.

By this last sentence, I immediately introduce another need in COPD science, namely the quest to drug therapies that actually reduce the mortality in COPD patients. In **chapter 6**, we will investigate whether statins have a beneficial effect on the mortality in subjects with COPD. Because statins possess pleiotropic anti-inflammatory and immunomodulating properties, we hypothesize that potential effects are modulated by the baseline levels of high-sensitivity C-reactive protein (hsCRP), a validated biomarker of systemic inflammation in COPD. In chapter 6, we will also identify the most frequent causes of death in COPD, including death to cardiovascular causes. After exploring the systemic inflammation in COPD, this leads us to the aim of studying comorbidities in COPD. A great advantage of the Rotterdam Study is that cardiovascular, dermatological, endocrine, hepatic, neurological, oncological, ophthalmic and psychiatric diseases are very well documented.[20] For this thesis in particular, an extensive assessment of sudden cardiac death, carotid artery plaques, cerebral microbleeds and stroke is crucial.

Additionally in Part II, we will investigate the association of COPD and cardiovascular mortality. Regarding the cardiovascular comorbidities, there is a need for longitudinal studies which explore the link between COPD, systemic inflammation and cardiovascular morbidity and mortality in the general population. Therefore, we will determine in **chapter 7** whether COPD is an independent risk factor for sudden cardiac death in the general population.

In Part III, we will further explore the cerebrovascular comorbidities in COPD by starting with a general overview of the current literature in **chapter 8**. In the subsequent chapters, the aim is to further explore the epidemiology of vascular dysfunction in subjects with COPD. In **chapter 9**, we want to investigate the relationship between COPD and macroangiopathy. Herein, the aim is to reveal noninvasively the composition of atherosclerotic plaques in the carotid arteries of subjects with COPD. In all participants of the Rotterdam study, ultrasonographic assessments of carotid intima-media thickness and carotid plaques are conducted.[20] Since 2007, carotid plaque components are measured using high-resolution magnetic resonance imaging (MRI) in all participants with carotid wall thickening on conventional carotid ultrasound.[55] Through the MRI scans, we have the ability to distinguish calcification, lipid and hemorrhagic components in carotid artery plaques.

In **chapter 10**, we want to investigate the relation of COPD and microangiopathy. Herein, the aim is to explore whether COPD is associated with an increased risk of cerebral microbleeds which is a marker of cerebral small-vessel disease. Furthermore, we want to explore whether the microbleed location differs from subjects with COPD to subjects without COPD. For this study, we will combine the lung function tests with the extensive brain imaging which is performed from 2005 onwards in all study participants without contra-indications using the 1.5 Tesla MRI scanner. This will allow us to identify the presence and location of cerebral microbleeds.(*Figure 5*) Finally in **chapter 11**, we like to explore whether vascular dysfunction in subjects with COPD leads to an increased risk of stroke. Within the Rotterdam Study, the history of stroke was assessed through interview and verified in medical records at baseline.[20] In addition, incident strokes are identified and further characterized and validated through the linkage of the study database with files from general practitioners, nursing home physicians, the municipality and hospital records.[20]

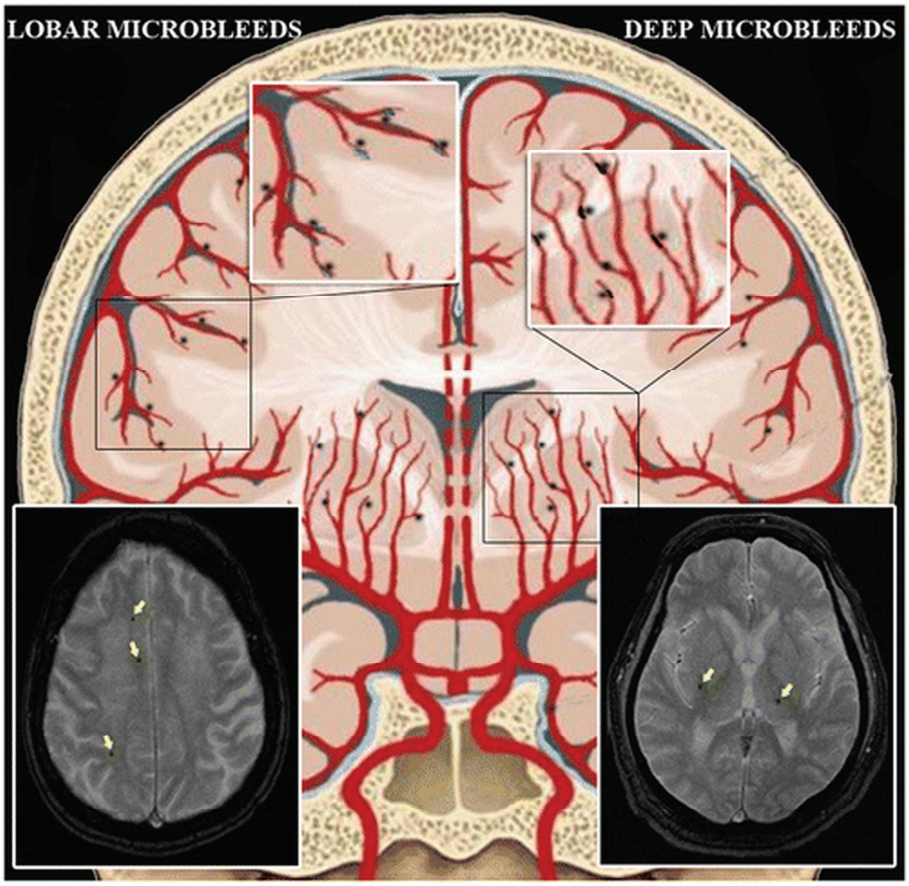


Figure 5: "The topography of cerebral microbleeds and their magnetic resonance imaging (MRI) features. Microbleeds are small collections of blood-breakdown products (hemosiderin laden macrophages) that can be seen in vivo through blood-sensitive MRI sequences. Depending on their location, microbleeds are defined as "lobar" or "deep" and reflect different pathological processes affecting the brain's small vessels. Lobar microbleeds (left side) affect small arteries, arterioles, and capillaries in the cerebral cortex, overlying leptomeninges and the gray–white matter junction; are caused by cerebral amyloid angiopathy; reflect the accumulation of amyloid β in the vessel walls (short blue lines in the upper left box); and appear as black spots in cortical or subcortical areas in the MRI images (yellow arrows in the left lower box). Deep and infratentorial microbleeds (right side) affect deep small arterioles; are caused by hypertensive arteriopathy, including lipohyalinosis and atherosclerosis; reflect damage to small perforating end arteries (short black lines in the upper right box); and appear as black spots in the basal ganglia in the MRI images (yellow arrows in the right lower box). The prevalence of deep and infratentorial microbleeds is associated with age and with cardiovascular risk factors. Also, they seem to have effects on cognition and increase the risk of stroke. Image courtesy of Amicus Visual Solutions." *Reprinted with permission of the American Thoracic Society. Copyright © 2014 American Thoracic Society. Rodriguez-Roisin R, Llufriu S, Fabbri LM. 2013 Changes in your breathing can change your brain. Am J Respir Crit Care Med. Oct 1;188(7):763-4*
Official Journal of the American Thoracic Society.

Part IV of this thesis elaborates on the potential associations between COPD and frailty. Both frailty and COPD are prevalent syndromes in the general elderly population, associated with substantial morbidity, disability and mortality. Conceptually, it is important to distinguish frailty from disability and comorbidity.(*Figure 6*) Although these three different entities are related, they are not identical.[56] Therefore, it is important not to use an instrument integrating disability or comorbidity items to measure frailty.[57] An unambiguous definition of frailty is of great importance for clinicians to identify those at an increased risk of adverse health outcomes, but also for policy makers to make cost-effective decisions in health care.[58] We will define frailty according to the physical definition wherein the nutritional status, physical activity, gait velocity, grip strength and exhaustion are evaluated.[38] In **chapter 12**, the aim is to assess frail persons according to this definition within the Rotterdam Study. Both frailty and COPD are moreover associated with a decline in function across multiple systems that in composite contribute to geriatric syndromes, including osteoporosis, cognitive decline, anemia, immune deficiency, weight loss and muscle wasting. Investigating the interrelationship between COPD and frailty may contribute to the general knowledge of both and even could help identify a new COPD phenotype. Therefore, the objective in **chapter 13** is to investigate the interrelationship between COPD and frailty. Regarding the effects on the musculoskeletal system (although vascular dysfunction might also play a role) and in this context of disability and the gait velocity assessment within frailty, we want to investigate in **chapter 14** whether COPD is associated with various gait domains and explore a potential link with falling.

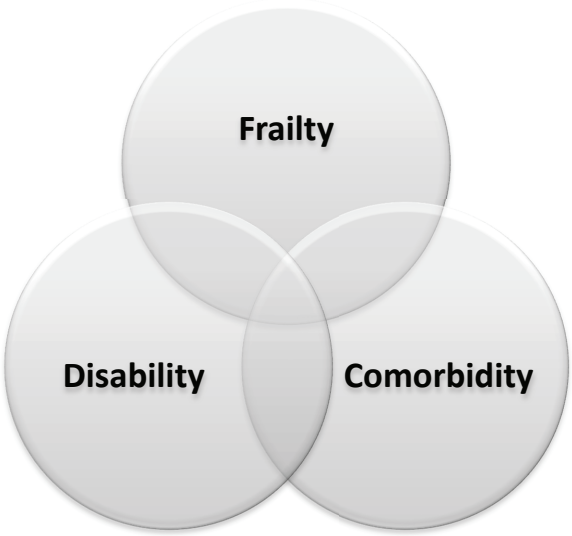


Figure 6: Although frailty, disability and comorbidity overlap, they are not identical.

PART II: COPD, SYSTEMIC INFLAMMATION AND CARDIOVASCULAR MORTALITY

ABSTRACT

Background

Cross-sectional studies have demonstrated that increased levels of interleukin 6 (IL6) are present in the airways and blood samples of patients with chronic obstructive pulmonary disease (COPD).

Objectives

To investigate the association between IL6 and the risk of COPD using a Mendelian randomization approach.

Methods

Eight common SNPs in the region of the IL6 gene were genotyped by using both TaqMan and Illumina in the Rotterdam Study, a prospective population-based cohort study consisting of 7983 participants aged 55 years or older, including 928 COPD patients. At baseline, blood was drawn in a random sample of 714 subjects to measure the IL6 plasma level. Analysis of variance, logistic regression and cox proportional hazard models -adjusted for age, gender, pack years and body mass index- were used for analyses.

Results

High levels of IL6 (>2.4 pg/ml, the highest tertile) were associated with a threefold increased risk of developing COPD, in comparison to low levels (<1.4 pg/ml, the lowest tertile). The rs2056576 SNP was associated with a 10% increase in the risk of COPD per additional T-allele. However, the association was no longer significant after adjustment. No association was found with other common SNPs in the *IL6* gene and COPD.

Conclusions

Although increased IL6 plasma levels at baseline are associated with the risk of developing COPD during follow-up, there was no strong evidence for an association between common variation in the IL6 gene and the risk of COPD.

van Durme YMTA, Lahousse L*, Verhamme KMC, Stolk L, Eijgelsheim M, Loth DW, Uitterlinden AG, Breteler MMB, Joos GF, Hofman A, Stricker BH, Brusselle GG. 2011 Mendelian Randomization Study of Interleukin 6 in Chronic Obstructive Pulmonary Disease. Respiration. 82:530–53.*

5. The role of Interleukin-6 in COPD development

INTRODUCTION

Worldwide, chronic obstructive pulmonary disease (COPD) is a major and still increasing health problem. The disease is characterized by progressive airflow limitation, driven by an abnormal inflammatory response of the airways to inhaled particles and fumes.[59, 60] Recently, COPD has been associated with elevated levels of several inflammatory markers in the systemic circulation including interleukin 6 (IL6) and C-reactive protein (CRP).[61-65] This may be a key link to various extrapulmonary effects which have been described in relation to COPD, such as cachexia, osteoporosis, skeletal muscle wasting, diabetes mellitus, cardiovascular disease and depression.[30, 66, 67]

Interleukin 6 (IL6) is a powerful stimulant of the immune system and has been shown to be present in sputum, broncheoalveolar lavage fluid and in blood samples of stable patients with mild COPD after a hypoxic challenge and during COPD exacerbations.[68-70] Elevated plasma levels of IL6 also appear to correlate with disease severity.[71] IL6 is a protein with a helical structure, mainly secreted by monocytes and macrophages, but can also be released in the airways by bronchial epithelial cells and fibroblasts.[72] IL6 is a multifunctional cytokine, originally identified as a B-cell differentiation factor, with a central role in the induction of hepatically derived acute-phase proteins such as CRP.[73-76] In addition, IL6 also mediates the activation, growth, differentiation and survival of T-cells and has emerged as a key regulatory signal in the development of the new T-helper17 subset.[75, 77]

The rs1800795 single nucleotide polymorphism (SNP) in the promoter region of the *IL6* gene influences plasma levels of IL6 and CRP.[78, 79] Although variation in this SNP has been associated with the risk and outcome of several inflammatory diseases, three studies could not find any association between this SNP and the risk of COPD.[80-82] In contrast, a recent report associated the rs1800795 SNP with rapid decline of FEV_1 and susceptibility to COPD in smokers.[83] In addition, other variants in the *IL6* gene have been linked with the risk of COPD.[81, 84]

In this Mendelian randomization study we hypothesized that common variation in the *IL6* gene that raises plasma levels of IL6, would modify the susceptibility of an individual to develop COPD, implicating that increased plasma levels of IL6 would be causally related to incident COPD. We tested these hypotheses in a large prospective population-based cohort with more than 15 years of follow-up.

METHODS

Study design and setting

The present study is part of the Rotterdam Study, a population-based cohort study to assess the occurrence of, and risk factors for chronic diseases in the elderly. Objectives and methods of the Rotterdam Study have been described elsewhere.[21, 85] In short, the Rotterdam Study cohort includes 7983 participants aged ≥ 55 years, living in Ommoord, a

well-defined suburb of the city of Rotterdam, the Netherlands. Almost all participants (99.8%) are of Caucasian descent. Baseline data were collected from 1989 until 1993. Participants were visited at home at the start of the study for a standardized interview on their health status. Since the start of the Rotterdam Study, each participant visited the research center every 2 to 3 years, during which several measurements were performed. In addition, participants were continuously monitored for the onset of major events which occurred during follow-up through automated linkage with files from general practitioners. Trained research assistants collected information from medical records of the general practitioners, medical specialists (e.g. respiratory physicians), hospitals and nursing homes. The medical ethics committee of the Erasmus Medical Center, Rotterdam, and the review board of The Netherlands Ministry of Health, Welfare and Sports, approved the study. Participants gave written informed consent and permission to retrieve information from treating physicians.

Study participants

Information about the COPD cohort definition and spirometry examination has been described previously.[5] In short, the diagnosis of COPD was classified as definite or probable. Definite COPD was defined by a moderate-to-severe obstructive spirometry ($FEV_1/FVC < 0.7$ and $FEV_1 < 80\%$ predicted), or as COPD diagnosed by a specialist in internal medicine (mainly respiratory physicians or internists with a subspeciality in respiratory medicine) based upon the combination of clinical history, physical examination and spirometry. Probable COPD was defined by a mild obstructive spirometry ($FEV_1/FVC < 0.7$ and $FEV_1 \geq 80\%$ predicted) or as COPD diagnosed by a physician in another medical speciality (e.g. a general practitioner). For the risk analyses, participants with indication of asthma in the medical files or a spirometry report suggestive of restrictive respiratory disease ($FEV_1/FVC \geq 0.7$ and $FEV_1 < 80\%$ predicted), were excluded. Spirometry was performed by using a SpiroPro® portable spirometer (Erich Jaeger GmbH, Hoechberg, Germany), according to the ATS/ERS guidelines by trained paramedical personnel.[22] Forced expiratory volume in one second (FEV_1), forced vital capacity (FVC) and FEV_1/FVC ratio were measured; the spirogram (volume-time curve) and maximal expiratory flow-volume curve were also recorded. Spirometries that yielded results which did not meet ATS/ERS criteria for acceptability and reproducibility were classified as “not interpretable” (9.6% of spirometries). The interpretation of spirometries was performed independently by two researchers; in case of discordance (6.1% of spirometries), the final protocol was made by a senior respiratory physician.[5]

The index date was defined as the date of diagnosis of COPD found in the medical reports or the date of a first prescription of COPD medication in someone with definite or probable COPD or the date of the obstructive lung function examination, whichever came first. Follow-up time was defined as the time period between cohort entry and the diagnosis of COPD, death, loss to follow-up or the end of the study period on 31 December 2004, whichever came first.

Measurement of IL6 plasma levels and common variation in the IL6 gene

Plasma IL6 and high-sensitivity (hs)CRP levels, were measured as previously described.[61, 86, 87] IL6 plasma levels were measured in 714 subjects, a 10% random subset of 7081 participants who gave permission to give blood. As previously described, a venapuncture was performed at the start of the study (1990-1993) by application of minimal stasis with a 21-gauge butterfly needle tube (Surflo winged infusion set, Terumo).[86, 88] Non-fasting blood was collected in tubes containing 0.129 mol/L sodium citrate at 4°C. Plasma was collected after centrifugation for 10 min at 3000 rpm. Subsequently, platelet-free plasma was obtained by centrifugation for 10 min at 10000 rpm, immediately frozen in liquid nitrogen, and stored at -80°C. IL 6 levels were measured by using a commercially available ELISA (Quantikine HS IL6 kit from R&D Systems Europe). The inter assay coefficient of variation for IL 6 was 8.7%.

The rs1800795 (-174G/C) SNP was genotyped in 6071 participants by using a TaqMan allelic discrimination assay (Applied Biosystems, Foster City, California, USA). In short, DNA was isolated using standard procedures and genotyping was performed using baseline samples stored at -80°C. Genotypes were determined in 2 ng genomic DNA with the TaqMan allelic discrimination assay. Primer and probe sequences were optimized by using the SNP-assay-by design service of Applied Biosystems. Reactions were performed with the TaqMan Prism 7900HT 384 wells format in 2 µL of reaction volume.

In addition, seven common tagging SNPs within the *IL6* gene ± 10 kb and minor allele frequency (MAF) > 0.10 (rs7801617, rs7805828, rs1880242, rs10499563, rs2056576, rs1554606 and rs10242595; chromosome 7, NCBI build) were extracted based on data from the Illumina Infinium II HumanHap550K BeadChip in 5639 participants (version 3) that genotyped participants with available DNA from the Rotterdam Study, as previously described.[87] Genotyping was performed at the Department of Internal Medicine, Erasmus Medical Center using BeadStudio software (version 0.3.10.14) for genotyping calling. Participants with call rate < 97.5%, excess autosomal heterozygosity, sex mismatch, or outlying identity-by-state clustering estimates were excluded.

Statistical analyses

Demographic and clinical characteristics of the study population are expressed as the median and the interquartile range (IQR) for description in the text. The eight SNPs in the *IL6* gene were tested for Hardy Weinberg Equilibrium with χ^2 statistics.

First, we studied the association between the eight SNPs in the *IL6* gene and COPD. A logistic regression model -adjusted for age at baseline, gender, total amount of pack years smoked and BMI- was used to calculate odds ratios (OR) and the 95% confidence intervals (CI) for the association between the different genotypes and COPD. Overall, additive genetic models in which persons homozygous for the common allele were used as the reference category were applied. All prevalent and incident COPD cases were used for analyses.

Second, hazard ratios (HR) and the 95% CI for the association between plasma levels of IL6 and incident COPD were evaluated by use of Cox proportional hazard analyses. Hereto, prevalent cases -subjects with a follow-up time of less than three years since the index date- were excluded. The Cox models were adjusted for covariates that changed the point estimate by more than 10% or that were biologically plausible according to previous literature. The following covariates were considered as potential confounders: age, gender, body mass index, the total amount of pack years smoked, diabetes and cardiovascular comorbidity. Cigarette pack years were computed as duration of smoking (years) multiplied by the number of smoked cigarettes, divided by 20. Missing pack years values were imputed for the analyses by predicted values, based on a logistic regression analysis adjusted for age and gender. Diabetes mellitus was defined as a non-fasting or 2-hour post load glucose level of ≥ 11.1 mol/l or antidiabetic medication use at baseline. Cardiovascular disease included myocardial infarction, coronary artery bypass graft, percutaneous transluminal coronary angioplasty, atrial fibrillation and heart failure. Interaction terms and subanalyses were introduced to explore for potential effect modification.

Differences in the IL6 and hsCRP levels according to the *IL6* genotypes were tested by means of analysis of variance (ANOVA). All participants with a blood sample were used for analyses. Because of the skewed absolute values of hsCRP and IL6 in our cohort, the natural logarithmic transformation of these markers was entered as a continuous variable in the models.

The difference between hazard ratios was interpreted by reference to 95% confidence intervals for each of the parameters of interest. P-values below the conventional level of significance ($p < 0.05$) were considered as statistically significant. All statistical analyses were performed using PASW Statistics for Windows version 18 (SPSS Inc, Chicago, IL).

RESULTS

Baseline characteristics

Of the 7983 participants in the Rotterdam Study, 7081 donated a blood sample. 288 subjects were excluded because of a diagnosis of asthma in the medical files or a spirometry report suggestive of a restrictive syndrome. The rs1800795 SNP was successfully genotyped by the Taqman method in 6071 participants of whom 779 had COPD. Regarding the other seven SNPs, Illumina genotyping data were available in 5639 participants of whom 732 had COPD. (*Figure 1*) With these data we cover 76%, 84% and 92% of the common variation in the *IL6* gene as present in the Caucasion HapMap reference population at r^2 tresholds of 1, ≥ 0.7 and ≥ 0.5, respectively.

Among the participants who were randomly selected from the cohort for IL6 plasma level measurement, subjects with prevalent COPD or a follow-up time of less than 3 years were excluded from the analyses. In the remaining subgroup of 572 participants, 55 subjects developed COPD during a median follow-up time of 12 years (IQR, 4) (*Figure 1*). *Table 1*

shows the baseline characteristics of the study populations. Overall, the COPD cases were more often male and (current) smokers.

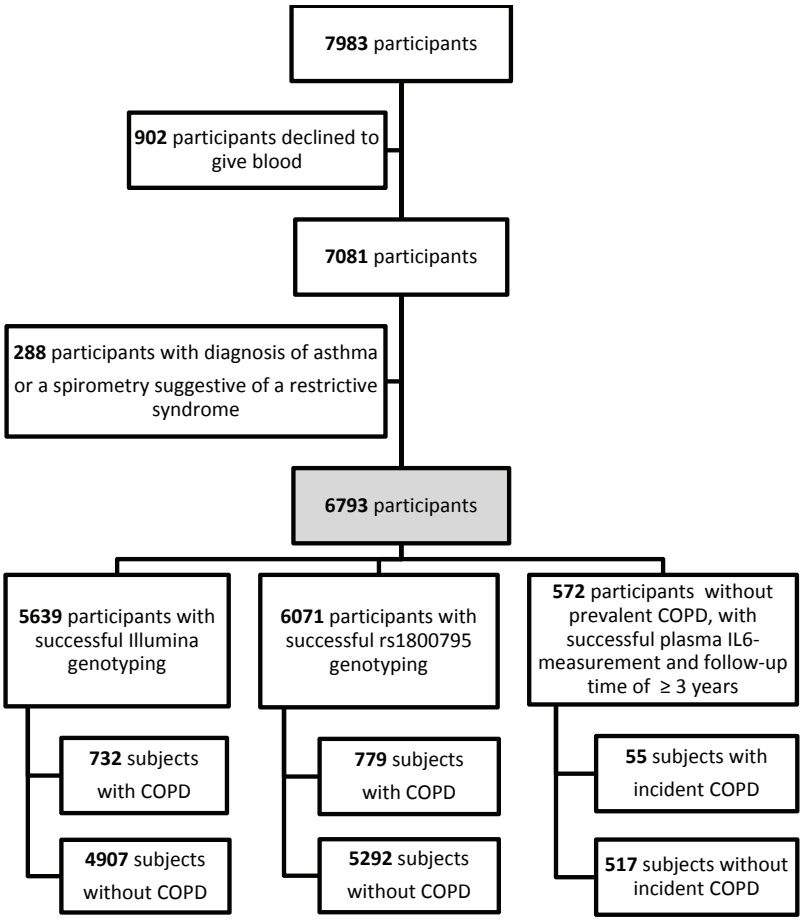


Figure 1: Cohort definition.

Table 1: Baseline characteristics of the study population.

Baseline characteristics	rs1800795 genotyped by Taqman			7 SNPs genotyped by Illumina			Random sample of subjects with IL6 plasma		
	COPD	Controls	Overall	COPD	Controls	Overall	COPD	Controls	Overall
Number of participants	779	5292	6071	732	4907	5639	55	517	572
Median age at entry (years)	67 (62-72)	69 (62-76)	68 (62-76)	67 (62-72)	69 (62-76)	68 (62-76)	66 (61-72)	70 (62-77)	69 (62-77)
Male	448 (58)	2031 (38)	2479 (41)	422 (58)	1879 (38)	2301 (41)	37 (67)	230 (45)	267 (47)
Smoking behaviour									
Never smoker	121 (16)	1953 (37)	2074 (34)	109 (15)	1812 (37)	1921 (34)	8 (15)	186 (36)	194 (34)
Current smoker	313 (40)	1042 (20)	1355 (22)	296 (40)	980 (20)	1276 (23)	22 (40)	93 (18)	115 (20)
Former smoker	340 (44)	2136 (40)	2476 (41)	322 (44)	1970 (40)	2292 (41)	25 (46)	229 (44)	254 (44)
Missing	5 (1)	161 (3)	166 (3)	5 (1)	145 (3)	150 (3)	0 (0)	9 (2)	9 (2)
Median pack years	26 (9-45)	4 (0-25)	6 (0-29)	26 (9-45)	4 (0-25)	7 (0-29)	30 (15-49)	6 (0-26)	9 (0-30)
Body mass index (kg/m²)	25 (23-28)	26 (24-28)	26 (24-28)	25 (23-28)	26 (24-28)	26 (24-28)	26 (23-28)	26 (24-29)	26 (24-28)
Diabetes	64 (8)	566 (11)	630 (10)	60 (8)	526 (11)	586 (10)	4 (7)	38 (7)	42 (7)
Cardiovascular comorbidity	246 (32)	1625 (32)	1871 (32)	226 (31)	1501 (32)	1727 (32)	14 (26)	142 (28)	156 (28)
Median IL6 plasma level (pg/ml)	2.0 (1.4-3.5)	1.9 (1.2-3.1)	1.9 (1.2-3.1)	2.0 (1.3-3.3)	1.9 (1.2-3.0)	1.9 (1.2-3.0)	2.0 (1.3-3.6)	1.9 (1.2-2.9)	1.9 (1.2-3.0)

Categorical variables are expressed as count (percentage). Values of continuous variables are expressed as median (25-75 percentiles).
Abbreviations: COPD=Chronic Obstructive Pulmonary Disease; IL6= Interleukin 6; SNPs = Single Nucleotide Polymorphisms.

Association between common variants in the IL6 gene and COPD

The rs1800795 TaqMan genotyped SNP was in Hardy Weinberg equilibrium ($\chi 2 = 1.79$, $p = 0.181$). The C-allele of the rs1800795 SNP was positively associated with higher hsCRP levels. (*Table 2*). No association was found between the rs1800795 SNP and the total group of COPD patients.(*Table 3*)

All seven common SNPs genotyped on Illumina arrays were in Hardy-Weinberg equilibrium. None of the seven SNPs were significantly associated with plasma IL6 levels. Three SNPs (rs10499563, rs1554606 and rs10242595) correlated significantly with plasma hsCRP levels .(*Table 2*) Only the rs2056576 SNP demonstrated an association with COPD (OR 1.1; 95% CI 1.01-1.27; *Table 3*); however, this was no longer significant after adjustment for age, gender, the total amount of pack years smoked at the start of the study and BMI. Prevalent diabetes or cardiovascular diseases did not substantially change the risk estimates. Additional subanalyses demonstrated that the increase in risk for this SNP was most pronounced in females (OR 1.2; 95% CI 1.02-1.43) and in never-smokers (OR 1.4; 95% CI 1.02-1.80).

Table 2: Association between common SNPs in the *IL6* gene and plasma IL6 and serum hsCRP levels.

***IL6* gene** Db SNP	**Genotype**	**IL6 level (pg/ml)**			**hsCRP level (mg/L)**		
		Number	**Ln IL6[a]**	**P-value[c]**	**Number**	**Ln CRP[b]**	**P-value[c]**
rs1800795	GG	238	0.72 (0.05)		2151	0.56	
	GC	311	0.72 (0.04)	0.990	2839	0.65	**0.006**
	CC	102	0.70 (0.07)	0.802	996	0.65	**0.026**
rs7801617	GG	466	0.71 (0.03)		4479	0.62	
	GA	120	0.67 (0.07)	0.675	1017	0.63	0.785
	AA	13	0.77 (0.17)	0.766	66	0.74	0.359
rs7805828	GG	191	0.64 (0.05)		1754	0.64	
	GA	306	0.73 (0.04)	0.171	2753	0.63	0.774
	AA	102	0.72 (0.08)	0.391	1054	0.57	0.087
rs1880242	TT	172	0.73 (0.06)		1648	0.59	
	TG	311	0.72 (0.04)	0.912	2726	0.64	0.078
	GG	113	0.63 (0.07)	0.255	1176	0.63	0.323
rs10499563	TT	330	0.68 (0.04)		3263	0.65	
	TC	237	0.70 (0.04)	0.719	1989	0.58	**0.036**
	CC	30	0.91 (0.16)	0.091	307	0.61	0.569
rs2056576	CC	281	0.69 (0.04)		2473	0.62	
	CT	258	0.71 (0.04)	0.689	2446	0.61	0.690
	TT	60	0.73 (0.11)	0.688	645	0.67	0.347
rs1554606	GG	197	0.69 (0.05)		1828	0.56	
	GT	297	0.72 (0.04)	0.658	2712	0.65	**0.003**
	TT	105	0.65(0.07)	0.589	1018	0.65	**0.031**
rs10242595	GG	295	0.68 (0.04)		2811	0.65	
	GA	241	0.74 (0.04)	0.348	2280	0.60	0.061
	AA	63	0.62 (0.08)	0.556	472	0.53	**0.017**

ANOVA was used for analyses. * Seattle SNP database number.

[a] Ln IL6 values are mean (standard error). [b] Ln CRP-values are mean (standard error).

[c] P-values (2-tailed) are calculated by a contrast test of the first genotype versus the second genotype; the first genotype versus the third genotype.

Abbreviations: hsCRP= High-sensitivity C-reactive protein; IL6= Interleukin 6; Ln= Logaritmus Naturalis; SNPs = Single Nucleotide Polymorphisms.

Table 3: Association between common SNPs in the *IL6* gene and COPD.

***IL6* gene** Db SNP No.*	**No. Patients/ cohort**	**Model 1**			**Model 2**		
		OR	**95% CI**	**P-value**	**OR**	**95% CI**	**P-value**
rs1800795	779/6071	1.0	0.87-1.08	0.602	1.0	0.88-1.10	0.779
rs7801617	731/5637	1.0	0.87-1.24	0.678	1.1	0.89-1.29	0.460
rs7805828	732/5637	1.1	0.95-1.18	0.339	1.0	0.91-1.15	0.694
rs1880242	732/5625	0.9	0.84-1.05	0.267	1.0	0.85-1.07	0.425
rs10499563	731/5634	0.9	0.82-1.07	0.334	0.9	0.81-1.06	0.246
rs2056576	732/5639	**1.1**	**1.01-1.27**	**0.037**	1.1	0.99-1.25	0.076
rs1554606	731/5633	1.0	0.89-1.11	0.925	1.0	0.91-1.14	0.752
rs10242595	732/5638	1.1	0.93-1.19	0.404	1.0	0.92-1.17	0.560

Additive genetic models were used for analyses. * Seattle SNP database number.

Model 1: not adjusted for confounders.

Model 2: adjusted for age, gender, total amount of pack years smoked at the start of the study and BMI.

Abbreviations: CI=Confidence Interval; COPD= Chronic Obstructive Pulmonary Disease; IL6= Interleukin 6; OR= Odds Ratio; SNPs = Single Nucleotide Polymorphisms.

Association between IL6 plasma levels and incident COPD

The median IL6 plasma level was 2.0 pg/ml (IQR 2.3) in COPD cases and 1.9 (IQR 1.7) in control subjects (*Table 1*). Female COPD cases had a significantly lower log mean IL6 plasma level (1.8 pg/ml) than men (2.7 pg/ml) (p=0.011). When stratified according to smoking, current smokers showed the highest log mean IL6 level (2.4 pg/ml) in comparison to participants who had never smoked (1.9 pg/ml, p=0.004) or who were former smokers (2.0 pg/ml, p=0.011).

An elevated plasma level of IL6 (>2.4 pg/ml, the highest tertile) was associated with a threefold increased risk to develop definite COPD (95% CI, 1.20 - 8.13, p = 0.020), in comparison to a low level of IL6 (<1.4 pg/ml, the lowest tertile) (*Table 4*). The risk remained significantly increased when the analyses were adjusted (95% CI 1.11 - 8.76, p = 0.031). The increase in risk was less pronounced and no more significant in the total group of definite and probable COPD patients (HR 1.9; 95% CI 0.97-3.53; p=0.062). Subanalyses suggested that the increase in risk was most pronounced in men and smokers; however, comparative numbers of women and never-smokers were low. When analyses were restricted to participants with a study acquired spirometry, the association between an elevated plasma level of IL6 and the risk to develop definite COPD remained significant (HR 3.7; 95% CI 1.19-11.27; p = 0.024; *Table 5*). Again the increase in risk was less pronounced but still significant in the total group of definite and probable COPD participants (HR 2.7; 95%CI 1.22-6.06; p=0.014).

Table 4: Association between plasma IL6 levels and incident COPD.

COPD Definition	No. cohort	IL6 plasma level	Model 1			Model 2		
			HR	95% CI	P-	HR	95% CI	P-
Definite COPD	6 / 182	< 1.40	1.0	*Reference*		1.0	*Reference*	
	8 / 182	1.40 - 2.40	1.6	0.55 - 4.57	0.393	1.6	0.54 - 4.75	0.397
	14 / 181	> 2.40	**3.1**	**1.20 - 8.13**	**0.020**	**3.1**	**1.11 - 8.76**	**0.031**
Definite & Probable COPD	16 / 191	< 1.39	1.0	*Reference*		1.0	*Reference*	
	17 / 191	1.39 - 2.40	1.3	0.65 - 2.55	0.469	1.2	0.61 - 2.51	0.563
	22 / 190	> 2.40	1.9	0.97 - 3.53	0.062	1.6	0.80 - 3.29	0.178

Cox proportional hazard models were used for analyses.
Model 1: not adjusted for confounders.
Model 2: adjusted for age, gender, total amount of pack years smoked at the start of the study and BMI.
Abbreviations: CI=Confidence Interval; COPD=Chronic Obstructive Pulmonary Disease; IL6= Interleukin 6; HR= Hazard Ratio; SNPs = Single Nucleotide Polymorphisms.

Table 5: Association between plasma IL6 levels and incident COPD confirmed by spirometry performed in the Rotterdam Study.

COPD Definition	No. cohort	Log IL6 level	Model 1			Model 2		
			HR	95% CI	P-	HR	95% CI	P-
Definite COPD*	6 / 66	< 1.40	1.0	*Reference*		1.0	*Reference*	
	4 /33	1.40 - 2.40	1.4	0.40 - 5.07	0.579	1.1	0.29 - 3.94	0.913
	11 /26	> 2.40	**5.6**	**2.07 – 15.16**	**0.001**	**3.7**	**1.19 -11.27**	**0.024**
Definite & Probable COPD*	12 /72	< 1.39	1.0	*Reference*		1.0	*Reference*	
	7 / 35	1.39 - 2.40	1.3	0.51 - 3.31	0.578	1.0	0.38 - 2.62	0.994
	15 / 31	> 2.40	**3.5**	**1.63 - 7.47**	**0.001**	**2.7**	**1.22 - 6.06**	**0.014**

Cox proportional hazard models were used for analyses. *Only participants with a study acquired spirometry were included.

Model 1: not adjusted for confounders.

Model 2: adjusted for age, gender, BMI and total amount of pack years smoked at the start of the study.

Abbreviations: CI=Confidence Interval; COPD=Chronic Obstructive Pulmonary Disease; IL6= Interleukin 6; HR= Hazard Ratio

DISCUSSION

The results of this study demonstrate that increased IL6 plasma levels at baseline were associated with a higher risk to develop COPD during follow-up. This effect remained significant after adjustment for several confounders and despite the exclusion of incident cases in the first three years of follow-up. In contrast, we found no strong evidence for an association between various common variants in the *IL6* gene and the risk of COPD. Using the Mendelian randomization approach previously used for the association with CRP, we demonstrate that also elevated plasma IL6 is not causally related to COPD.[61, 64] The rs1800795 SNP has previously been associated with susceptibility and outcome to several acute and chronic inflammatory diseases, such as juvenile chronic polyarthritis, type I diabetes mellitus, atherosclerosis and Alzheimer disease.[78, 89-91] In COPD, patients carrying the *IL6* GG genotype of this SNP showed higher plasma levels of IL6 and higher pulmonary artery pressure than those carrying the CC or CG genotype.[92] These results raise the possibility that individuals homozygous for the C-allele may have a better prognosis from respiratory inflammatory diseases because of a less pronounced IL6 mediated acute phase response. The results of our study demonstrate that the most frequently studied SNP in the promoter region of the *IL6* gene, rs1800795, did not influence the risk of COPD. Regarding other common variants in the *IL6* gene, the rs2056576 SNP demonstrated a small increase in the risk for COPD. However, the associations were no longer significant after adjustment for age, gender, total amount of pack years smoked and BMI. In accordance with these results, we recently demonstrated in a murine model of COPD that - although IL6 levels were significantly increased after cigarette smoke exposure - the cigarette smoke-induced pulmonary and systemic manifestations were *IL6* independent.[54]

We did not find an association between the eight investigated common variants in the *IL6* gene and the development of COPD, confirming preliminary evidence from earlier reports.[80-

[82] More recently, Smolonska *et al.* and Castaldi *et al.* both published a meta-analysis regarding genetic association studies of COPD using the candidate gene approach. Also, in these meta-analysis, they could not demonstrate a significant effect of the -174 G/C IL6 polymorphism on the risk of COPD.[93, 94] In contrast, a recent study by He *et al.* demonstrated that the rs1800795 SNP was associated with an accelerated decline in FEV_1 and susceptibility to COPD in smokers.[83] In addition, Cordoba-Lanus *et al.* did associate another SNP (rs1800796) with COPD and Yanbaeva *et al.* showed that a haplotype of the *IL6* gene was associated with an increased risk of moderate-to-severe smoking-induced COPD.[81, 84] However, the design of these studies has several limitations. A first limitation is the small sample size of both the COPD and the control groups, which may lead to spurious results and can compromise the power for the genetic association analysis. Secondly, several studies had a cross-sectional design with its difficulty to judge the sequence of cause and effect. Thirdly, the studies were not population-based which makes them prone to selection bias. Lastly, the studies of He *et al.* and Yanbaeva *et al.* only included smoking subjects.[83, 84]

There was no clear functional link between the different *IL6* gene variants and the plasma IL6 level although rs1800795 and three other SNPs (rs10499563, rs1554606 and rs10242595) correlated significantly with plasma hsCRP levels. IL6 plasma concentrations show considerable interindividual variability in acute and chronic inflammation. It has been speculated that these differences may be determined genetically by variations in the *IL6* gene, such as the rs1800795 polymorphism.[73] However, there is disagreement concerning the question whether any genotype is associated with higher IL6 plasma concentrations.[78, 95-97] It is possible that the effect is dependent on interactions between several SNPs in the *IL6* gene and that one SNP is not an optimal marker in this respect. Nevertheless there is agreement between our results and previous data by Vickers *et al.* and a genome-wide association study of Okada *et al.* which also show significant associations between SNPs in the promoter region of *IL6* and serum CRP levels.[79, 98]

The strengths of our study are the prospective, population-based cohort design and the large number of study subjects -almost 8000- with similar data collection procedures for every participant. In this large ongoing cohort study, more than 900 well-defined COPD cases were identified over a total follow-up time of 15 years (1989-2004), as previously described.[5] All data collection during follow-up of the cohort was performed in a prospective manner, without knowledge of future disease or research hypothesis, making selection and information bias unlikely. The prospective nature of this cohort study makes the results less prone to bias and made it possible to adjust analyses for established risk factors and confounders.

Possible limitations of the study are the limited sample size of the plasma IL6 measurements, since they were only analyzed in a (random) subgroup of the total cohort, the absence of repeated measurements of the IL6 level and the absence of a study acquired spirometry and high resolution computed tomography of the chest of each participant. Therefore, we

cannot completely exclude the presence of bronchiectasis and/or bronchial asthma, which could affect the blood IL6 levels of COPD participants and controls. The limited sample size of plasma IL6 measurements might explain the lack of association between the *IL6* genotypes and IL6 plasma levels in our study cohort. Still, we observed a significant association between hsCRP levels and the rs1800795 *IL6* genotype, as previously described.[79] Regarding the genotype data, there is a chance of missing associations with variants in low linkage disequilibrium with the SNPs. Furthermore, the results of this study are restricted to an European ancestry population, since they were based on data from the HapMap CEU sample.

In conclusion, previous evidence has identified the presence of increased levels of IL6 in the plasma and airways of patients with COPD. However, it was not known if IL6 was causally involved in the pathogenesis of COPD or just an innocent bystander of the disease, related to smoking or other risk factors. The answer to this question became of clinical relevance with the recent development of tocilizumab, a humanized monoclonal IL6 receptor antagonist, which is already successfully used in refractory rheumatoid arthritis. In this Mendelian randomization study, we have demonstrated that elevated plasma levels of IL6 are significantly associated with an increased risk of incident COPD. In contrast, there was no strong evidence for an association between common variants in the *IL6* gene and the risk of developing COPD.

ABSTRACT

Background

Studies suggest that statins decrease mortality in COPD patients but it is unknown which patients might benefit most.

Objectives

We investigated whether statins were associated with reduced mortality in COPD patients and whether effects differed according to baseline high-sensitivity C-reactive protein (hsCRP) concentration, a marker of systemic inflammation.

Methods

This nested case-control study was part of the Rotterdam Study, a prospective population-based cohort study among 7983 subjects ≥ 55 years. Using automated pharmacy records, we evaluated statin use of 363 cases (COPD patients who died during follow-up of 17 years) with 2345 age and sex matched controls (COPD patients who survived the follow-up period of the index case).

Results

Compared to never use, long-term statin use (> 2 years) was associated with a 39% decreased risk of death in COPD patients. Stratified according to the level of systemic inflammation, long-term statin use was associated with a 78% reduced mortality if hsCRP level > 3 mg/L, versus a non significant 21% reduced mortality if hsCRP level ≤ 3 mg/L.

Conclusions

Statin use is associated with a beneficial effect on all-cause mortality in COPD, depending on the baseline level of systemic inflammation.

Lahousse L, Loth DW, Joos GF, Hofman A, Leufkens HG, Brusselle GG, Stricker BH. 2014 Statins, systemic inflammation and risk of death in COPD: the Rotterdam Study Pulm Pharmacol Ther.26(2):212-7

6. The role of high-sensitivity C-reactive protein in statin treatment

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is currently the fourth leading cause of morbidity and mortality worldwide. In industrialized countries about 400,000 COPD deaths occur each year and the mortality is expected to increase further.[99] The disease is characterized locally by a chronic inflammation of small airways and destruction of alveoli and systemically, in a subset of COPD patients, by increased markers of inflammation, including high-sensitivity C-reactive protein (hsCRP) and interleukin 6 (IL6).[13, 61, 100] The subset of COPD patients with persistent systemic inflammation has recently been shown to be associated with poor clinical outcomes despite similar lung impairment.[34] The chronic low-grade inflammation might be the key link to the occurrence of various comorbidities in COPD including cardiovascular diseases and lung cancer. Moreover, these comorbidities are the main causes of death in mild to moderate COPD; therefore, with the increased recognition of the prognostic role of comorbidities in COPD, all-cause mortality has become one of the major endpoints in the evaluation of novel therapies.[29]

Recent observational studies suggest that statins [3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors] may reduce morbidity and mortality in COPD patients.[101-103] Statins are a class of drugs mainly used to treat hypercholesterolemia and to prevent cardiovascular events. Statins reduce cholesterol synthesis by inhibition of HMG-CoA reductase in the liver and increase low density lipoprotein-cholesterol uptake from the circulation. In addition to their lipid-lowering effect, statins also possess pleiotropic anti-inflammatory and immunomodulating properties, and are able to reduce levels of inflammatory markers such as CRP.[104] CRP is a validated biomarker of systemic inflammation in COPD and increased CRP levels in patients with COPD are associated with increased mortality.[105, 106] Lee *et al.* recently showed in a randomized controlled trial with COPD patients, that pravastatin treatment significantly decreased CRP and IL6 levels compared to placebo and that the improvement of exercise tolerance was greater in those with a greater decrease of hsCRP levels and higher baseline CRP levels.[107] However, to our knowledge, studies exploring whether the beneficial effect of statins on all-cause mortality in COPD is greater in those with increased systemic inflammation, have not yet been published.

Therefore, the objective was to investigate whether statins have a beneficial effect on mortality in COPD patients with increased baseline hsCRP-levels in the Rotterdam Study, a large prospective population-based cohort study with long-term follow-up.

METHODS

Study population and design

We performed a nested case-control analysis in all COPD cases within the Rotterdam Study, a population-based cohort study aimed at assessing the occurrence of and risk factors for chronic diseases in the elderly.[108] The Rotterdam study cohort includes 7983 participants aged ≥ 55 years, living in Ommoord, a well-defined suburb of Rotterdam, the Netherlands. Almost all participants (99.8%) are of Caucasian descent. Baseline data were collected from 1989 until 1993 and each participant visits the research center every 2 to 3 years. In addition, participants are continuously monitored for the onset of major events which occur during follow-up through automated linkage with files from general practitioners. Nearly all participants (99.7%) are registered at one or more of seven automated pharmacies serving the Ommoord area. From these pharmacies, records of all filled prescriptions were available as of January 1st, 1991. The medical ethics committee of the Erasmus Medical Center, Rotterdam, and the review board of The Netherlands Ministry of Health, Welfare and Sports, approved the study. Participants gave written informed consent.

Definition of cases and controls

Cases and controls were nested in all participants of the source population of whom hsCRP was measured at baseline and of whom COPD was diagnosed by an obstructive spirometry [proportion of the forced vital capacity exhaled in the first second (FEV_1/FVC) < 0.7] during the research center or pulmonologist visits or by a general practitioner. Physician diagnosed asthma patients were excluded. The incident COPD date was defined as the date of COPD diagnosis in the medical records, or the date of a first COPD medication prescription or the date of obstructive lung function examination, whichever came first. To ensure at least three months medication history for every subject, participants of whom study follow-up started before April 1st, 1991 were excluded. Cases were COPD subjects who died between April 1st, 1991 and January 1st, 2008. The mortality date was taken as the index date. Controls were COPD subjects matched on sex and age (+/- 1 year) who were still alive on the same day of follow-up as their matched case. The duration of COPD was determined as the time between the incident COPD date and the index date.

Statin exposure

Complete information on all filled prescriptions on a day-to-day basis was obtained in automated format from the pharmacies. Subjects were classified as statin users if they had received at least one prescription for statins between start and index date. The duration of a prescription was calculated as the total number of delivered units divided by the prescribed daily number of units. The studied statins were simvastatin, pravastatin, fluvastatin, atorvastatin, cerivastatin and rosuvastatin. All drugs under study are only available on prescription in the Netherlands.

All-cause mortality & primary cause of death

Information on vital status of the Rotterdam Study participants was obtained from general practitioners and from municipal records. Mortality follow-up started at baseline and was complete until January 1st, 2008. Causes of death during follow-up in the Rotterdam Study were coded according to the International Classification Of Diseases (ICD)-10.[109] The following categories were applied for description in the text: cardiac mortality (ICD-10: I21-I73, R96), pulmonary mortality (ICD-10: J15-J44), death from bronchial carcinoma (ICD-10: C34), death from other malignancies (ICD-10: C15-C96 except C34) and other causes of death (ICD-10: all other used codes).

Covariables

The nested case-control approach makes it possible to account for age and gender by matching and to adjust for other drug use at the index date. Therefore, we adjusted mortality risk estimates for use of cardiovascular drugs (antihypertensives, diuretics, β-blockers, calcium channel blockers and agents acting on the renin-angiotensin system, ATC C02, C03, C07, C08 & C09 respectively), antidiabetics (ATC A10) and corticosteroids for systemic use (ATC H02) on the index date. Furthermore, the duration of COPD at index date and the following covariables at baseline were considered as potential confounders: pack years of cigarette smoking, total serum cholesterol, hsCRP, systolic blood pressure, body-mass index (BMI), diabetes mellitus and cardiovascular covariables (myocardial infarction, coronary artery bypass graft, percutaneous transluminal coronary angioplasty, atrial fibrillation and heart failure); and their assessment has been described previously.[61, 100] One prerequisite to be a confounder is that the variable is associated with the exposure, here statin use. Therefore, the relationship between the potential confounder and statin use (yes/no) was evaluated for categorical variables with a Chi Square test and for continuous variables with a Mann-Whitney U test.

Statistical analysis

Crude and adjusted odds ratios for mortality were estimated using conditional logistic regression. All models were adjusted for covariates that were significantly related to the exposure and changed the point estimate by more than 10%. Statin drug use was categorized as no use (0 days), short-term (1-30 days), mid-term (31 days-2 year) and long-term use (≥ 2 year). Because every participant should have the opportunity to be a long-term user, COPD subjects with an incident date from January 1st, 2006 onwards, were excluded from analyses. Never use of statins was the reference category in all analyses. To gain power in the subdivided cause-specific mortality analyses, the groups mid-term and long-term use were combined. hsCRP serum levels were categorized as high versus average and low, based on the American Heart Association classification.[110] Total serum cholesterol levels were categorized as high versus borderline and desirable, based on the Adults Treatment Panel III classification.[111] All statistical analyses were performed using SPSS version 18 (SPSS Inc, Chicago, IL). P-values below the conventional level of significance ($p < 0.05$) were considered as statistically significant.

RESULTS

Baseline characteristics of the study population

Within the source population of 7983 subjects, hsCRP was successfully measured in 6658 participants at baseline.*(Figure 1)* Of these, 758 COPD patients were identified with a study start date after April 1st, 1991 to ensure at least three months of medication history. The vast majority (82,5%) of COPD patients was confirmed by an obstructive spirometry. Seventy-one patients with an incident COPD date after January 1st, 2006 were excluded from analyses because at least two years of follow-up between incident COPD and death were required to study the association of long-term statin use on mortality. During the potential follow-up of 17 years (1991-2008), 363 COPD patients deceased and were determined as cases. For each case, an average of six age- and sex-matched controls with COPD who survived the follow-up period of their matched case, were selected for a total of 2345 controls. Because non-deceased participants with COPD could serve as a control in several case-control sets, the total number of controls is larger than the total number of incident cases of COPD. *Table 1* shows the baseline characteristics of cases and controls. Cases were more frequently current smokers and had a higher prevalence of cardiovascular disease at baseline in comparison to the controls. Although age- and sex-matched, cases seem older and less often males as a consequence of the fact that there were more controls for males and lower age-categories.

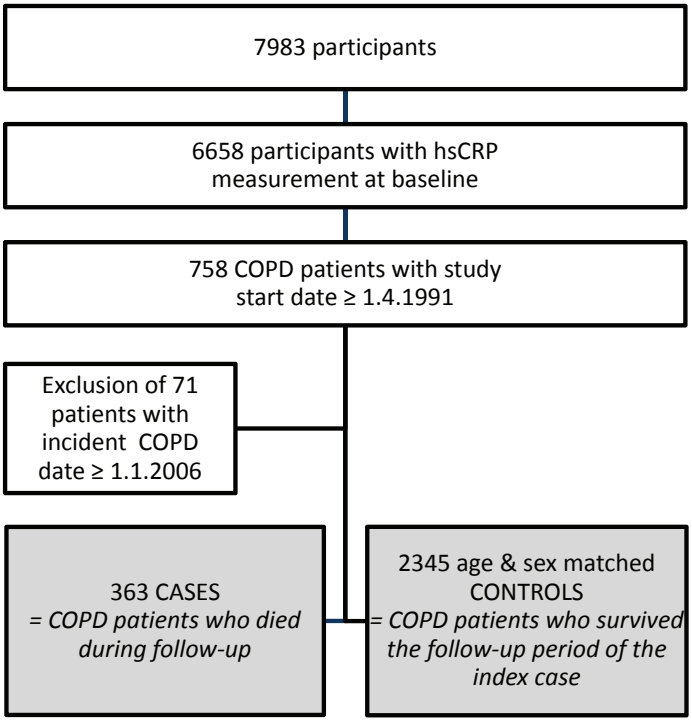


Figure 1: Study flowchart.

Table 1: Baseline characteristics.		**Cases (n=363)**	**Controls (n=2345)**
Age at index date (years)		81 (75-85)	78 (74-81)
Male		249 (69%)	1722 (73%)
Smoking behaviour			
Never smoker		39 (11%)	252 (11%)
Current smoker		145 (40%)	820 (35%)
Former smoker		164 (45%)	1170 (50%)
Missing		15 (4%)	103 (4%)
Median pack years		30 (14-50)	28 (13-47)
Body mass index (kg/m²)		25 (23-28)	26 (24-28)
Diabetes		43 (12%)	161 (7%)
Systolic blood pressure (mmHg)		139 (123-155)	137 (124-152)
Cardiovascular covariables[§]		163 (45%)	724(31%)
hsCRP (mg/L)		2.1 (1.1-4.0)	2.7 (1.3-4.8)
hsCRP categories[a]	≤ 3 mg/L	202 (56%)	1524 (65%)
	> 3 mg/L	161 (44%)	821 (35%)
Total serum cholesterol (mmol/l)		6.4 (5.6-7.2)	6.4 (5.7-7.3)
Total cholesterol categories[†]	< 240 mg/dL	166 (46%)	1055 (45%)
	≥ 240 mg/dL	197 (54%)	1290 (55%)
Drug use at index date			
Antidiabetics		41 (11%)	194 (8%)
Cardiovascular drugs[b]		209 (58%)	1249 (53%)
Oral corticosteroids		138 (38%)	303 (13%)
Duration of statin use			
None		299 (82%)	1856 (79%)
1-30 days		5 (1%)	22 (1%)
31 days-2 years		18 (5%)	197 (8%)
Duration of COPD at index date (years)		7 (3-11)	5 (2-8)

Categorical variables are expressed as count (percentage). Values of continuous variables are expressed as median (25-75 percentiles). Cases: COPD patients who deceased during follow-up; controls: COPD patients who survived the follow-up period of the index case. §Cardiovascular covariables included myocardial infarction, coronary artery bypass graft, percutaneous transluminal coronary angioplasty, atrial fibrillation and heart failure. [a] hsCRP categories based on the American Heart Association[110], total cholesterol categories based on the Adults Treatment Panel III classification[111] [b]Cardiovascular drugs include antihypertensives, diuretics, β-blockers, calcium channel blockers and agents acting on the renin-angiotensin system.

Mortality in COPD patients

The cumulative survival of COPD patients (n=758) was worse in those with a baseline hsCRP of more than 3 mg/L than in those with an hsCRP of 3 mg/L or less (*Figure 2*; log-rank: $p < 0.0001$). In contrary, COPD patients with a total cholesterol of 240 mg/dL or more had a better survival than those with less than 240 mg/dL at baseline (*data not shown*; log-rank: $p = 0.008$). The most frequent causes of death in COPD patients are listed in *Table 2*. Most cases died due to cardiovascular causes (38.3%), followed by pulmonary complications of COPD (exacerbations, emphysema or pneumonia; 19.6%) and bronchial carcinoma (10.5%).

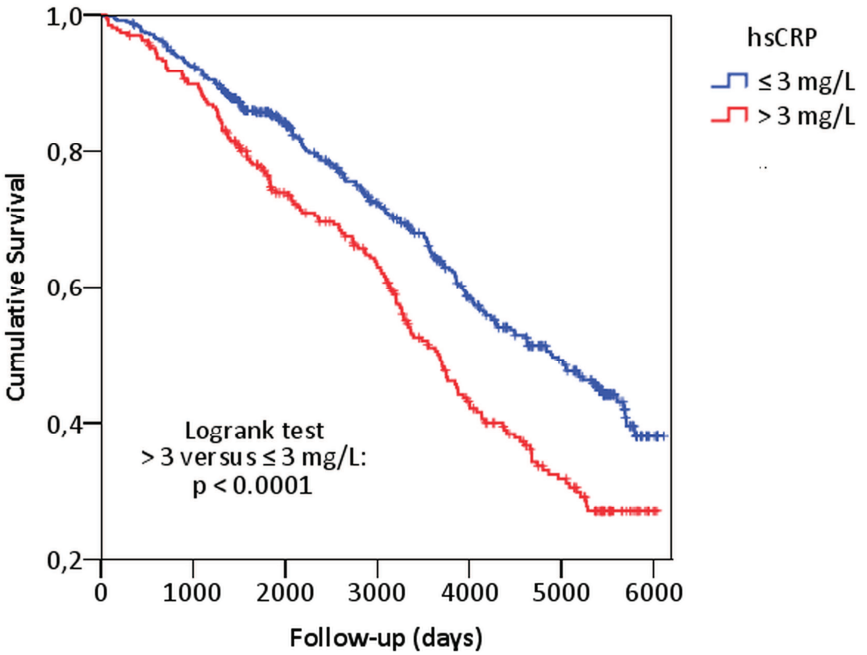


Figure 2: Kaplan-Meier survival curve of COPD patients (n=758) according to the hsCRP level at baseline. hsCRP= high-sensitivity C-reactive protein

Table 2 : Causes of death in the COPD study population.

		n	% of Total
Pulmonary		71	19.6
	COPD	59	16.3
	Pneumonia	12	3.3
Cardiovascular		139	38.3
Mainly	Heart failure	31	8.5
	Stroke	30	8.3
	Sudden (cardiac) death	30	8.3
	Cardiac arrest	18	5.0
	Acute myocardial infarction	12	3.3
	Chronic ischaemic heart disease	6	1.7
Cancer		88	24.2
Mainly	Malignant neoplasm of bronchus and lung	38	10.5
	Malignant neoplasm of colon	8	2.2
	Malignant neoplasm of pancreas	6	1.7
	Malignant neoplasm of prostate	6	1.7
Other		65	17.9
Mainly	Other ill-defined and unspecified causes of mortality	19	5.2
	Dementia	8	2.2
	Fracture of femur	7	1.9
		Total: 363	

Statin use and the risk of mortality in COPD

Table 3 shows the association between statin use and the risk of all-cause mortality in COPD in a first model which is already adjusted for age and sex by matching and in a second model adjusted for all confounders related to the exposure which changed the point estimate by more than 10%. Baseline hsCRP-level was ruled out as confounder because it was not associated with statin prescriptions (nor as categorical variable neither continuously); however, hsCRP was evaluated as a potential effect modifier. Compared to never use, long-term statin use (> 2 years) was associated with a 39% decreased risk of (all-cause) death in COPD patients (95%CI, 0.38-0.99) independent of age, sex, other drug use, duration of COPD, pack years, total serum cholesterol, BMI and cardiovascular covariables.

Table 3 : Association between statin use and the risk of (all-cause) death in COPD.

	Model 1				**Model 2**			
	OR	95% CI		p-value	OR	95%CI		p-value
		Lower	Upper			Lower	Upper	
No statin use	*Reference*				*Reference*			
1 - 30 days	1.34	0.49	3.67	0.572	1.61	0.48	5.48	0.443
31 days - 2 years	0.68	0.40	1.16	0.156	0.64	0.35	1.15	0.136
> 2 years	0.82	0.55	1.22	0.331	**0.61**	**0.38**	**0.99**	**0.045**

Model 1: adjusted for age and sex by matching.
Model 2: adjusted for age and sex by matching; adjusted in the analyses for the use of cardiovascular drugs, antidiabetics, oral corticosteroids and duration of COPD at index date, and pack years of cigarette smoking, total serum cholesterol, body-mass index and cardiovascular covariables at baseline.
Abbreviations : CI = confidence interval; OR = Odds ratio

Regarding cause-specific mortality in COPD, statin use (>30 days) compared to never use was associated with a significant 64% decrease in risk of pulmonary mortality (95%CI, 0.13-0.97). *(Table 4)* In model 2, there was a trend of decrease in risk of pulmonary and cardiovascular mortality. Statin use did not affect cancer mortality in both, crude and adjusted analyses.

Table 4 : Association between statin use and the risk of cause-specific mortality in COPD.

	Model 1				Model 2			
	OR	95% CI		p-value	OR	95% CI		p-value
Pulmonary mortality		Lower	Upper			Lower	Upper	
No statin use	*Reference*				*Reference*			
>30 days of statin	**0.36**	**0.13**	**0.97**	**0.044**	0.37	0.13	1.08	0.068
Cardiovascular mortality								
No statin use	*Reference*				*Reference*			
>30 days of statin	0.87	0.54	1.41	0.579	0.58	0.33	1.01	0.053
Cancer mortality								
No statin use	*Reference*				*Reference*			
>30 days of statin	0.89	0.49	1.61	0.700	0.92	0.48	1.75	0.790

Model 1: adjusted for age and sex by matching.
Model 2: adjusted for age and sex by matching; adjusted in the analyses for the use of cardiovascular drugs at index date, and total serum cholesterol and cardiovascular covariables at baseline.
Abbreviations : CI = confidence interval; OR = Odds ratio

Statin use and the risk of (all-cause) death in COPD according to the level of systemic inflammation

When stratified according to the level of systemic inflammation, long-term statin use was associated with a 78% reduced risk of death in COPD patients with a hsCRP level > 3 mg/L (95% CI, 0.06-0.74), versus a non significant 21% reduced risk of death in COPD patients with a hsCRP level ≤ 3 mg/L (95% CI, 0.41-1.55).*(Table 5)* When we excluded COPD patients exclusively diagnosed by GP, the point estimator did not change substantially (OR 0.49 in model 1 and OR 0.31 in model 2, investigating the effect of long-term statin use compared to no use in COPD subjects with hsCRP > 3mg/L). A sensitivity analysis restricting the subjects to smoking COPD patients with a hsCRP level > 3 mg/L, confirmed that long-term statin use compared to no use was associated with a 85% reduced risk of death (95% CI, 0.04-0.61, model 2). In *Table 6,* analyses were stratified according to the total serum cholesterol level at baseline. The reduced risk of death in COPD patients by long-term statin use was significant in both categories (<240 mg/dl and ≥ 240 mg/dl).

Table 5: Association between statin use and the risk of death in COPD, stratified according to the serum level of hsCRP (at baseline).

		Model 1				Model 2			
			95% CI		p-value		95% CI		p-value
		OR	Lower	Upper		OR	Lower	Upper	
hsCRP ≤ 3 mg/L	No statin use	*Reference*				*Reference*			
	1 - 30 days	0.66	0.14	3.16	0.606	0.77	0.14	4.09	0.756
	31 days - 2 years	0.70	0.35	1.43	0.335	0.60	0.27	1.34	0.212
	> 2 years	0.87	0.51	1.50	0.620	0.79	0.41	1.55	0.496
hsCRP > 3 mg/L	No statin use	*Reference*				*Reference*			
	1 - 30 days	1.83	0.29	11.49	0.520	*NA*			
	31 days - 2 years	0.77	0.30	1.96	0.584	0.95	0.33	2.73	0.917
	> 2 years	**0.44**	**0.20**	**0.97**	**0.042**	**0.22**	**0.06**	**0.74**	**0.015**

Model 1: adjusted for age and sex by matching.
Model 2: adjusted for age and sex by matching; adjusted by model for the use of cardiovascular drugs, antidiabetics, oral corticosteroids and duration of COPD at index date, and pack years of cigarette smoking, total serum cholesterol, body-mass index and cardiovascular covariables at baseline.
Abbreviations : hsCRP, high-sensitivity CRP; CI, confidence interval; OR, Odds ratio; NA, Not applicable (due to low numbers)

Table 6: Association between statin use and the risk of death in COPD, stratified according to the total serum cholesterol level (at baseline).

		Model 1				Model 2			
			95% CI		p-value		95% CI		p-value
		OR	Lower	Upper		OR	Lower	Upper	
Total cholesterol < 240 mg/dL	No statin use	*Reference*				*Reference*			
	1 - 30 days	1.99	0.36	10.85	0.428	5.68	0.38	84.61	0.208
	31 days - 2 years	0.32	0.07	1.40	0.130	0.17	0.02	1.42	0.102
	> 2 years	0.41	0.13	1.30	0.129	**0.16**	**0.03**	**0.91**	**0.039**
Total cholesterol ≥ 240 mg/dL	No statin use	*Reference*				*Reference*			
	1 - 30 days	1.24	0.22	7.01	0.806	1.22	0.07	21.19	0.891
	31 days - 2 years	0.69	0.37	1.32	0.266	0.65	0.32	1.33	0.238
	> 2 years	0.79	0.47	1.31	0.355	**0.50**	**0.26**	**0.95**	**0.034**

Model 1: adjusted for age and sex by matching.
Model 2: adjusted for age and sex by matching; adjusted by model for the use of cardiovascular drugs, antidiabetics oral corticosteroids and duration of COPD at index date, and pack years of cigarette smoking, body-mass index and cardiovascular covariables at baseline.
Abbreviations : CI, confidence interval; OR, Odds ratio

DISCUSSION

This is the first prospective study in a general population showing that the beneficial effect of long-term statin use on the risk of mortality in COPD patients is modified by the baseline level of systemic inflammation. The results suggest that the subset of COPD patients characterized by increased markers of systemic inflammation might benefit most from long-term statin therapy. One in three COPD patients in our study died from cardiovascular causes - figures which have also been described by other authors.[29, 112] Although the protective effect of statins in COPD patients could represent solely an indirect effect on the cardiovascular comorbidities associated with COPD, our results also suggest an effect on respiratory mortality by statin use compared to never use.

Increasing insights into the pleiotropic effects of statins unravel several possible mechanisms for the beneficial effects seen in COPD patients. Beyond their known ability to inhibit endogenous cholesterol synthesis, statins exert immunomodulating effects in both systemic and pulmonary cytokine driven inflammation by inhibiting guanosine triphosphatase proteins.[113] Statins down regulate the expression of adhesion molecules involved in the recruitment of inflammatory cells, and of chemokines which are increased in COPD, such as CCL2 and CXCL8.[114] Furthermore, simvastatin reduces the expression of matrix metalloproteinases (MMPs) involved in COPD matrix remodelling, such as MMP2, MMP9 and MMP12.[115, 116] In support of a direct effect of statins in COPD, Lee *et al.* demonstrated in a rat model of smoking-induced emphysema that simvastatin ameliorated the structural and functional derangements of the lungs partly by suppressing inflammation and matrix MMP9 induction.[117] Statins may even reduce oxidative stress, related to their ability to scavenge oxygen derived free radicals.[118]

The 39% reduced risk of death in COPD patients by using (long-term) statin therapy is consistent with findings of previous (retrospective) observational studies.[102, 103] Similar to the retrospective study of Frost *et al.*, we found a protective effect of statin use on the risk of pulmonary mortality in COPD.[119] Furthermore, our results showed that having an hsCRP baseline level of more than 3 mg/L was associated with increased mortality and therefore we expected, if statins were effective, that the pre-specified subset of COPD patients with high hsCRP levels would benefit most of treatment. Surprisingly but consistent with other prospective population-based studies in the elderly, we found that COPD patients with a total cholesterol of 240 mg/dL or more had a better survival.[120, 121] Selective survival or changes in the arterial wall by aging might yield individuals resistant to the effects of high cholesterol concentrations in the blood or high cholesterol levels might be associated with less frailty.

Importantly, we demonstrate a significant interaction between statin use and the degree of systemic inflammation because the protective effect of statins was only significant in COPD patients with the highest hsCRP serum level. There was no significant effect in COPD patients with a low-to-moderate degree of systemic inflammation, which cannot be explained due to lack of power as numbers were even higher in this stratum. The same phenomenon has also been reported in cardiovascular studies and one RCT in COPD patients. Kinjo *et al.* described a decreased hazard by statin therapy for 1-year mortality in patients with a CRP level above 2.9 mg/L who had an acute myocardial infarction.[122] In addition, the JUPITER trial of apparently healthy persons without hyperlipidemia but with elevated hsCRP levels,

demonstrated that rosuvastatin significantly reduced the incidence of all cause mortality and was associated with a 60% decreased risk for cardiovascular endpoints.[123] However, a recent RCT in persons at high risk of vascular events, could not confirm that these vascular benefits of statin therapy were affected by the baseline CRP concentration.[116] The RCT in COPD patients by Lee *et al.* demonstrated that the improvement in exercise time by statin treatment is modified by plasma hsCRP-levels.[107] Because both the inflammation and therapeutic interventions in COPD have been studied merely in (ex)smoking subjects, we furthermore restricted the analyses to smoking COPD patients, which confirmed the beneficial effects of long-term statin use in the subgroup with greater inflammation.

Because of its observational character, a possible limitation of our study is that treatment with statins was not randomly assigned. Our results could therefore be flawed by confounding by indication. However, because statins are selectively prescribed in subjects who have substantial comorbidities such as diabetes mellitus and cardiovascular disease, this kind of confounding would only underestimate the protective effect of statins on the risk of death. Consequently, this would mean that the true protective effect is even stronger than we measured. Secondly, we cannot exclude a healthy user bias if statins are prescribed more readily in health-conscious, medical-attention-seeking patients or in those patients who are expected to live long enough to benefit from statin treatment. Importantly, however, this would not explain effect modification by baseline hsCRP as this measurement was not used for usual patient care. Finally, of a minority of COPD patients diagnosed by GP (17,5%), we do not have the certainty that diagnosis was confirmed by an obstructive spirometry. However, exclusion of this subgroup did not change the point estimator substantially.

The strengths of this study are the high quality information available about exposures prior to outcome with a prospective data collection, the general population based setting, the large number of subjects that participated in the Rotterdam Study and the long duration of follow-up. The high response rate and virtually complete follow-up for every participant makes information and selection bias for these data unlikely. An important advantage is the availability of continuous pharmacy dispensing data with complete pharmacy records of all filled prescriptions on practically all members of the cohort, providing very specific and detailed information on drug use and thus minimizing the risk of exposure misclassification. Furthermore, the nested case-control approach made it possible to account for other drug use at index date, a major confounder in the association between statins and mortality. It is important that these results should be further investigated in randomized controlled trials before recommending widespread statin use in COPD patients. According to Clinicaltrials.gov, several randomized clinical trials are ongoing.

In conclusion, statin use is associated with a decreased risk of all-cause mortality in patients with COPD, depending on the degree of systemic inflammation. These results suggest that in an older population of COPD patients, CRP levels might guide the clinician better than total cholesterol levels in his decision to start lipid lowering therapy. This study may provide a rationale for undertaking more definitive randomized clinical trials to confirm the impact of statin use on the outcome of COPD and to elucidate the mechanisms by which they may work.

ABSTRACT

Background

Sudden cardiac death (SCD) and chronic obstructive pulmonary disease (COPD) are both common conditions in the elderly. Previous studies have identified an association between COPD and cardiovascular disease, and with SCD in specific patient groups.

Objectives

To investigate whether there is an association between COPD and SCD in the general population.

Methods

The Rotterdam Study is a population-based cohort study among 14 926 subjects aged 45 years and older with up to 24 years of follow-up. Analyses were performed with a (time-dependent) Cox proportional hazard model adjusted for age, sex and smoking.

Results

Of the 13 471 persons included in the analysis; 1615 had a diagnosis of COPD and there were 551 cases of SCD. COPD increased the risk of SCD (age- and sex adjusted HR 1.34, 95%CI 1.06-1.70). The risk particularly increased in the period 2000 days (5.48 years) after the diagnosis of COPD (age- and sex adjusted HR 2.12, 95%CI: 1.60-2.82) and increased further to a more than threefold higher risk in COPD subjects with frequent exacerbations during this period (age- and sex adjusted HR 3.58, 95%CI 2.35-5.44). Analyses restricted to persons without prevalent myocardial infarction or heart failure yielded similar results.

Conclusions

COPD is associated with an increased risk for SCD, independent of prevalent coronary heart disease. The risk especially increases in persons with frequent exacerbations five years after the diagnosis of COPD. This risk indicator could provide new directions for better-targeted actions to prevent SCD.

7. COPD and Sudden Cardiac Death

INTRODUCTION

Sudden cardiac death (SCD) forms a substantial part of cardiovascular mortality, which is the leading cause of death globally[124], with a currently estimated incidence of 4 to 5 million cases worldwide per year.[125] SCD is primarily caused by ventricular arrhythmias[126, 127] and risk factors for SCD include male gender, increasing age[125], a history of heart failure or cardiac ischemia, cardiomyopathies, long QT syndrome (LQTS) and alcohol and drug use.[128] However, SCD is a heterogeneous outcome with multiple etiological pathways of which many have not been established. This complicates an adequate risk assessment and prevention plan for SCD.

Chronic obstructive pulmonary disease (COPD) is the third leading cause of death.[2] COPD is characterized by a progressive airflow limitation and is associated with a chronic inflammatory response in the airways and lungs. Besides these local pulmonary manifestations, systemic effects and comorbidities are frequently present and determine the prognosis of patients with COPD.[1, 129, 130] Patients with COPD have a two to threefold higher risk of developing cardiovascular disease and half of COPD deaths can be attributed to cardiovascular disease.[131, 132] Although the underlying mechanisms for this high cardiovascular morbidity and mortality in patients with COPD are not fully established to date, a role for the accompanied hypoxia and hypoxemia[133], higher heart rate[134], systemic inflammation[133] or recurrent exacerbations has been suggested. Moreover, patients with COPD suffer more frequently from ventricular arrhythmias than controls.[135-137] In patients hospitalized for an exacerbation of COPD, atrial fibrillation and ventricular arrhythmias are independent predictors of death at one year.[138]

Furthermore, COPD has also been shown to be an independent predictor of SCD in high-risk patients after a percutaneous coronary intervention and coronary artery bypass graft.[138, 139] However, this association has not yet been assessed in the general population. Therefore, our objective was to assess whether COPD is an independent risk factor for SCD in the general population, and whether COPD exacerbations and systemic inflammation modulate the association between COPD and SCD.

METHODS

Setting

The Rotterdam Study is a prospective population-based cohort study in 14 926 people aged ≥45 years, which started in 1990 in the Ommoord district, in the city of Rotterdam, the Netherlands.[20, 21] All inhabitants of the Ommoord district, aged 55 years of age and older were invited to participate (n=10 215). At baseline, from 1990 through 1993, 7 983 participants (response rate 78%) were included. In 2000, an additional 3 011 participants, out of 4 472 invitees, were enrolled (response rate 67%). This extension consisted of all persons living in the study district who had become 55 years of age or had moved into the study district. A second similar extension of the cohort was initiated in 2006, in which 3 932 participants (out of 6 057 invitees; response rate 65%), aged 45 years and older were included. Follow-up examinations are conducted periodically, approximately every 4 to 5 years. Examinations consist of a home interview and an extensive set of tests at a specially built research facility in the study district. Besides this, participants are continuously monitored for major morbidity and mortality through linkage of general practitioners and municipality records to the study base. The Rotterdam Study was approved by the medical ethics committee according to the Population Study Act Rotterdam Study executed by the Ministry of Health, Welfare and Sports of the Netherlands. The study population consisted of participants who gave informed consent for follow-up.

COPD

COPD was diagnosed by an obstructive spirometry ($FEV_1/FVC < 0.70$) performed at the research center visit or, if not available, by a physician based on the combination of clinical history, physical examination and spirometry.[140] Asthma patients were excluded. The date of incident COPD was defined as the date of obstructive lung function measurement, the date of COPD diagnosis in the medical records or the date of a first COPD medication prescription in someone with established COPD, whichever came first. Medication use was obtained through pharmacy-filled prescription data from seven pharmacies in the region which are all on one computer network. Moderate exacerbations were defined as needing a course of steroids and/or antibiotics and severe exacerbations as needing hospitalization. Frequent exacerbators were defined as COPD participants who had during follow-up on average two or more moderate or severe exacerbations a year.[141]

Sudden Cardiac Death (SCD)

SCD cases were validated independently by two researchers and confirmed by a cardiologist through details from the medical records. In witnessed deaths, SCD was diagnosed according to Myerburg's definition endorsed by the European Society of Cardiology: "a natural death due to cardiac causes, heralded by abrupt loss of consciousness within one hour from onset of acute symptoms; pre-existing heart disease may have been known to be present, but the time and mode of death are unexpected".[142, 143] Unwitnessed deaths were coded as SCD if death was unexpected in persons found death, while they were in a stable medical condition 24 hours before they were found and without evidence of a noncardiac cause.[144]

High-sensitivity C-reactive protein (hsCRP) serum level measurement

HsCRP serum levels were measured as previously described.[61] Non-fasting blood was collected at baseline and stored at -80°C until CRP measurement. Storing temperature did not affect stability of CRP. HsCRP measurements were performed on all blood samples using a rate near-infrared particle immunoassay (Immage Immunochemistry System; Beckman Coulter, San Diego, CA).

Covariables

Information on covariables was obtained through interview (i.e. smoking and alcohol use), laboratory, medical files, or physical examination (i.e. height, weight, systolic and diastolic blood pressure, myocardial infarction, heart failure, revascularization procedures, diabetes mellitus, atrial fibrillation, heart-rate corrected QT (QTc) interval and statin use) at the regular visits of the study participants to the Rotterdam Study research center. Pack years of cigarette smoking were computed as duration of self-reported smoking (years) multiplied by the number of daily smoked cigarettes, divided by 20. Body mass index was calculated as weight in kilograms divided by height in meters squared. Systolic (SBP) and diastolic blood pressure (DBP) were measured in sitting position at the right upper arm. The average of 2 consecutive measurements was taken. Hypertension was defined as a SBP >140 mmHg, DBP >90 mmHg, or use of blood pressure lowering medication for the indication hypertension. Myocardial infarction was adjudicated based on a combination of symptoms, ECG measurements and enzyme markers. Heart failure diagnosis was adjudicated in accordance with the guidelines of the European Society of Cardiology and included typical signs or symptoms of heart failure confirmed by objective evidence of cardiac dysfunction, as described in more detail previously.[145, 146] A revascularization procedure is either a percutaneous coronary intervention or a coronary artery bypass graft. Diabetes mellitus was defined as fasting glucose above 6.9 mmol/L, non-fasting glucose above 11.0 mmol/L, the use of blood glucose lowering medication, or a previous diagnosis of diabetes mellitus. Atrial fibrillation is ascertained from electrocardiogram measurements as well as medical records. QTc interval according to Bazett's formula was derived from a standard electrocardiogram. More detailed information on the methods of data collection and definitions of cardiac outcomes have been previously described.[146]

Statistical analysis

Differences between subjects with and without COPD were studied using Mann-Whitney U and Chi-Square tests. Time-dependent cox proportional hazard models were performed to assess the association between COPD and SCD. All models were adjusted for age and sex, and additionally for covariables which changed the risk estimate by more than 10%. Potential confounders were age, sex, height, weight, body mass index, smoking behaviour, pack-years, myocardial infarction, heart failure, coronary revascularisation procedures, systolic blood pressure, diastolic blood pressure, hypertension, total serum cholesterol, diabetes mellitus, atrial fibrillation, heart-rate corrected QT interval according to Bazett's

formula, and statin use at baseline. High-sensitivity CRP (hsCRP) serum levels were categorized as high versus moderate/low, based on the American Heart Association classification.[110] We constructed cumulative survival curves using the Kaplan-Meier method and used Log-rank to determine significant survival differences.

We performed a sensitivity analysis by exclusion of persons with prevalent heart failure and myocardial infarction. Since we were interested in how COPD affects SCD in general terms, Cox models for the hazard on SCD that treat other death as censored were perfectly valid.[147] Nevertheless, with regard to the competing risk of non-SCD, we performed a sensitivity analysis using the data augmentation method and unstratified model described by Lunn & McNeil[148] to estimate the hazard ratio (HR) of SCD in comparison with non-SCD. Although it should be mentioned that if the proportionality assumption is not satisfied, then the stratified Lunn-McNeil model should be used, which is identical to the Cox cause-specific model.[149] Furthermore, since we were also interested in the incidence of SCD, we estimated the cumulative incidence function (CIF)[150] from the competing risk data and performed the Gray's test for equality of CIFs.[151, 152]. The CIF is a summary curve, showing the cumulative event rates over time due to a particular cause[150, 153]. The Gray's test is developed for the comparison of the cumulative incidence of events among different groups[151].

Follow-up time was determined as the time between study entry (i.e. for subjects without COPD or with prevalent COPD) or incident COPD date (i.e. for subjects with incident COPD) and death or end of study (January 1st 2011). This definition includes the time at risk for sudden cardiac death. However, we also performed a sensitivity analysis adding the time between cohort entry and incident COPD to the control follow-up time. A two-sided p-value below 0.05 was considered statistically significant. Statistical analyses were performed using R Statistical Software (Foundation for Statistical Computing, Vienna, Austria) and SPSS, version 20.0 (IBM Corp., Somers, NY, USA).

RESULTS

General characteristics

Table 1 shows the baseline characteristics of the study population (n=13 471) with a median age of 64 years (interquartile range (IQR)=16) at the start of follow-up. COPD subjects were older, more often male, more (current) smokers, had a slightly lower body mass index, higher levels of hsCRP at baseline and had more frequently a medical history of myocardial infarction and coronary artery bypass graft. During a median follow-up of 3 229 days (8.84 years; IQR=3 651 days; total of 123 024 person years of follow-up), 5 197 (39%) of the participants died of whom 551 died suddenly. 82 (5%) COPD subjects and 469 (4%) subjects without COPD died due to SCD (*Figure 1*). The SCDs were witnessed in about half of the cases (54%) and the ratio of witnessed versus unwitnessed SCD was not different for COPD subjects versus subjects without COPD (p=0.975).

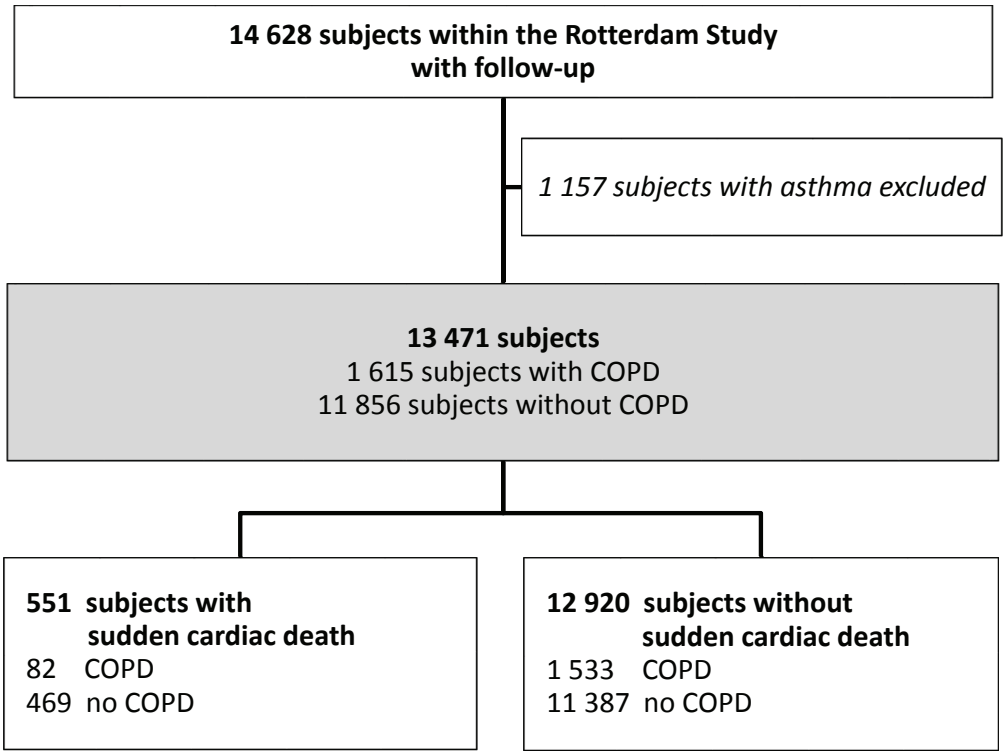


Figure 1: Flow chart of the study population.

Table 1: Baseline characteristics of the population at cohort entry (n=13 471).

	COPD (n= 1 615)	No COPD (n=11 856)	P-value
Age, in years	70 (13)	63 (15)	<0.001
Males	909 (56)	4,727 (40)	<0.001
Smoking status Never smoker Former smoker Current smoker	 251 (16) 689 (44) 632 (40)	 4,361 (38) 4,903 (43) 2,209 (19)	<0.001
Pack-years of cigarette smoking	26 (37)	3 (23)	<0.001
Height (cm)	170 (13.2)	167 (13.6)	<0.001
Weight (kg)	74.9 (17.0)	74.6 (17.8)	0.773
BMI, in kg/m²	25.8 (4.7)	26.4 (4.9)	<0.001
hsCRP, in mg/L	1.9 (3.0)	1.5 (2.5)	<0.001
Myocardial infarction	118 (7)	581 (5)	<0.001
Heart failure	46 (3)	224 (3)	0.090
Coronary revascularization CABG* PTCA*	57 (4) 41 (3) 19 (1)	334 (3) 212 (2) 155 (1)	0.141 0.048 0.608
Systolic blood pressure	137.0 (29.0)	137.0 (29.0)	0.399
Total serum cholesterol (mmol/l)	6.2 (1.6)	6.1 (1.6)	0.036
Diastolic blood pressure	75.0 (16.0)	77.0 (85.0)	<0.001
Hypertension	741 (55)	5344 (56)	0.499
Atrial fibrillation	71 (5)	438 (4)	0.268
QTc interval	429.5 (31.0)	429.0 (30.0)	0.618
Diabetes mellitus	154 (10)	1196 (10)	0.488

Categorical variables are expressed as numbers (percentage). Values of continuous variables are expressed as median (interquartile range). * Numbers overlap

There were missings in baseline smoking status (n=426), pack-years (n=728), height (n=1447), weight (n=1438), BMI (n=1455), hsCRP (n=1922), myocardial infarction (n=161), heart failure (n=297), coronary revascularization (n=583), systolic/ diastolic blood pressure (n=1317), cholesterol (n=1439), hypertension (n=2547), atrial fibrillation (n=1876) and QTc interval (n=1659).

Abbreviations: BMI = body mass index; CABG=coronary artery bypass graft; COPD=chronic obstructive pulmonary disease; hsCRP= high-sensitivity C-Reactive Protein; QTc=heart-rate corrected QT interval according to Bazett's formula; PTCA=percutaneous transluminal coronary angioplasty

Association between COPD and SCD

Figure 2 illustrates the higher probability of SCD for COPD subjects compared to subjects without COPD. Adjusted for age and sex, COPD was significantly associated with an increased risk of dying due to SCD (HR 1.34, 95%CI: 1.06-1.70). A stratified analysis in patients without prevalent myocardial infarction nor heart failure nor CABG nor PCI resulted in a comparable age and sex adjusted HR of 1.38 (95%CI: 1.03-1.85). A sensitivity analysis using the stratified Lunn-McNeil method to model the competing risk effect of other causes of death, resulted in an age and sex adjusted HR of COPD on SCD of 1.43; 95%CI: 1.19-1.67. The cumulative incidence function (CIF) in the online supplement represents the higher incidence of SCD and other causes of death for subjects with COPD compared to subjects without COPD (see *Figure 3*). The CIFs for COPD versus no COPD were statistically significant for SCD (Gray's test; p=0.0006) and for non-SCD (Gray's test; p<0.0001). Another sensitivity analysis adding the time between cohort entry and incident COPD to the control follow-up time did not substantially change the estimate.

Figure 2 further illustrates that the distinction of SCD risk according to COPD status occurred about 2000 days (5.48 years) after the study start (incident COPD date or cohort entry for participants without COPD), while the difference for other causes of death was seen much earlier (*Figure 3*). Since the proportional hazard assumption was not satisfied, we evaluated the effect of COPD on SCD using a time-dependent Cox regression analysis. The interaction between COPD and the dichotomous time indicator (more or less than 2000 days) was significant (p<0.001) and adjusted for age and sex, only the HR for the period 2000 days after the COPD incident date was significant (HR 2.12, 95%CI: 1.60-2.82; *Table 2*). Furthermore, COPD was associated with an almost twofold increased risk to develop SCD, independent of age, sex and pack years of cigarette smoking (HR 1.93, 95%CI 1.44-2.59; *Table 2*). The other potential confounders mentioned in the methods did not significantly influence the effect.

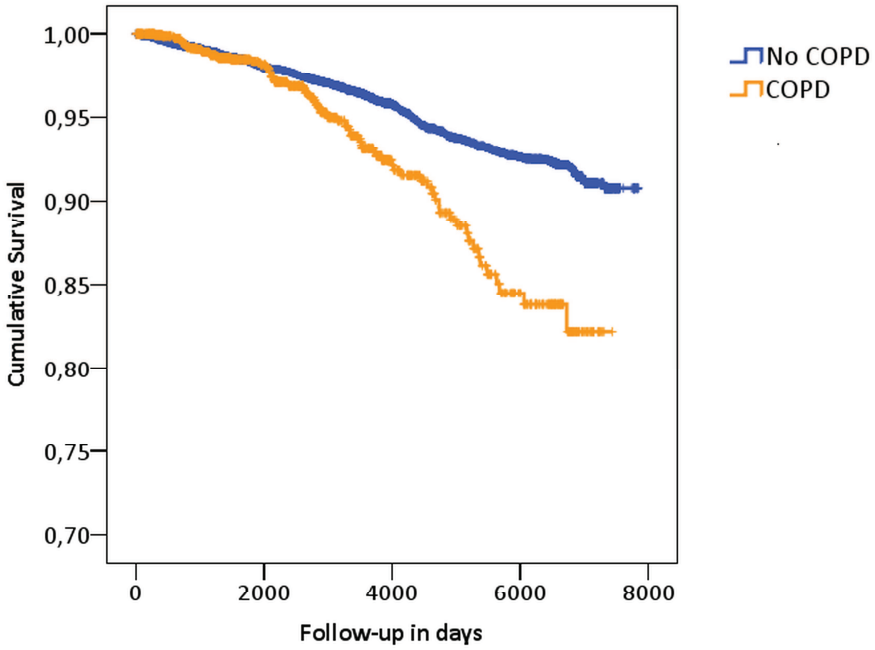


Figure 2: Kaplan-Meier curve of sudden cardiac death according to COPD status (Log-rank p<0.001).

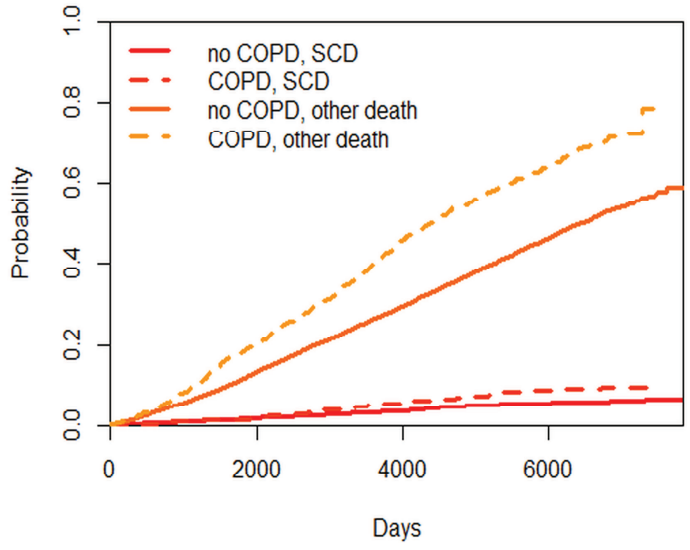


Figure 3: Cumulative incidence function of sudden cardiac death (Gray's test; p=0.0006) and other death (Gray's test; p<0.0001) according to COPD status.
Abbreviations: COPD= Chronic Obstructive Pulmonary Disease; SCD= Sudden Cardiac Death

Table 2: COPD and the hazard on sudden cardiac death for the period >2,000 days (5.48 years) of follow-up.

	Model 1			Model 2		
	HR	**95% CI**	**P-value**	**HR**	**95% CI**	**P-value**
COPD	2.12	1.60-2.82	<0.001	1.93	1.44-2.59	<0.001
COPD *without frequent exacerbations*	1.66	1.16-2.37	0.005	1.52	1.06-2.19	0.023
COPD *with frequent exacerbations*	3.58	2.35-5.44	<0.001	3.21	2.08-4.95	<0.001

Model 1: age and sex adjusted.
Model 2: adjusted for age, sex and pack years of cigarette smoking.
Time-dependent Cox Regression analysis for the period >2,000 days of follow-up.
Frequent exacerbations were defined as having at least two moderate or severe exacerbations a year, averaged over the total years of follow-up.
Abbreviations: CI= Confidence Interval; COPD= Chronic Obstructive Pulmonary Disease; HR= Hazard Ratio

Influence of exacerbations

Regarding the influence of COPD exacerbations, 366 (23%) of 1 615 COPD subjects had frequent exacerbations. 33 (9%) frequent exacerbators compared to 49 (4%) COPD subjects without frequent exacerbations died due to SCD. The Kaplan Meier curve for SCD in *Figure 4* illustrates the poorer survival for COPD subjects with frequent exacerbations (Log-rank p<0.001). Adjusted for age, sex and pack years of cigarette smoking, the COPD frequent exacerbator phenotype was significantly associated with a more than threefold increased risk of developing SCD (HR 3.21; 95%CI: 2.08-4.95; *Table 2*). A sensitivity analysis using the stratified Lunn-McNeil method to model the competing risk effect of other causes of death, resulted in an age and sex adjusted HR of the frequent exacerbators on SCD of 2.51 (95%CI: 2.15-2.86). Finally, the cumulative incidence of SCD was significantly higher when COPD subjects had frequent exacerbations (*Figure 5*).

Influence of baseline systemic inflammation (hsCRP)

Among the 13 471 subjects, 11 549 (85.7%) had a baseline hsCRP measurement. A linear regression model adjusted for age and sex, demonstrated that the natural logarithm of hsCRP concentration was significantly increased in subjects with COPD and SCD compared to subjects with COPD without SCD (p<0.05). When stratified according to the level of baseline systemic inflammation, the HR for SCD was significantly increased in COPD subjects with frequent exacerbations having a hsCRP level > 3 mg/L (HR 3.67; 95% CI: 1.97-6.85; *Table 3*). In contrast, in all subjects with a low-to-moderate degree of systemic inflammation, the HR for SCD was significantly increased in COPD subjects without frequent exacerbations (HR 1.84; 95% CI: 1.16-2.91; *Table 3*).

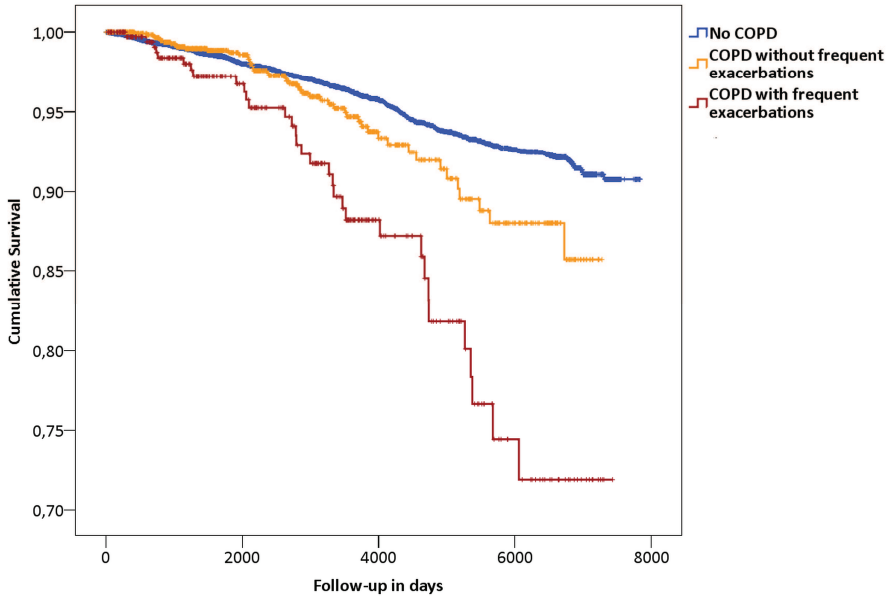


Figure 4: Kaplan-Meier curve of sudden cardiac death according to COPD status with or without frequent exacerbations (Log-rank p<0.001).

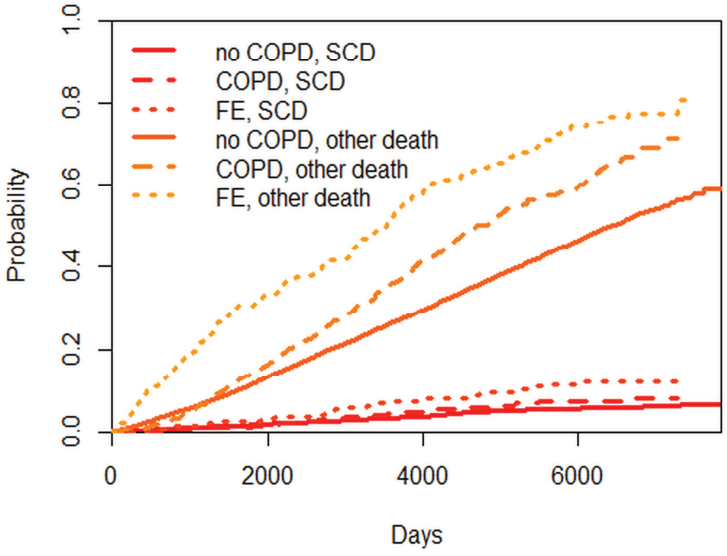


Figure 5: Cumulative incidence function of sudden cardiac death (Gray's test; p=0.0002) and other death (Gray's test; p<0.0001) according to COPD exacerbator status (no COPD, COPD without frequent exacerbations, COPD with frequent exacerbations).
Abbreviations: COPD= Chronic Obstructive Pulmonary Disease; FE= COPD frequent exacerbator; SCD= Sudden Cardiac Death

Table 3: COPD and the hazard on sudden cardiac death for the period >2 000 days (5.48 years) of follow-up, stratified according to the serum level of hsCRP.

		Model 1			Model 2		
		HR	**95% CI**	**P-value**	**HR**	**95% CI**	**P-value**
hsCRP ≤ 3 mg/L	COPD	1.98	1.32-2.98	0.001	1.76	1.15-2.68	0.009
	COPD, *no frequent exacerbations*	2.09	1.34-3.26	0.001	1.84	1.16-2.91	0.010
	COPD *with frequent exacerbations*	1.60	0.65-3.90	0.304	1.48	0.60-3.64	0.391
hsCRP > 3 mg/L	COPD	1.88	1.16-3.05	0.010	1.93	1.18-3.16	0.008
	COPD *no frequent exacerbations*	1.21	0.62-2.35	0.573	1.24	0.63-2.42	0.535
	COPD *with frequent exacerbations*	3.51	1.90-6.49	<0.001	3.67	1.97-6.85	<0.001

Model 1: age and sex adjusted.
Model 2: adjusted for age, sex and pack years of cigarette smoking.
Time-dependent Cox Regression analysis for the period >2,000 days of follow-up.
Frequent exacerbations were defined as having at least two moderate or severe exacerbations a year, averaged over the total years of follow-up.
Abbreviations: CI= Confidence Interval; COPD= Chronic Obstructive Pulmonary Disease; hsCRP= high-sensitivity C-Reactive Protein; HR= Hazard Ratio

DISCUSSION

In this population-based study in community dwelling middle-aged and elderly persons, we show an increased risk for SCD in subjects with COPD. The risk almost doubles in the period 2 000 days (5.48 years) after the diagnosis of COPD and there was a more than threefold increase in the risk for SCD in patients with frequent exacerbations having a higher degree of baseline systemic inflammation.

An increased risk for SCD has been previously described in specific patient populations at high risk of SCD and a diagnosis of COPD.[138] From previous studies we already know that COPD increases the risk of ventricular arrhythmias and cardiovascular morbidity and mortality.[131, 132, 135-137, 154-156] Our prospective cohort study for the first time demonstrates that COPD is associated with an increased risk of SCD in the general population.

There are several factors in persons with COPD that could play a role in the mechanism which ultimately leads to SCD. COPD is associated with a prolongation of the QTc interval, currently the most well-known electrocardiogram-derived risk indicator for SCD.[157, 158] Other factors that are present in persons with COPD are a higher resting heart rate, hypoxia and hypoxemia, cardiac ischemia and heart failure; all known risk factors for SCD.[128, 131-134, 154, 159, 160] On top of this, exacerbations could provide an additional mechanism through which SCD is caused. Suissa *et al.* recently described that with every exacerbation both the risk of subsequent COPD exacerbations and the risk of mortality is increased.[161] Elevated biomarkers of cardiovascular morbidity, such as troponins, midregional-pro atrial natriuretic peptide and N-terminal-pro brain natriuretic peptide (the latter two are both indicators of heart failure) are associated with exacerbation mortality and a higher risk for myocardial infarction and stroke after a COPD exacerbation has been observed.[162-165] Wedzicha *et al.* described that COPD exacerbations may lead to increased cardiovascular morbidity through systemic inflammation.[25] This hypothesis is endorsed by another study that showed increased levels of C-reactive protein and interleukin-6 following COPD exacerbations.[166] Previous studies have also shown associations between a high CRP and cardiovascular disease and SCD.[167-169] Interestingly, our results demonstrate that the effect of frequent exacerbations was modified by the baseline degree of systemic inflammation (hsCRP). In the absence of increased systemic inflammation, COPD without frequent exacerbations significantly increased the risk on SCD. Since the Kaplan-Meier curve suggests that a latency period was more pronounced for COPD patients without frequent exacerbations, one might speculate two different mechanisms underlying SCD in COPD. Possibly, COPD patients with substantial systemic inflammation develop more atherosclerosis increasing the risk of coronary heart disease whereby each exacerbation amplifies these mechanisms an results in an acute increased risk on SCD. While in COPD patients without these factors, COPD might lead via hyperinflation to remodeling, pulmonary hypertension, cardiac arrhythmias and subsequently SCD.

The association between COPD and SCD provides various directions for further research. The first is to unravel the underlying mechanisms. A second is to assess the role of respiratory medication in the risk for SCD.[170] Thirdly, further research is needed to identify effective strategies to prevent SCD in COPD patients.

Our study has several strengths and some limitations. First, in previous studies the association between COPD and SCD has been assessed only in specific patient populations who already have an increased risk of SCD, but not in a general population setting with adjustment for all known confounders. Second, SCD was validated by research physicians blinded to the COPD status of the subject. Another strength of this study is that we have prospectively gathered data on morbidity and mortality events, and on covariables, limiting the risk of information bias. However, some potential misclassification may have occurred by inclusion of unwitnessed deaths, though the percentage of unwitnessed deaths in our study was not different for COPD subjects compared to subjects without COPD. It has previously been suggested that participants in a prospective cohort study are more healthy than non-participants.[171, 172] The Rotterdam Study has an overall response rate of 72%. Persons with more severe COPD could have had lower participation rates. However, in our study this would have only resulted in an underestimation of the effect.

Conclusion

COPD is an independent risk factor for SCD in the general population, especially in the period 2,000 days (5.48 years) after the diagnosis of COPD. The risk further increases in COPD patients with frequent exacerbations having a higher degree of baseline systemic inflammation.

PART III: COPD, ANGIOPATHY AND CEREBROVASCULAR MORBIDITY

ABSTRACT

Increasing evidence associates lung impairment with brain disorders in the elderly. The most prevalent chronic lung disease in elderly is COPD, a major public health problem with significant economic burden. Despite the clinical relevance and the important impact of cerebrovascular disease and neurodegenerative disorders in respiratory impaired elderly, the evidence on potential explanatory mechanisms is scarce.

The aim of the present review is therefore to summarize current scientific knowledge to provide a better understanding of the interplay between the lung and the aging brain and to define remaining knowledge gaps.

This review article 1) provides a general overview of the epidemiology of stroke, cerebral small vessel disease, cognitive impairment, depression and anxiety in respiratory impaired elderly; 2) discusses potential underlying mechanisms including aging, smoking, systemic inflammation, vasculopathy, hypoxia and genetic susceptibility; and 3) highlights areas requiring further research.

8. COPD and brain disorders

INTRODUCTION

The main task of the lungs is to provide oxygen to the blood and eliminate carbon dioxide from the blood. 20% of the total body oxygen is consumed by the brain.[173] Inevitably, impairment of the lungs will affect structure and functioning of the brain. Aging of the brain mainly affects high metabolic demanding neurons and the brain reserve capacity is reduced in elderly.[44, 174] Therefore, particularly elderly are prone to a distorted supply of oxygen to the brains. By focusing on mortality rates rather than disability rates, the large burden of neurological disorders has been underestimated in Europe.[175]

Chronic Obstructive Pulmonary Disease (COPD), partially called accelerated lung aging, has been most extensively studied in the context of lung-brain interactions.[176] Worldwide, COPD is the third leading cause of mortality, and the overall severity and prognosis of patients with COPD is strongly influenced by associated concomitant diseases.[1, 2] Stroke, cognitive impairment, anxiety and depression are frequently described in patients with COPD.[177-179] Despite the impact on wellbeing and functional capacity, mental health in COPD is not frequently assessed.[180] However, there is increasing recognition that COPD extends beyond the lungs.

Increasing evidence demonstrates that accumulation of brain pathology associates with cognitive decline and can lead to clinical outcomes, such as stroke and dementia. However, a large part of neurological diseases remains subclinical and may lead to subtle deficits, only measurable with cognitive testing and visualized with magnetic resonance imaging (MRI). Early clinical manifestations of these diseases include Transient Ischemic Attacks (TIAs) for stroke and Mild Cognitive Impairment for dementia.

In this review article, we provide a general overview of the link between the brain and the lung in elderly with chronic respiratory diseases, including the epidemiology, pathophysiology and impact of concurrent brain disorders. We will focus on stroke, cerebral small vessel disease, cognitive impairment, depression, anxiety and panic disorders. These disease entities will be described mainly in relation to COPD. Nevertheless, information regarding the influence of the brain on other lung diseases including asthma, obstructive sleep apnea syndrome (OSAS) and interstitial lung disease is included. Lung cancer (and brain metastases or paraneoplastic neurological syndromes) and pulmonary arterial hypertension are out of the scope of the review. Further in this review, we describe potential underlying mechanisms -adding the novel information on cerebral small vessel disease- and we summarize the impact of aging and smoking on the association between diseases of the brain and lung. Finally, we address the current knowledge gaps and indicate potential future research directions.

BRAIN DISORDERS AND POTENTIAL BRAIN-LUNG INTERACTIONS

A) Stroke

Stroke is one of the most common neurological diseases worldwide. Since 2010, stroke is the second leading cause of death worldwide.[2] A stroke is defined as the sudden death of brain cells in a localized area due to inadequate blood supply. The underlying cause can be a blockage in one of the blood vessels supplying the brain of oxygen and nutrients, called a ischemic stroke, or a weakened or ruptured brain vessel causing bleeding in (intracerebral) or around (subarachnoidal) the brain, called a hemorrhagic stroke. Ischemic strokes are further subtyped into large vessel stroke, small vessel stroke and cardioembolic stroke and they are mainly caused by atherosclerotic disease, cerebral hypoperfusion and cardiac embolism. A transient ischemic attack (TIA) is defined as a brief episode of neurological dysfunction caused by focal temporary cerebral ischemia without cerebral infarction.[181]

Increasing evidence relates impaired pulmonary function, particularly COPD, to stroke and stroke-associated mortality.[182-190] Recent studies have shown that carotid arterial plaque burden is increased in patients with COPD and that these plaques are more prone to rupture, due to an increased lipid content, leading to ischemic stroke.[141, 191, 192] Also, an increased carotid intima-media thickness might explain the high risk of stroke in women with adult onset asthma.[193, 194] Finally, there is some evidence of obstructive sleep apnea syndrome (OSAS) as a risk factor for ischemic stroke.[195, 196]

B) Cerebral small vessel disease

Cerebral small vessel disease refers to a group of pathological processes with various etiologies that affect small arteries, arterioles, venules and capillaries of the brain; and can be subtyped into age or hypertension-related small vessel diseases and cerebral amyloid angiopathy.[197] Magnetic resonance imaging (MRI) is commonly used to visualize effects of small vessel disease. Among the MRI markers, we can distinguish the following: A. focal markers, such as white matter lesions, lacunar infarcts, and microbleeds (collectively called small vessel disease); B. atrophy markers, such as brain atrophy/lobar atrophy/hippocampus atrophy and C. emerging markers, such as diffusion tensor imaging (microstructural integrity) or amyloid imaging (using positron emission tomography [PET] tracers that bind to amyloid plaques). In addition, MRI can also be used to quantify total brain perfusion (e.g. phase-contrast MRI) or regional perfusion (e.g. arterial spin labeling).

Small vessel disease can lead to thrombosis and subsequent stroke due to lipohyalinosis, fibrinoid degeneration or atheroma formation. Patients with COPD have a higher prevalence of neurological disease other than stroke, including cerebral vascular disease without hemiplegia, peripheral neuropathy and seizure disorder.[198] Elderly subjects with impaired pulmonary function (measured by FEV_1) have decreased regional white matter volume in the cerebellum.[199] Moreover, recent evidence has linked COPD to cerebral small vessel disease through an increased volume of cerebral white matter lesions[200, 201] and a higher prevalence

of cerebral microbleeds (45% in COPD subjects compared to 31% in controls without COPD).[141]

Cerebral small-vessel disease is common among elderly, and cerebral microbleeds are a relatively new suitable marker of cumulative cerebrovascular damage.[197, 202, 203] Furthermore, COPD would preferentially lead to the development of deep or infratentorial microbleeds which are thought to occur by arteriolosclerosis on the basis of hypertensive vasculopathy and lipohyalinosis.[141, 202] Microbleeds occurring in strictly lobar brain sites which are indicative of cerebral amyloid angiopathy, were not differentially affected, suggesting that COPD might be less involved in beta-amyloid pathology, which also underlies Alzheimer's dementia.[141, 202, 204]

C) Cognitive impairment and dementia

Cognitive impairment is a broad term that encompasses all deficits in the intellectual process of information handling, attention and concentration, memory, executive functioning and self-control.[205] Mild cognitive impairment (MCI) refers to measurable deficit(s) in cognition beyond normal aging in the absence of dementia, although it frequently leads to dementia. MCI is a clinical diagnosis based on a specified threshold on the continuum of cognitive impairment and subjective complaints. It is unclear from literature which cognitive domains are preferentially and consistently affected by lung impairment. However, cognitive deterioration appears to be highly prevalent in COPD, ranging from 10% to 49%.[206, 207] Approximately 42% of the patients with COPD in the nocturnal oxygen therapy trial (NOTT) demonstrated moderate-to-severe cognitive impairment compared to 14% amongst controls. Moreover, the prevalence of cognitive impairment correlated to the severity of COPD, ranging from 27% in COPD patients with mild hypoxemia to 62% in severe hypoxemia.[208, 209] The association of cognitive impairment with COPD severity might be restricted to patients with severe to very severe COPD.[210, 211] Overall, the cognitive functioning in patients with COPD would be impaired generally but more specifically in the domains of information processing and speed, memory and learning abilities, and coordination and motor functions.[177, 205, 210] Regarding clinical diagnosed MCI, 36% of patients with COPD had MCI compared to 12% of healthy controls.[212] Patients with COPD had mainly prevalent nonamnestic MCI with predominant attention and executive dysfunctions in this study.[212] In line with these results, COPD was associated with incident nonamnestic MCI in a dose-dependent manner in the Mayo Clinic Study on Aging.[213] Further research is needed to define whether patients with COPD with MCI return to normal, remain stable over time or mainly progress towards dementia.[212] A pitfall could be the underdiagnosis of COPD in cognitive impaired patients, since cognitive and functional impairment have a negative effect on the quality of spirometry needed to confirm the diagnosis of COPD.[214, 215]

D) Depression

Depression is a common mental disorder and is defined as a state of low mood characterized by sadness, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, feelings of tiredness and poor concentration. Community-dwelling elderly demonstrate depression rates ranging from 2% to 10%.[216] The prevalence of depression is significantly higher in COPD patients (approximately 40%) than in the general population.[217] Chronic diseases are frequently associated with psychological distress. Depression might be even more prevalent in patients with COPD than in patients with other chronic devastating illnesses such as cancer, stroke, arthritis, diabetes, heart disease, and renal disease.[217-221] However, prevalence figures of depression in COPD vary widely between 7% and 79% according to the studied COPD population and the method of assessment.[179, 222] A meta-analysis by Yohannes *et al.* reported a prevalence of depression of 40% (95% CI 36–44%), which is consistent with the observed 40% prevalence of depression in a large, nationally representative sample of COPD patients in the US population.[221, 222] The prevalence of depression in adult asthma was found to be 10%.[223, 224] Patients with idiopathic pulmonary fibrosis had significant levels of depressive symptoms in 24 to 49% of the patients.[225, 226] Very little is known on the causal direction between depression and respiratory diseases. Feelings of dyspnea and anticipatory anxiety limit physical activity in patients with COPD which can lead to mood changes and social impairment. Interestingly, within a large longitudinal cohort study, depression was recently been found to be a marker of risk for incident adult-onset asthma, but in contrast, prevalent asthma was not associated with incident adult-onset depression.[227]

E) Anxiety

Anxiety is an emotion characterized by feelings of tension, worried thoughts and physical changes like sweating, trembling, dizziness or a rapid heartbeat. Persons with anxiety or panic disorders usually have recurring intrusive thoughts or concerns which impact their behavior. The prevalence of anxiety in COPD patients in the United States was 36% (95% CI 31–41%).[228] However, as for depression, figures on the prevalence of anxious symptomatology ranged widely from 6% to 74%[222] or even up to 100%.[179] Regarding anxiety disorders, clinical anxiety was found in around 55% of COPD patients.[222] Panic attacks or panic disorders occurred in 8% to 67% of COPD patients.[179, 229] In patients with adult asthma, the prevalence of clinical anxiety was found to be 30%.[224] Depression and anxiety scores were found to be comparable for patients with COPD and asthma, but were significantly higher than for patients with tuberculosis.[223] In contrast, a study by Carvalho *et al.* observed that patients with asthma had significantly higher anxiety scores than COPD patients without recent exacerbation.[230] However, this might be influenced by a much higher presence of females in the asthma group, since females are more likely to have an anxiety disorder than men. Information on the incidence of anxiety and panic disorders and longitudinal population-based studies to explore the direction of the associations are lacking.

PATHOPHYSIOLOGY

Multiple interconnected factors associated with lung impairment, specifically COPD, might lead to brain dysfunction and/or damage.(Figure 1) Some factors (i.e. genetic susceptibility, smoking and aging) might be causally related with COPD and can induce a multimorbidity state affecting both lung and brain. Other factors (i.e. physical inactivity, inflammation, atherosclerosis, disturbed oxygenation and cardiovascular disease) might be instead aggravated by COPD.

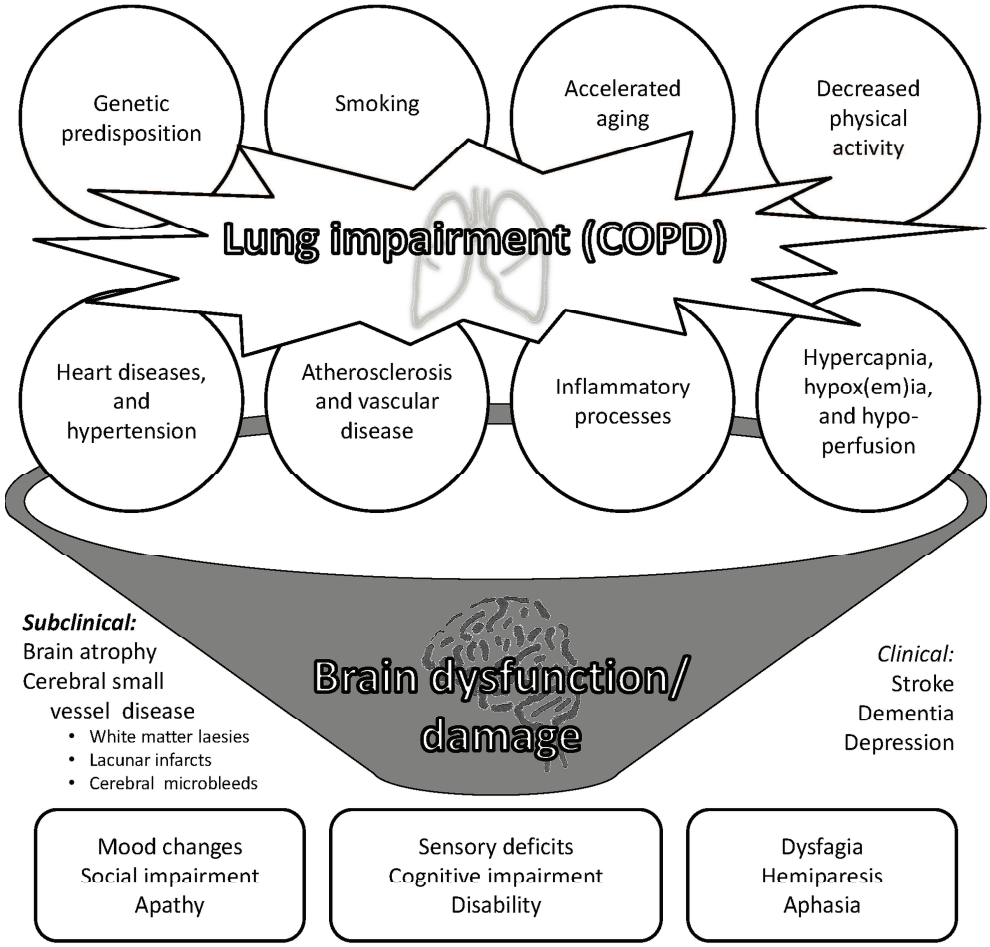


Figure 1: Potential mechanisms contributing to clinical and subclinical brain dysfunction and/or damage in elderly subjects with COPD.
COPD = Chronic Obstructive Pulmonary Disease

Effects of aging and smoking on brain and lung

Aging and smoking are common risk factors for brain disorders and lung diseases. Although the prevalence of some brain disorders was still higher in COPD after controlling for aging and smoking[141, 200, 201, 231-235], it remains to be further investigated whether COPD enhances the development of brain disorders independent of any combined risk factors. Since few studies adequately control for the total amount of smoking or environmental tobacco smoke, residual smoking confounding could still exist. Undisputably, both aging and smoking have an influence on the associations and can accelerate or amplify contributing mechanisms. Firstly, pulmonary and vascular inflammation may develop simultaneously in response to smoking, as with aging. Accumulating cellular defects during aging can initiate a vicious circle of inflammatory reactions further increasing damage.[236] Moreover, local and systemic inflammation activate microglial cells in the brain which become hyperresponsive with aging.[37]

Secondly, glucocorticoid concentrations are chronically elevated during aging and the hippocampus might lose its ability to repress glucocorticoid release through the hypothalamus, which exposes the entire organism to elevated stress hormone concentrations.[44] Through reduced glucocorticoid sensitivity, the aging brain might be a regulator of organismal aging, including accelerated lung aging.[44, 176] Thirdly, at the cellular level, several hallmarks of the aging process have been associated with impaired lung function or an increased risk of COPD, including telomere shortening, epigenetic alterations, loss of proteostasis and an altered intercellular communication.[176, 237, 238] The proteostasis might also be impaired in OSAS due to an activation of endoplasmic reticular stress pathways in the brain.[239] Importantly, the ability to handle proteostatic stress caused by environmental stressors like smoking declines with age.[176, 239]

Several studies have associated cigarette smoking to an altered brain structure.[199, 240-246] However, whilst smoking appears to be an independent risk factor in cognitive dysfunction, studies have found associations between impaired lung function and cognition that were independent of current and lifetime smoking status.[247-249] Smoking can exacerbate cerebral hypoxia due to chronically elevated carbon monoxide levels causing a leftward shift of the oxyhaemglobin dissociation curve.[208] In addition, cigarette smoke components such as heavy metals and nicotin are thought to have a direct neuromodulating effect.[250, 251] Nicotin upregulates nicotinic cholinergic receptors [252] and makes the cerebellum, believed to be rich in nicotinic cholinergic receptors[253], vulnerable to the chronic effects of smoking.[199] Nicotine might also affect mood via cholinergic receptors.[254] On the other hand, acetylcholine might act as a systemic mediator between lung and brain.[255] Both affective disorders and airway constriction have been associated with cholinergic activation.[227, 256] Hypoxemia can elevate brain choline levels which are associated with brain tissue breakdown, myelin damage and increased turnover of neuronal membrane precursors.[257-259] Since white matter microstructural damage was already observed in stable nonhypoxemic patients with COPD, these manifestations might not be limited to advanced COPD.[200] Finally, the biomarker plasma clusterin or apolipoprotein J might be a mediator of the relationship between COPD

and MCI since clusterin levels have been shown to be elevated in patients with COPD and are associated with cognitive decline in elderly.[260, 261]

Effects of systemic inflammation, vasculopathy and hypoxia on brain and lung

The mechanisms underlying the link between lung impairment and brain disorders have not yet entirely been elucidated, although different hypotheses encompass systemic inflammation, vasculopathy and hypoxia (hypoxemia) and hypercapnia. In patients with COPD, the hypoxia due to progressive airflow limitation and emphysema, as well as the chronic low-grade systemic inflammation might contribute to vessel wall changes resulting in endothelial dysfunction, stiffening of arteries and arterioles, and an impaired vascular reactivity.[32, 141, 232, 262-265] The inflamed endothelium overexpresses surface adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), facilitating the adherence of white blood cells to damaged endothelial surfaces.[29]

In addition, interleukin 6 can stimulate the release of acute phase proteins by hepatocytes including C Reactive Protein (CRP), serum amyloid A, fibrinogen, and procoagulant factors which further promote or amplify the inflammatory process.[29, 266] CRP fosters the uptake of low density lipoproteins (LDL) by macrophages, which can contribute to the increased prevalence of arterial plaques containing a lipid core in patients with COPD.[29, 232] Progression of atherosclerosis and instability of plaques in the carotid arteries can thereby lead to stroke. In OSAS, the increased risk of stroke is thought to result from an accelerated atherogenesis, impaired endothelial function, and a prothrombotic and proinflammatory state in addition to blood pressure swings, reduction in cerebral blood flow and an altered cerebral autoregulation.[195] Similarly, a vascular inflammation state is hypothesized to lead to microvascular dysfunction or cerebral small vessel disease which results in observable cerebral microbleeds.[32, 197, 202] Arteriolosclerotic microangiopathy or cerebral small-vessel disease is linked with (vascular) depression, cognitive and functional impairment.[177, 178, 197] Moreover, the role of vascular pathology including atherosclerosis is increasingly being recognized in the etiology of dementia and Alzheimer's disease in addition to beta-amyloid and tau pathology.[267] The fact that COPD is preferably associated with nonamnestic MCI also suggests a role of inflammation and vascular disease.[213] Cerebral small vessel disease is known to cause cognitive decline and functional loss in the elderly.[197] Vascular risk factors and chronic ischemic brain changes are associated with vascular dementia and vascular depression.[268-271] Patients with COPD who had cognitive impairment showed an altered cerebral perfusion.[272] Especially brain regions fed by the posterior cerebral circulation (e.g. cerebellum, hippocampus, and deep nuclei) are more vulnerable to hypoxia, which is consistent with the preferential deep or infratentorial cerebral microbleeds seen in patients with COPD.[141, 273, 274] Hypoxia is moreover thought to cause an altered brain bioenergetic metabolism, brain dysfunction and hippocampal atrophy as well as generalized cerebral atrophy.[275-277] However, in order to draw more definitive conclusions on COPD, depression and dementia and its subtypes, further research is required.

Effects of genetic susceptibility and epigenetic mechanisms on brain and lung

Shared genetic factors might predispose subjects to COPD and brain disorders.[278, 279] Most respiratory and neurological diseases in the elderly are complex and involve interactions between genetic susceptibility, decreased reserve capacity by aging and cumulative exposure to environmental factors such as smoking, air pollution, unhealthy diet or lifestyle. Although research into common genetic and epigenetic mechanisms underlying lung and brain diseases is still in its infancy, several clues are emerging.

First, subjects with lung disease and stroke might share genetic variations that lead to an altered histone deacetylase (HDAC) expression.[280-283] In addition, sirtuines (type III HDACs) are associated with aging, neurodegenerative diseases and COPD.[284, 285] Especially sirtuin 1 (SIRT1) is of interest since it is downregulated in COPD and the posttranslational modification is modified by smoking and oxidative stress.[286] Downregulation of SIRT1 is involved in proinflammatory pathways, impairs mechanisms of neuronal repair and limits cognitive function processes.[280, 285-287]

Second, genome-wide association studies of asthma and depression both suggested involvement of Retinoic Acid Receptor-Related Orphan Receptor Alpha (RORα).[288, 289] Moreover, RORα has been suggested to play a role in chronic inflammation, hypoxia signaling and the pathogenesis of emphysema and premature aging. [290-292]

Finally, genetic variations affecting serotonin and nicotinic acetylcholine pathways are associated with COPD, nicotine dependence and depression.[11, 279, 293] An increased genetic risk of nicotine addiction might therefore indirectly link COPD to depression, on top of the psychosocial effects of a chronic debilitating disease as COPD.

IMPACT OF THE BRAIN DISORDERS ON LUNG DISEASE

The impact of brain damage on the quality of life in survivors of a clinical stroke is clearly observable through the functional loss of body parts or abilities (e.g. hemiparesis, sensory deficits, dysphagia or aphasia, Figure 1). The impact of subclinical events on the quality of life such as silent stroke, brain atrophy or small vessel disease can be more subtle and become only clinically apparent after a certain amount of damage has been reached. Early assessment of cerebrovascular diseases, cognitive impairment and mental illness in respiratory impaired patients is a first important step to favorably influence the large health burden. A reduced pulmonary function is associated with an increased risk of stroke and stroke-associated mortality, but even in stroke survivors the lung function is often affected due to weakness of respiratory muscles associated with stroke or central diaphragmatic impairment.[183, 186, 294, 295] Furthermore, stroke survivors are at increased risk of dysphagia and aspiration pneumonia.[296]

Several studies in mostly selected patient populations show that cognitive dysfunction reduces the level of functioning as assessed by activities of daily living [297-300] and is associated with increased length of hospital stay, mortality, discharge destination [301] and poor compliance with both medication and oxygen therapy.[302] This poor compliance in turn increases the risk of acute exacerbation.[303, 304] During an exacerbation of COPD, cognitive function is further impaired but the impairment and excitability changes may be (initially) reversible.[177, 305]

Several associations between cognitive dysfunction in COPD and health outcomes were not consistent between studies.[177, 306-310] Methodological limitations of the performed studies including limited or self-reported assessment of COPD and/or cognitive dysfunctioning, might explain the discrepancies.[205] Depressive symptoms and anxiety have been associated with reduced functional capacity, increased risk of COPD exacerbations and increased mortality.[311-317] Depression and cognitive impairment can place COPD patients in a vicious cycle since it may influence their attempt to stop smoking, make them less compliant to the maintenance medication and raise the exacerbation and mortality risk.[313, 318-320]

Finally, multiple comorbidities in elderly with respiratory impairment result in polypharmacy.[321] Regarding the brain-lung interaction, it is important to note that some drugs affecting the central nervous system (like opiates, narcotics and tranquillizers) might conversely cause lung injury or respiratory depression (*http://www.pneumotox.com*). Especially elderly COPD patients are at increased risk of adverse drug reactions due to polypharmacy and overdosage of drugs with renal clearance.[322]

FUTURE RESEARCH DIRECTIONS

Because the influence of comorbidities on disability and mortality is especially important at an older age, prevalence figures should be more precisely estimated and large prospective studies conducted to estimate the incidence of brain disorders in elderly with lung impairment. Unified definitions of both brain disorders and respiratory diseases herein are crucial. In addition to COPD, brain disorders in lung disease entities as obstructive sleep apnea syndrome (OSAS), interstitial lung disease and frequently underdiagnosed asthma in the elderly should be investigated too.[323] Many different definitions are used for cognitive dysfunction and many different strategies for screening (using different cut-offs) exist for cognitive impairment and diagnosis of depression in patients with chronic lung diseases such as COPD.[210, 324, 325] This makes it difficult to ascertain the consequences of cognitive dysfunction and depression on the daily living in respiratory impaired patients.

Definitely, more research is needed to investigate the relation between COPD and dementia. Regarding the underlying mechanisms, a link with the different subtypes of dementia might be interesting to explore. Another interesting topic might be research into emerging MRI markers as diffusion tensor imaging to investigate associations with early microstructural white matter changes. [326]

In addition, there is need for studies further investigating the mechanisms contributing to the development or influencing the course of the described brain disorders, including ethnic or gender differences, epigenetic mechanisms and genetic predisposition.[327] Longitudinal studies are required to elucidate the pathophysiological mechanisms underlying brain-lung diseases in elderly and clinical trials are awaited to evaluate potential preventive or treatment strategies for the brain disorders resulting from the brain-lung interaction. The routinely exclusion of COPD patients with cognitive impairment, depression, physical limitations or comorbidities in general from large pharmacological trials, precludes optimal daily care for the majority of elderly COPD patients.[322] Besides pharmacological therapies, more research is needed to explore the potential benefits of cognitive behavioral therapy or pulmonary rehabilitation in this population.

In conclusion, increasing evidence associates lung impairment with brain disorders in the elderly. Considering that neurological disorders have a major impact on the daily lives of patients with respiratory diseases, time is running for more investigations towards interactions between the lung and the brain in the elderly.

ABSTRACT

Background

Chronic obstructive pulmonary disease (COPD) is an independent risk factor for ischemic stroke and the risk increases with severity of airflow limitation. Even though vulnerable carotid artery plaque components, such as intraplaque hemorrhage and lipid core, place persons at high risk for ischemic events, the plaque composition in COPD patients has never been explored.

Objectives

To investigate the prevalence of carotid wall thickening, the different carotid artery plaque components, and their relations with severity of airflow limitation in elderly patients with COPD.

Methods

This cross-sectional analysis was part of the Rotterdam Study, a prospective population-based cohort study performed in subjects aged 55 years and older. Diagnosis of COPD was confirmed by spirometry. Participants with carotid wall intima-media thickness (IMT) ≥ 2.5 mm on ultrasonography underwent high-resolution magnetic resonance imaging (MRI) for characterization of carotid plaques. Data were analyzed using logistic regression.

Results

COPD cases (n = 253) had a twofold increased risk (OR 2.0, 95%CI 1.44-2.85, p<0.0001) of presentation with carotid wall thickening on ultrasonography compared to controls with a normal lung function (n = 920). Moreover, the risk increased significantly with severity of airflow limitation. On magnetic resonance imaging, vulnerable lipid core plaques were more frequent in COPD cases than in control subjects (OR 2.1, 95%CI 1.25-3.69, p=0.0058).

Conclusions

Carotid artery wall thickening is more prevalent in COPD patients than in controls. In elderly subjects with carotid wall thickening, COPD is an independent predictor for the presence of a lipid core, and therefore of vulnerable plaques.

Lahousse L, van den Bouwhuijsen Q, Loth DW, Joos GF, Hofman A, Witteman JC, van der Lugt A, Brusselle GG, Stricker BH. 2013 Chronic Obstructive Pulmonary Disease and Lipid Core Carotid Artery Plaques in the Elderly: the Rotterdam Study. Am J Respir Crit Care Med. 187(1):58-64
Official Journal of the American Thoracic Society.

9. COPD and macroangiopathy: carotid artery plaques

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterized by progressive airflow limitation and various systemic manifestations which significantly impact mortality.[325, 328, 329] Increasing evidence demonstrates that COPD is an independent risk factor for ischemic stroke and that the risk increases by severity of airflow limitation.[183, 184] Moreover, the risk of stroke-associated mortality has also been shown to correlate with degree of airflow limitation.[184] The main underlying causes of cerebral ischemia are atherosclerotic disease and cardiac embolism.

Vulnerable carotid artery plaques place persons at high risk for ischemic stroke through thromboembolism arising from a disrupted plaque surface. Besides size and degree of obstruction, plaque properties are recognized to be crucial to identify high risk patients.[330] Unlike the extensively used ultrasound and Computed Tomography (CT), high-resolution magnetic resonance imaging (MRI) has the ability to distinguish between lipid and hemorrhagic components in plaques and is therefore very powerful to reveal noninvasively the composition of atherosclerotic plaque.[331] Regarding the different carotid artery plaque components, calcified plaques are generally known to be more stable than noncalcified plaques. Intraplaque hemorrhage and lipid core are both recognized as vulnerable plaque components and associated with the risk of cerebrovascular disease.[332] Intraplaque microhemorrhages initiate phagocytosis of erythrocytes which may lead to lipid accumulation in macrophages.[333] Plaques with a lipid core contain fat-laden macrophages and extracellular lipids and are along with a thin fibrous cap more prone to rupture.[334]

Even though increasing evidence indicates that impaired lung function is an important risk factor for the formation of carotid plaques on ultrasonography, the association between COPD and the different plaque components on MRI has not yet been investigated. Therefore, the aim of this study was to examine in a large, prospective population-based cohort study of elderly, whether carotid atherosclerosis is indeed more prevalent in subjects with COPD compared to subjects with a normal lung function, whether plaque components differ between both groups, and whether plaque prevalence or components relate to severity of airflow limitation.

METHODS

Study design

This cross-sectional analysis was part of the Rotterdam Study, a population-based cohort study aimed at assessing the occurrence of, and risk factors for chronic diseases in the elderly.[108] The study started in 1990 among 7983 persons aged ≥ 55 years, and all participants are invited every 3 to 4 years to the research center for follow-up examinations, including spirometry and carotid ultrasonography. The medical ethics committee of the

Erasmus Medical Center, Rotterdam, and the review board of The Netherlands Ministry of Health, Welfare and Sports, approved the study. Participants gave written informed consent.

Carotid artery atherosclerosis

Ultrasonography of the common carotid artery, bifurcation and internal carotid artery of the left and right carotid arteries was performed using a 7.5-MHz linear-array transducer. Participants, in whom ultrasonography revealed carotid intima-media wall thickness (IMT) ≥ 2.5 mm in the left, right or both carotid arteries, were selected for carotid MRI scanning from October 2007 till August 2010. MRI of the carotid arteries was performed on a 1.5-T MRI scanner with a bilateral phased-array surface coil. Participants were recorded as positive for the presence of any plaque component if the component was identified in one or both carotid arteries. The ultrasonography and carotid MRI protocol, reading and reproducibility were previously described.[331, 335]

In detail, ultrasonography was performed by careful searching all interfaces of the near and far walls of the distal common carotid artery, the carotid bifurcation and the internal carotid artery of the left and right carotid arteries using a 7.5-MHz linear-array transducer in accordance with the Rotterdam Study ultrasound protocol.[335, 336] Optimal longitudinal, two-dimensional ultrasound images of the carotid artery were frozen on the R-wave of the ECG and stored on videotape. The actual measurements of intima–media thickness were performed off-line, averaged from three frozen images of each arterial segment from the videotape. The interfaces of the common carotid artery, the carotid bifurcation and the internal carotid artery were marked across a length of 10 mm. Then the maximum carotid intima–media thickness value was determined as the mean of the maximum intima–media thickness over the marked length of anterior (near)- and posterior (far)-wall measurements of both the left and right side arteries for each of the three arterial segments. When an atherosclerotic plaque was present at the measurement site, it was included in the measurement. If data on one of the walls or one of the sides was missing, maximum thickness of the available wall and side was used. Readers of the ultrasound images were unaware of the case status of the subject.

Results from a reproducibility study of ultrasound intima-media thickness measurements of the common carotid artery among 80 participants of the Rotterdam Study who underwent a second ultrasound of both carotid arteries within 3 months of the first scan, showed low mean differences (SD) in far-wall intima–media thickness of the common carotid artery between paired measurements of sonographers, readers, and visits of 0.005 mm (0.09), 0.060 mm (0.05), and 0.033 mm (0.12), respectively.[337]

MRI high-resolution images were obtained using a standardized protocol.[331] First, both carotid bifurcations were identified by means of two-dimensional (2D) time-of-flight MR angiography. Thereafter, high-resolution MRI sequences were planned to image the carotid

bifurcations on both sides: four sequences in the axial plane: (1) a proton density weighted (PDw)-fast spin echo (FSE)-black blood (BB) sequence; (2) a PDw-FSE-BB with an increased in-plane resolution; (3) a PDw-echo planar imaging (EPI) sequence, and (4) a T2w-EPI sequence; and two 3D sequences: (1) a 3D-T1w-gradient echo (GRE) sequence; and (2) a 3D phased-contrast MR angiography.

Carotid plaque characteristics were assessed with an online PACS viewer. All scans were reviewed by a trained physician with three years of experience in carotid MRI under supervision of a neuro-radiologist with more than six years of experience in MRI plaque analysis, who were both unaware of the research hypothesis. Calcification was defined as the presence of a hypointense region in the plaque on all sequences.[331, 338, 339] Intraplaque haemorrhage was defined as the presence of a hyperintense region in the atherosclerotic plaque on 3D-T1w-GRE.[331, 340, 341] Lipid core presence was defined as a hypointense region, not classified as intraplaque haemorrhage or calcification, in the plaque on PDw-FSE or PDw-EPI and T2w-EPI images, or a region of relative signal intensity drop in the T2w-EPI images compared with the PDw-EPI images.[331, 338, 339, 342] Results from a reproducibility study of MRI measured plaque composition among 40 participants of the Rotterdam Study who underwent a second MRI scan (average time between scans 15±9 days), showed excellent inter-observer and intra-subjects agreement.[331] The Kappa values for inter-observer agreement were 0.86 (95% CI 0.72-0.99) for intraplaque hemorrhage; 0.86 (95% CI 0.72-0.99) for lipid core presence and 0.94 (95% CI 0.86-0.99) for calcification. The Kappa values for intra-subjects agreement were 0.95 (95% CI 0.88-0.99) for presence of intraplaque hemorrhage; 0.85 (95% CI 0.74-0.96) for lipid core and 0.91 (95% CI 0.82-0.99) for calcification. Furthermore, the non-contrast-enhanced MRI technique for plaque characterization has been shown to have good accuracy and reproducibility in other validation studies.[338, 339, 342]

Diagnosis and staging of COPD

The diagnosis of COPD was based on an obstructive spirometry examination according to the modified Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria [proportion of the forced vital capacity exhaled in the first second (FEV_1/FVC) < 0.7] and classified into mild, moderate or severe by forced expiratory volume in one second (FEV_1)% predicted of ≥ 80%, 50-80% or < 50% respectively. Participants with a spirometry report suggestive of restrictive respiratory disease [FEV_1/FVC ≥ 0.7 and forced expiratory vital capacity (FVC) and/or FEV_1 < 80% predicted], and patients with asthma were excluded. No reversibility tests were conducted. Spirometry was performed between March 2009 and January 2011 using a Master Screen® PFT Pro (CareFusion, San Diego, CA) by trained paramedical personnel according to the ATS/ERS guidelines.[22]

Statistical analyses

Differences between COPD and control subjects were compared using t-test or Mann-Whitney U test for continuous variables and Chi-Square test for categorical variables. Wilson score method for a binomial proportion was used to calculate prevalence plus 95% confidence intervals. A logistic regression model was used to calculate the risk of COPD on carotid artery wall thickening and the different plaque components. Age, sex, body mass index (BMI), smoking behaviour, hypertension, hypercholesterolemia [total serum cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides], anemia [haemoglobin, hematocrit], diabetes mellitus, kidney function [creatinin] and maximum wall thickness were considered as potential confounders and comprise the previously identified risk factors for the different plaque components.[331] Information on smoking behaviour was collected using home interviews. Smoking status was thus self-reported and classified as current, past, and never. Cigarette pack years were computed as duration of smoking (years) multiplied by the number of smoked cigarettes, divided by 20. Hypertension was identified as the use of antihypertensive medication and/or an average systolic blood pressure of 160 mmHg or above and/or an average diastolic blood pressure of 100 mmHg or above (Grade 2 according to European Society of Cardiology criteria).[343] Medication use was assessed through automated linkage to pharmacies with computerized records. Blood pressure, total cholesterol, HDL cholesterol, and triglycerides were measured at study center visits as described previously.[344] Diabetes mellitus was defined as the use of blood glucose-lowering medication and/or a non-fasting serum glucose level of ≥11.1 mmol/L and/or fasting serum glucose levels ≥7 mmol/L.

Covariables were included in the models if they changed the risk estimate by more than 10% or if they were biologically plausible according to previous literature. Statistical analyses were performed using SPSS, version 20.0 for Windows (IBM, North Castle, NY).

RESULTS

Subject flow and baseline characteristics

Until February 2011, 1386 participants of the Rotterdam study had an interpretable spirometry test.*(Figure 1)* Of them, 253 (18.3%) COPD patients and 920 (66.4%) controls underwent ultrasonography of both carotid arteries. *Table 1* shows the baseline characteristics of the study population (n=1173) with median age 78 (IQR=6). COPD subjects were more often male and (current) smokers. 694 out of 1173 participants (59.2%) participants had carotid wall thickening (IMT ≥ 2.5 mm in the left, right or both carotid arteries) determined by ultrasonography.*(Figure 1)* 216 subjects were not invited for carotid MRI scanning due to contraindications for MRI (n=17), dementia (n=3), physical immobility (n=19), history of carotid endarterectomy (n=3), living in a nursing home or because they moved outside the area (n=30) or could not be scheduled during the study period (n=144). Of the 478 subjects who were invited during the study period, 407 agreed to participate

(response rate 85.1%). Due to physical inabilities (e.g. back pain) or claustrophobia, imaging could not be performed or completed in 26 individuals (6.4%). In total, 358 subjects of the 381 participants who underwent a complete scan, had scans of good quality (94.0%); 88 patients were COPD cases and 270 control subjects with normal lung function. *Table 2* shows that the clinical, demographic and physiological characteristics of the subjects excluded from the MRI examination were similar to those from the 358 subjects included in the MRI examination.

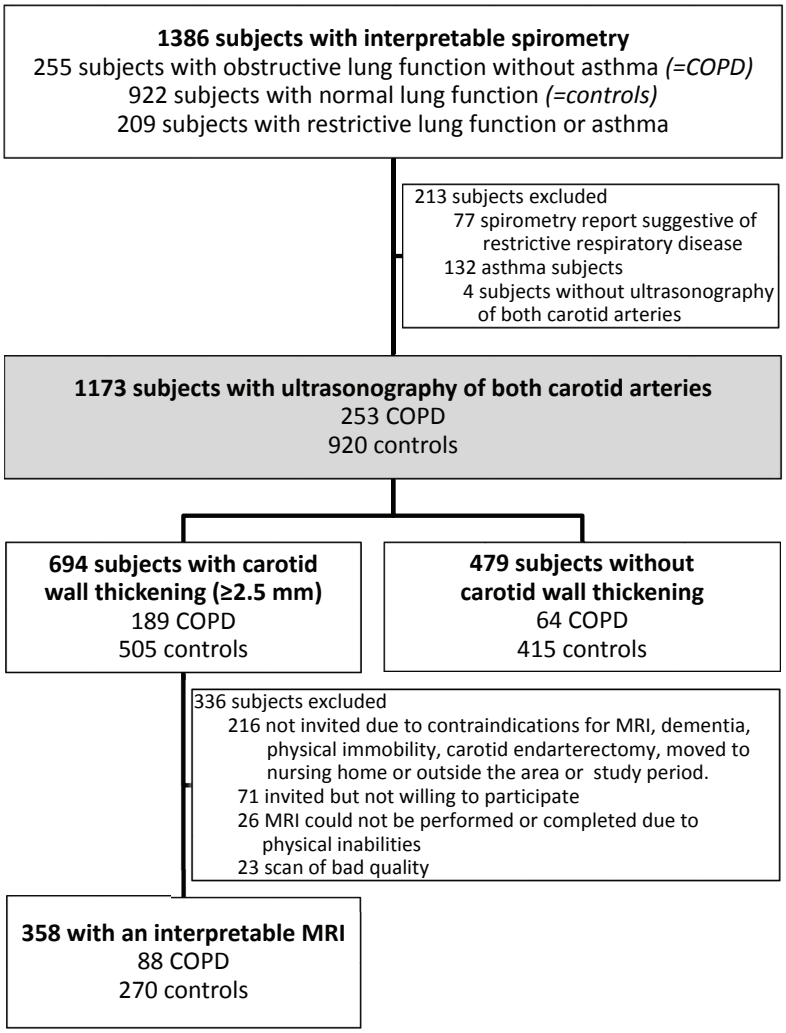


Figure 1: Study profile.

Table 1: Baseline characteristics of the study population (n=1173).

	COPD (n=253)	Controls (n= 920)	p-value
Age (years)	79 (7)	78 (6)	0.006
Males	142 (56.1)	366 (39.8)	<0.001
Smoking status[1]			<0.001
Never smoker	50 (19.8)	345 (37.5)	
Former smoker	159 (62.8)	520 (56.5)	
Current smoker	44 (17.4)	55 (6.0)	
Pack years for smokers	27.5 (36.0)	14.4 (25.2)	<0.001
Body mass index (kg/m²)	25.9 (5.0)	27.0 (4.8)	0.001
Hypertension[2]	136 (53.8)	468 (50.9)	0.416
Myocardial infarction	20 (7.9)	64 (7.0)	0.610
Coronary revascularization[3]	23 (9.1)	80 (8.7)	0.852
Diabetes[4]	40 (15.8)	122 (13.3)	0.301
Glucose (serum, mmol/l)	5.5 (0.9)	5.5 (0.9)	0.378
Total cholesterol (serum, mmol/l)	5.2 (1.4)	5.3 (1.5)	0.299
HDL-cholesterol (serum, mmol/l)	1.4 (0.5)	1.4 (0.5)	0.648
Creatinin (serum, µmol/l)	79.5 (27.0)	80.0 (23.5)	0.156
Hemoglobin (mmol/l)	8.6 (1.1)	8.5 (0.9)	0.173
Hematocrit (%)	44 (5)	43 (4)	0.025
Leucocytes (#,*10^3)	7.1 (2.3)	6.8 (2.0)	0.004
Granulocytes (#,*10^3)	4.4 (1.8)	4.1 (1.5)	<0.001
FEV_1 (% predicted)	80.5 (27.6)	111.6 (24.1)	<0.001
FEV_1/FVC (%)	65.6 (8.8)	78.1 (6.2)	<0.001

Categorical variables are expressed as numbers (percentage). Values of continuous variables are expressed as median (IQR). [1]Smoking status was self-reported. [2]Hypertension was defined as antihypertensive medication use and/or an average systolic blood pressure of ≥ 160 mmHg and/or an average diastolic blood pressure of ≥ 100 mmHg. [3]Coronary revascularization was defined as coronary artery bypass grafting and percutaneous coronary intervention. [4]Diabetes mellitus was defined as blood glucose-lowering medication use and/or a non-fasting serum glucose level of ≥11.1 mmol/L and/or fasting serum glucose levels ≥7 mmol/L.
Abbreviations: COPD=Chronic Obstructive Pulmonary Disease; FEV_1= forced expiratory volume in one second; FEV_1/FVC= proportion of the forced vital capacity exhaled in the first second; HDL= High-Density Lipoprotein

Table 2: Comparison of the subjects included in the MRI component and excluded from the MRI component.

	IMT ≥ 2.5 mm, MRI performed (n=358)	IMT ≥ 2.5 mm, MRI not performed (n=336)	p-value
Age (years)	78 (6)	79 (7)	0.127
Males	169 (47.2)	178 (53.0)	0.129
Smoking status[1] Never smoker Former smoker Current smoker	 91 (25.4) 236 (65.9) 31 (8.7)	 89 (26.5) 215 64.0) 32 (9.5)	0.853
Pack years for smokers	21 (31.7)	17 (32.0)	0.585
Body mass index (kg/m²)	27.0 (5.0)	27.0 (5.4)	0.374
COPD	88 (24.6)	101 (30.1)	0.105
Hypertension[2]	204 (57.0)	204 (60.7)	0.318
Myocardial infarction	37 (10.4)	32 (9.5)	0.703
Coronary revascularization[3]	51 (14.3)	35 (10.4)	0.119
Diabetes[4]	57 (15.9)	52 (15.5)	0.885
Glucose (serum, mmol/l)	5.6 (0.8)	5.6 (1.1)	0.455
Total cholesterol (serum, mmol/l)	5.1 (1.6)	5.2 (1.7)	0.701
HDL-cholesterol (serum, mmol/l)	1.4 (0.5)	1.4 (0.5)	0.529
Creatinin (serum, µmol/l)	82.0 (25.3)	82.0 (24.0)	0.540
Hemoglobin (mmol/l)	8.5 (1.1)	8.6 (1.1)	0.446
Hematocrit (%)	43.5 (5)	43.0 (5)	0.538
Leucocytes (#,*10^3)	7.0 (1.9)	6.9 (2.2)	0.702
Granulocytes (#,*10^3)	4.4 (1.7)	4.3 (1.6)	0.939
FEV_1 (% predicted)	103.9 (27.4)	101.3 (31.0)	0.172
FEV_1/FVC (%)	76.1 (10.1)	74.9 (10.6)	0.042

Categorical variables are expressed as numbers (percentage). Values of continuous variables are expressed as median (IQR). [1]Smoking status was self-reported. [2]Hypertension was defined as antihypertensive medication use and/or an average systolic blood pressure of ≥ 160 mmHg and/or an average diastolic blood pressure of ≥ 100 mmHg. [3]Coronary revascularization was defined as coronary artery bypass grafting and percutaneous coronary intervention. [4]Diabetes mellitus was defined as blood glucose-lowering medication use and/or a non-fasting serum glucose level of ≥11.1 mmol/L and/or fasting serum glucose levels ≥7 mmol/L.

Abbreviations: COPD=Chronic Obstructive Pulmonary Disease; FEV_1= forced expiratory volume in one second; FEV_1/FVC= proportion of the forced vital capacity exhaled in the first second; HDL= High-Density Lipoprotein

Carotid artery wall thickening as determined by ultrasonography

The prevalence of carotid artery wall thickening was higher in participants with COPD (189 out of 253; 74.7%; 95% CI: 69.0 to 79.7%) than in those without COPD (505 out of 920 controls; 54.9%; 95% CI: 51.7 to 58.1%) (p<0.0001). The prevalence of carotid wall thickening (≥ 2.5 mm in the left, right or both carotid arteries) was significantly associated with severity of airflow limitation.*(Figure 2)* Subjects with severe airflow limitation had a more than sixfold increased risk of carotid wall thickening compared to control subjects, independent of age, sex, BMI, smoking status, hypertension, HDL-cholesterol, triglycerides, hemoglobin, diabetes and serum creatinin.*(Table 3)* Evaluating the continuous measures FEV_1 and FEV_1/FVC, *Table 3* shows that per 10% predicted increase in FEV_1 the risk of carotid artery atherosclerosis decreases by 14% and that per percentage increase in FEV_1/FVC, the risk of carotid artery atherosclerosis decreases by 4%. Stratified on smoking status, the prevalence of carotid wall thickening (≥2.5 mm in the left, right or both carotid arteries) in COPD cases was significantly higher in both never, former, and current smokers compared to controls.*(Figure 3)* Additionally, a plaque score was measured on ultrasonography and reflects the total number of sites with plaques (left- and right-sided common carotid artery, bifurcation, and internal carotid artery); the score was significantly higher in subjects with COPD compared to controls (p<0.001). Volume of plaques was also measured on ultrasonography and categorically defined as stenosis more or less than 50%; 20.2% of subjects with COPD had a stenosis ≥50% left-, right- or both-sided compared to 13.7% of controls (p=0.011).

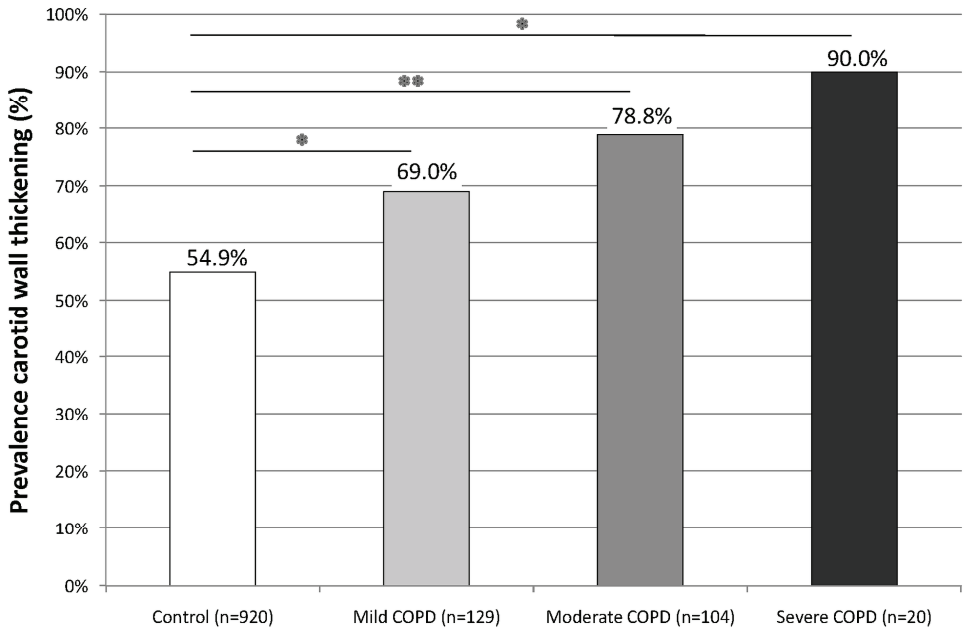


Figure 2: Prevalence (%) of carotid artery wall thickening (≥ 2.5 mm in the left, right or both carotid arteries) determined by ultrasonography, according to severity of airflow limitation. * p < 0.005 ; ** p < 0.0001 by Pearson Chi-Square

Table 3:

A) Risk of carotid artery wall thickening determined by ultrasonography.	**Model 1** (n=1173)			**Model 2** (n=1145)		
Categorical, all versus no COPD	OR	95% CI	P-value	OR	95% CI	P-value
COPD	2.1	1.53-2.90	**<0.0001**	2.0	1.44-2.85	**<0.0001**
COPD, *mild*	1.6	1.09-2.46	**0.0174**	1.6	1.06-2.49	**0.0258**
COPD, *moderate*	2.6	1.57-4.27	**0.0002**	2.4	1.42-4.04	**0.0011**
COPD, *severe*	6.1	1.36-26.95	**0.0180**	6.4	1.37-29.81	**0.0180**
COPD, *dyspnea score <2*	1.6	1.09-2.44	**0.0175**	1.6	1.04-2.46	**0.0328**
COPD, *dyspnea score ≥2*	3.1	1.89-5.04	**<0.0001**	2.8	1.71-4.71	**<0.0001**
COPD, *no chronic bronchitis*	2.0	1.42-2.77	**<0.0001**	1.9	1.35-2.73	**0.0003**
COPD, *chronic bronchitis*	4.0	1.35-11.80	**0.0122**	3.7	1.21-11.46	**0.0218**
Continuous, lung function	OR	95% CI	P-value	OR	95% CI	P-value
FEV_1 (per 10% predicted increase)	0.8	0.80-0.90	**<0.0001**	0.8	0.81-0.92	**<0.0001**
FEV_1/FVC (per 1 % increase)	0.9	0.95-0.98	**<0.0001**	0.9	0.94-0.98	**<0.0001**
B) Risk of lipid core carotid plaques determined by MRI.	**Model 1** (n=358)			**Model 2** (n=353)		
Categorical, all versus no COPD	OR	95% CI	P-value	OR	95% CI	P-value
COPD	2.2	1.31-3.58	**0.0025**	2.1	1.25-3.69	**0.0058**
COPD, *mild*	2.0	1.07-3.93	**0.0306**	2.0	0.97-3.98	0.0598
COPD, *moderate*	2.1	1.04-4.31	**0.0398**	2.3	1.08-4.94	**0.0317**
COPD, *severe*	3.8	0.72-20.17	0.1173	2.6	0.44-15.58	0.2930
COPD, *dyspnea score <2*	1.6	0.79-3.04	0.2006	1.7	0.81-3.47	0.1680
COPD, *dyspnea score ≥2*	2.9	1.52-5.66	**0.0013**	2.7	1.33-5.49	**0.0062**
COPD, *no chronic bronchitis*	2.3	1.37-3.99	**0.0018**	2.5	1.40-4.45	**0.0019**
COPD, *chronic bronchitis*	1.2	0.37-3.95	0.7595	0.7	0.17-2.52	0.5382
Continuous, lung function	OR	95% CI	P-value	OR	95% CI	P-value
FEV_1 (per 10% predicted increase)	0.8	0.81-0.99	**0.0256**	0.8	0.80-0.99	**0.0248**
FEV_1/FVC (per 1 % increase)	0.9	0.94-0.99	**0.0037**	0.9	0.94-0.99	**0.0142**

Model 1: age and sex adjusted.

Model 2: adjusted for age, sex, Body Mass Index, smoking status, hypertension, High-Density Lipoprotein-cholesterol (serum, mmol/l), triglycerides (serum, mmol/l), hemoglobin (mmol/l), diabetes and creatinin (serum, µmol/l).

COPD: defined as $FEV_1/FVC < 0.7$ and categorized according to the modified Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria into mild COPD (GOLD1; $FEV_1 \geq 80\%$pred), moderate COPD (GOLD2; $50\% \leq FEV_1 < 80\%$pred) & severe COPD (GOLD3; $FEV_1 < 50\%$pred)

Dyspnea score: based on 5 dyspnea-questions and scored from 0 (all questions negative) to 5 (all positive)

Chronic bronchitis: defined as the self-reported presence of cough and sputum for at least 3 months in each of two consecutive years *(http://www.goldcopd.org)*

Abbreviations: OR= Odds Ratio; CI=Confidence Interval; COPD= Chronic Obstructive Pulmonary Disease; FEV_1= forced expiratory volume in one second; FEV_1/FVC = proportion of the forced vital capacity exhaled in the first second; MRI= magnetic resonance imaging

Bold values indicate significance at $P < 0.05$.

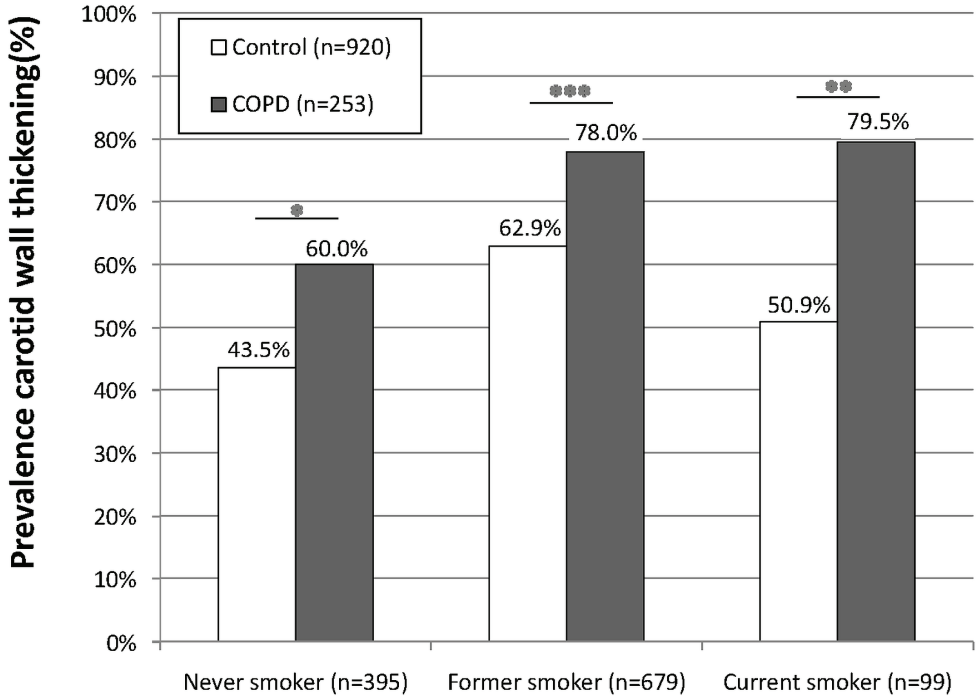


Figure 3: Prevalence (%) of carotid artery wall thickening (≥2.5 mm in the left, right or both carotid arteries) determined by ultrasonography, according to smoking status.
* p = 0.0284; ** p = 0.0032 ; *** p = 0.0004 by Pearson Chi-Square

Carotid artery plaque components as determined by MRI

358 subjects with carotid wall thickening on ultrasonography had an interpretable MRI scan result. The median maximum wall thickness on MRI was 3.6 mm (IQR: 1.1 mm) in 88 COPD subjects and 3.3 mm (IQR : 1.0 mm) in 270 controls (p=0.04). Median stenosis was 16.7% (IQR: 32.2 %) in COPD subjects and 12.6% (IQR: 25.6 %) in controls (p=0.102). The different prevalence of intraplaque hemorrhage, lipid core, and calcification between COPD subjects and controls is shown in *Figure 4*. Carotid artery plaques with a lipid core were significantly more prevalent in COPD subjects than in controls. No significant associations between COPD and the risk of intraplaque hemorrhage or calcification were observed.(*Table 4*). COPD cases have a more than twofold increased risk to present lipid core compared to control subjects independent of age, sex, BMI, smoking status, hypertension, HDL-cholesterol, triglycerides, hemoglobin, diabetes, creatinin, and maximum wall thickness.(*Table 3)* Especially COPD subjects with a dyspnea score ≥ 2 had a significantly increased risk of a lipid core plaque compared to control subjects (OR 2.7, 95%CI 1.33-5.49, p=0.0062). The risk of lipid core plaques was significantly inversely related to both FEV_1% predicted and FEV_1/FVC.

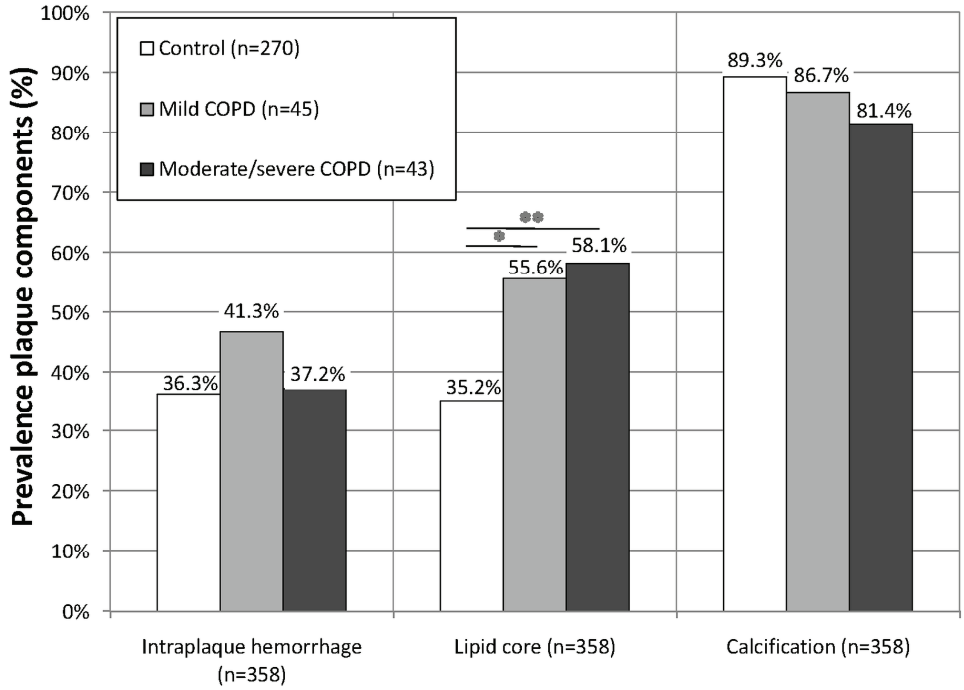


Figure 4: Prevalence of carotid artery plaque components (%) determined by magnetic resonance imaging (MRI) . * p = 0.0092 ; ** p = 0.0040 by Pearson Chi-Square

Table 4: COPD versus control subjects and the risk of different carotid artery plaque components (intraplaque hemorrhage, lipid core or calcification).

	Model 1 (n=358)			**Model 2** (n=353)		
	OR	**95% CI**	**P-value**	**OR**	**95% CI**	**P-**
COPD and risk of intraplaque hemorrhage	1.0	0.61-1.72	0.9386	0.8	0.45-1.50	0.5184
COPD and risk of lipid core	2.2	1.31-3.58	**0.0025**	2.1	1.25-3.69	**0.0058**
COPD and risk of calcification	0.6	0.31-1.27	0.1965	0.6	0.27-1.24	0.1637

Model 1: age and sex adjusted.
Model 2: adjusted for age, sex, Body Mass Index, smoking status, hypertension, High-Density Lipoprotein-cholesterol (serum, mmol/l), triglycerides (serum, mmol/l), hemoglobin (mmol/l), diabetes, creatinin (serum, µmol/l) and maximum wall thickness (mm). Abbreviations: OR= Odds Ratio; CI=Confidence Interval; COPD= Chronic Obstructive Pulmonary Disease. Bold values indicate significance at P < 0.05.

DISCUSSION

This population-based study in elderly demonstrates that COPD subjects have a twofold increased risk of carotid artery wall thickening on ultrasonography compared to controls with a normal lung function, and that COPD is an independent determinant for the presence of a lipid core, an indicator of a vulnerable plaque, as determined by high-resolution MRI. To our knowledge, the association between COPD and the presence of intraplaque hemorrhage, lipid core, and calcification has never been studied before. Therefore, our MRI-based results give more insight into COPD as an independent risk factor for stroke by progression of atherosclerosis. Since the risk of stroke and stroke-associated mortality is related with severity of airflow limitation, identification of risk factors for stroke is crucial to identify the subgroup of COPD patients at high risk, and to develop preventive, more personalized treatment strategies.[184, 345, 346]

Few studies have investigated the association between COPD and/or severity of airflow limitation and carotid wall thickening on ultrasound.[191, 192, 347-350] Consistent with our results, a study performed in vascular surgery patients detected an association between COPD and carotid wall thickening (IMT ≥ 1.25 mm) independent of age and smoking status.[347] Frantz *et al.* recruited participants of a respiratory questionnaire survey and observed a higher prevalence of COPD in subjects with than without plaques (IMT ≥ 2 mm), although they could not confirm that COPD was an independent predictor of carotid plaques.[348] In accordance with our lung function parameter results, an inverse association between FEV_1, FEV_1/FVC and internal carotid IMT has been recently reported.[192] Three other studies also found that the severity of airflow limitation measured by FEV_1 was significantly associated with continuously increased IMT.[191, 349, 350] In line with these three studies, our population-based study performed in older subjects demonstrates a significant association between severity of airflow limitation and carotid wall thickening (IMT ≥ 2.5 mm). Although severity of airflow limitation may not entirely reflect disease activity, it previously correlated well with clinical important outcomes as hospitalizations due to exacerbations, cardiovascular comorbidity, and mortality.[33, 351-353] Furthermore, our study adds to all previous studies that the risk of carotid wall thickening further increases when COPD subjects have clinical symptoms of dyspnea or chronic bronchitis, that plaques are more lipid-rich in COPD subjects compared to controls, and that lipid core plaques also relate to the severity of airflow limitation.

The regulatory pathway responsible for the association between COPD and plaque progression has not yet been elucidated, although several hypotheses have been proposed. COPD and plaque formation may coexist as a result of common risk factors such as smoking. However, our results demonstrate that COPD cases have an increased risk of carotid wall thickening independent of smoking status and that the prevalence of carotid wall thickening was consistently higher in COPD cases compared to controls, even in never smokers. In line with our results, two studies in smoking men found an increased susceptibility for

asymptomatic carotid and leg atherosclerosis by a higher degree of lung function impairment, independently of tobacco consumption, and a significantly higher mean carotid IMT in smokers with airflow limitation compared to smokers without airflow limitation.[191, 354] Furthermore, ex-smokers retain an increased risk of atherosclerosis even after a long period of smoking cessation.[355] Since the atherogenic effect proceeds despite smoking cessation, an associated process such as COPD might be causative.[356] COPD and plaque remodelling to vulnerable plaques can result from increased numbers of macrophages, interferon(IFN)-γ secreting Th1 lymfocytes, and metalloproteinase (MMP)-9 and MMP-12, observed in both disorders.[13, 325, 330, 357] Our observation that there was a gradual association between COPD and lipid core plaques, but not between COPD and intraplaque hemorrhage, suggests that the underlying mechanism differentially affects the presence of the two vulnerable plaque characteristics.

The strengths of this study are the high quality information derived from state of the art diagnostic imaging techniques, the prospective data collection, the general population based setting and the large number of elderly subjects that participated in the Rotterdam Study. Possible limitations are the lack of computed tomography findings of the lungs to corroborate emphysema in those with airflow limitation and the cross-sectional design. The latter implies that we cannot infer causal mechanisms between COPD and carotid plaques. However, the association is biologically plausible and the risk of carotid plaques increased according to severity of airflow limitation. As we were not able to administer contrast material because of the population-based setting, a fibrous cap of carotid artery plaques could not be identified. Although this could have provided additional information on assessing plaque stability, it is known that the size of lipid core and the presence of hemorrhage are both independently associated with a worse fibrous cap status.[358] In addition, contrast material would have improved lipid core detection.[331] Because misclassification by underestimation is often random, the association with COPD may be higher than we found.

In conclusion, this study shows an increased risk of carotid artery plaque formation and of presence of vulnerable plaques with a lipid core in population-based elderly patients with COPD. Clinicians should be aware that asymptomatic carotid atherosclerosis is more prevalent in subjects with COPD and that COPD as a systemic inflammatory disease might lead to vulnerable plaques by inducing or aggravating the presence of a lipid core. This important observation may advance further research in the prevention of ischemic strokes, a devastating complication of carotid atherosclerosis.

ABSTRACT

Background

Chronic obstructive pulmonary disease (COPD) is a common, complex multisystem disease in the elderly with multiple comorbidities that significantly impact morbidity and mortality. Although cerebral small-vessel disease is an important cause of cognitive decline and age-related disability, it is a poorly investigated potential systemic manifestation of patients with COPD.

Objectives

To examine whether COPD relates to the development and location of cerebral microbleeds, a novel marker of cerebral small-vessel disease.

Methods

Cross-sectional and longitudinal analyses were part of the Rotterdam Study, a prospective population-based cohort study in subjects aged ≥55 years. Diagnosis of COPD was confirmed by spirometry. Cerebral microbleeds were detected using high-resolution Magnetic Resonance Imaging (MRI).

Results

Subjects with COPD (n = 165) had a higher prevalence of cerebral microbleeds compared to subjects with normal lung function (n = 645) independent of age, sex, smoking status, atherosclerotic macroangiopathy, antithrombotic use, total cholesterol, triglycerides, and serum creatinin (OR 1.7, 95%CI 1.15-2.47, p=0.007). Regarding the specific microbleed location, COPD subjects had a significantly higher prevalence of microbleeds in deep or infratentorial locations (OR 3.3, 95%CI 1.97-5.53, p<0.001), which increased with severity of airflow limitation and are suggestive of hypertensive or arteriolosclerotic microangiopathy. Furthermore, in longitudinal analysis restricted to subjects without microbleed at baseline, COPD was an independent predictor of incident cerebral microbleeds in deep or infratentorial locations (OR 7.1, 95%CI: 2.1-24.5, p=0.002).

Conclusions

Our findings are compatible with an increased risk of COPD on the development of cerebral microbleeds in deep or infratentorial locations.

Lahousse L, Loth DW, Vernooij MW, Darweesh SKL, Akoudad S, Joos GF, Hofman A, Stricker BH, Ikram MA, Brusselle GG. 2013 Chronic Obstructive Pulmonary Disease and cerebral microbleeds: the Rotterdam Study. Am J Respir Crit Care Med. 188(7):783-8
Official Journal of the American Thoracic Society.

10. COPD and microangiopathy: cerebral microbleeds

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a multisystem disease characterized primarily by persistent airflow limitation that is associated with an abnormal inflammatory response of the lungs to noxious particles or gases.[13] Worldwide, COPD is a leading cause of morbidity and mortality, and the frequent comorbidities further impact the overall severity and prognosis of patients with COPD.[1] Depression, postural instability, cognitive and functional impairment are known consequences of cerebral small-vessel disease, and are frequently described extrapulmonary manifestations in patients with COPD.[177, 178, 197, 359] However, it is unclear whether COPD is associated with incident cerebral small-vessel disease.

Cerebral small-vessel disease is common among elderly, and cerebral microbleeds are a relatively new marker of the condition.[197, 202] These microbleeds, which consist of hemosiderin deposits in macrophages, can be visualized on Magnetic Resonance Imaging (MRI) as small areas of hypointensity.[360] Microbleeds rarely disappear, making them suitable markers for cumulative cerebrovascular damage.[203] The location of a cerebral microbleed appears to be associated with its underlying disease mechanism: microbleeds in deep or infratentorial locations are suggestive of hypertensive or arteriolosclerotic microangiopathy, whereas those occurring in strictly lobar brain sites are indicative of cerebral amyloid angiopathy.[202, 204]

Detection of cerebral microbleeds in patients with COPD and studying their location might increase insight into the pathology substantially. Furthermore, it might also pave the path for better prevention of cognitive and functional impairment in these vulnerable patients. The aim of this study was therefore to investigate whether cerebral microbleeds were more prevalent in subjects with (more severe) COPD and whether microbleed location differed compared to subjects without COPD. Moreover, we wanted to confirm our cross-sectional results into longitudinal analyses to further explore causality. We examined our hypotheses in a large, prospective population-based cohort study of elderly using state of the art MRI.

METHODS

Study design

The present study is embedded within the Rotterdam Study, a population based cohort study comprising almost 15.000 participants aimed at assessing the occurrence of, and risk factors for chronic diseases in the elderly.[108] The study started in 1990 and all participants are invited every 3 to 4 years to the research center for follow-up examinations, including spirometry. As a part of the Rotterdam Study, the Rotterdam Scan Study is investigating age-related brain changes on MRI from 2005 onwards.[55] The present study comprises a cross-sectional analysis performed within all participants taking part of the Rotterdam Scan Study

where spirometry was performed around the same time (2009-2010); and a longitudinal analysis performed within all participants with complete and reliable baseline (2005-2006) and follow-up MRI examinations (2008-2010).[55, 203] In the longitudinal analysis, only subjects without any microbleed at the time of the first MRI scan were included. The medical ethics committee of the Erasmus Medical Center, Rotterdam, and the review board of The Netherlands Ministry of Health, Welfare and Sports, approved the study. All participants gave written informed consent.

Assessment of COPD

Diagnosis of COPD was spirometry based as described previously.[232] Spirometry and diffusing capacity were performed using a Master Screen® PFT Pro (CareFusion, San Diego, CA) by trained paramedical personnel according to the ATS/ERS guidelines.[22, 23] The diffusing capacity of the lungs measured using carbon monoxide was corrected for the haemoglobin concentration ($DL_{CO,c}$).[23] The diagnosis of COPD was based on an obstructive spirometry examination according to the modified Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria [proportion of the forced vital capacity exhaled in the first second (FEV_1/FVC) < 70%] and classified into mild, moderate, or severe airflow limitation by forced expiratory volume in one second (FEV_1)% predicted of ≥ 80%, 50-80% or < 50% respectively.[24]

In addition, according to the GOLD 2011 update, the influence of respiratory symptoms was evaluated.[1] Dyspnea score was based on the following five dyspnea-questions: 1. Are you troubled by shortness of breath when climbing stairs? (i.e. at a normal speed) 2. Are you troubled by shortness of breath when walking on level ground? 3. Do you have shortness of breath? (i.e. during normal/daily life activities) 4. Are you troubled by shortness of breath when lying down, while this improves when you sit up or when you sleep on more pillows? 5. Are you short of breath at rest? Based on these five dyspnea questions, a dyspnea score was added from 0 (all questions negative, never dyspneic) to 5 (all positive, even dyspneic at rest).[232] Chronic bronchitis was defined as the self-reported presence of cough and sputum for at least 3 months in each of two consecutive years.

Participants with a spirometry report suggestive of a restrictive syndrome [FEV_1/FVC ≥ 70% and forced expiratory vital capacity (FVC) < 80% predicted], and asthma patients were excluded. No reversibility tests were conducted. Two COPD subjects within the longitudinal analysis had no interpretable spirometry at the research center within the study period, however, diagnosis was made by a pulmonologist. In longitudinal analyses, subjects who developed COPD between both MRIs, were excluded, and subjects who developed COPD after the second MRI were treated as controls.

Assessment of cerebral microbleeds on MRI

As described previously, all participants of the Rotterdam Scan Study underwent a multisequence MRI protocol on a 1.5-T scanner (GE Healthcare).[55] A custom-made accelerated three-dimensional T2*-weighted gradient-recalled echo sequence with high spatial resolution and long echo time was used for cerebral microbleed detection.[361] MRI scans were viewed by research physicians blinded to the COPD status of the subject, and presence, number and location of microbleeds was rated. Individuals who had dementia or MRI contraindications were not eligible. Furthermore, participants who had claustrophobia or motion artifacts or susceptibility artifacts on their MRI scans, were excluded from the study population.

Microbleeds were defined as focal areas of very low signal intensity on T2*-weighted imaging that were not accompanied by evident signal abnormality on other structural sequences.[55, 202] Intraobserver and interobserver reliabilities for microbleed rating were very good (κ = 0.85–0.87) and review of the initial ratings by an experienced neuroradiologist yielded a very high accordance.[204]

In accordance with previous literature, microbleed location was classified as lobar (cortical gray matter and subcortical or periventricular white matter), deep (deep gray matter: basal ganglia and thalamus, and the white matter of corpus callosum, internal, external, and extreme capsule) or infratentorial (brainstem and cerebellum).[202, 362, 363] According to the presumed underlying disease mechanism, microbleeds were classified as occurring in strictly lobar brain sites, or occurring in deep or infratentorial locations (whether or not additional to lobar microbleeds).[203]

Statistical analyses

Differences between subjects with and without COPD were studied using Mann-Whitney U test for continuous variables and Chi-Square test for categorical variables. Logistic regression models were performed to assess the effect of COPD on cerebral microbleeds, both overall and per location class (strictly lobar versus deep or infratentorial). All models were adjusted for age and sex, and additionally, for covariables which changed the risk estimate by more than 5%. Following covariables were considered as potential confounders: age, sex, body mass index (BMI), smoking behaviour, pack years, APOE genotype, hypertension [antihypertensive medication, systolic and diastolic blood pressure], hypercholesterolemia [total serum cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides], diabetes mellitus [blood glucose-lowering medication, glucose], kidney function [creatinin], atherosclerotic macroangiopathy [carotid intima-media wall thickness ≥ 2.5mm in the left, right or both carotid arteries on ultrasonography] and use of drugs for obstructive airway diseases, antithrombotic, and lipid lowering agents. These covariables include previously identified risk factors for cerebral microbleeds.[232, 364] Medication use was obtained through automated linkage with pharmacy filled prescription data. For the other factors, information was obtained through interview, laboratory, or physical examination at the latest regular visit of the study participant to the Rotterdam Study research center preceding the most recent

MRI in the cross-sectional analyses, and preceding the baseline MRI in the longitudinal analyses.[365]

With regard to cerebrovascular disease status, a known history of stroke was assessed on entry of study participants into the Rotterdam Study.[366] Subsequently, participants have been continuously monitored for incident stroke through automated linkage of the study database with files from general practitioners, and hospital discharge information. For all cases of stroke, subsequent validation by research physicians and neurologists was performed on the basis of clinical details from medical records as described earlier.[367]

Sensitivity analyses were done by re-examining the associations after exclusion of participants with a history of stroke, in order to assess the confounding effect of previously existent cerebrovascular disease. Individuals with at least one microbleed were consistently compared to individuals without any microbleed. Statistical analyses were performed using SPSS, version 20.0 for Windows (IBM, North Castle, NY).

RESULTS

Cross-sectional study of COPD, severity of airflow limitation and cerebral microbleeds

944 participants of the Rotterdam study had both an interpretable spirometry and brain MRI during the last center visit.*(Figure 1)* After exclusion of 134 subjects with asthma or a lung function suggestive of a restrictive syndrome, 165 subjects with COPD and 645 subjects without COPD were evaluated.

Table 1 shows the baseline characteristics of the cross-sectional study population (n=810) with a total median age of 78 years (inter-quartile range[IQR]=6). COPD subjects were slightly older, more often male, (current) smokers, had a lower body mass index and worse lung function. Of the 79 (47.9%) COPD subjects who took drugs for obstructive airway diseases (Anatomical Therapeutic Chemical (ATC) classification R03), 50 (63.3%) used inhaled anticholinergics (R03BB), 48 (60.8%) inhaled β2-sympathomimetics (R03AC), 33 (41.8%) inhaled corticosteroids (R03BA), 55 (69.6%) inhaled fixed combinations (R03AK), and 13 (16.5%) COPD subjects were on treatment with other agents for obstructive airway diseases including xanthines (R03BC, R03C and R03D). Because 57 (72.2%) COPD subjects were on multiple treatments, summed percentages exceed hundred percent.

The 165 COPD subjects were categorized according to the modified Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification of airflow limitation [24] into 84 (50.9%) mild, 73 (44.2%) moderate and 8 (4.8%) severe COPD; and according to the updated GOLD group categorization[1] into 81 (49.1%) group A, 58 (35.2%) group B, 7 (4.2%) group C and 19 (11.5%) group D. COPD categories did not vary significantly by age or sex.

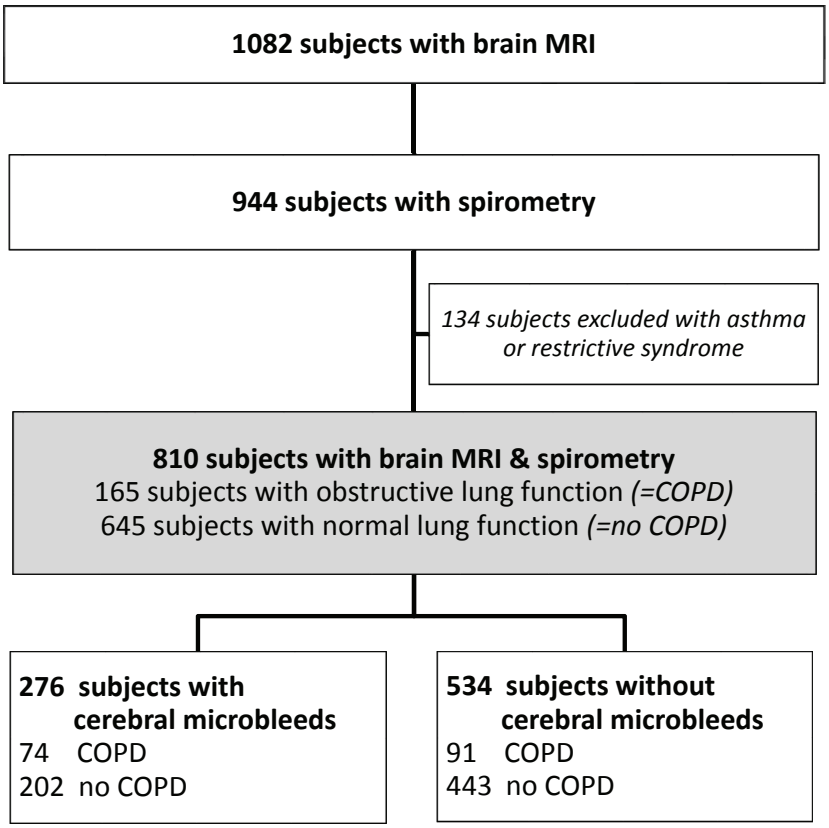


Figure 1: Study profile of cross-sectional analysis.

The prevalence of cerebral microbleeds was significantly higher in participants with COPD (74 out of 165; 44.8%; 95% CI: 37.5 to 52.5%) than in those without COPD (202 out of 645; 31.3%; 95% CI: 27.9 to 35.0%) (p=0.001). The difference remained statistically significant after controlling for age, sex, smoking status, carotid artery wall thickening, antithrombotic use, total cholesterol, triglycerides, and serum creatinin (OR 1.7, 95%CI 1.15-2.47, p=0.007). The prevalence of cerebral microbleeds in (ever) smoking COPD subjects (47.8%; 95% CI: 39.5 to 56.2%) was significantly higher than in (ever) smoking subjects without COPD (32.8%; 95% CI: 28.4 to 37.5%), even after controlling for age, sex, and pack years (p=0.003).

Location of cerebral microbleed

Because the location of a cerebral microbleed tends to be associated with its underlying pathogenetic mechanism, we further investigated the association of COPD with microbleeds occurring in strictly lobar brain sites (amyloid angiopathy), and with microbleeds in deep or infratentorial locations (arteriolosclerotic microangiopathy).*(Figure 2)* Out of 276 subjects who had a cerebral microbleed detected on MRI *(Figure 1),* 177 (64%) had microbleed(s) with a strictly lobar location. In addition, 99 (36%) subjects had microbleed(s) which were located deeply. COPD was not significantly associated with microbleeds occurring in strictly lobar brain sites.*(Figure 2)*

Table 1: Baseline characteristics of the cross-sectional study population (n=810).

	COPD (n=165)	**No COPD (n= 645)**	**p-value**
Age, years	79 (7)	77 (6)	0.023
Males	101 (61.2)	265 (41.1)	<0.001
Smoking status[1]			<0.001
Never smoker	31 (18.8)	242 (37.5)	
Former smoker	107 (64.8)	368 (57.1)	
Current smoker	27 (16.4)	35 (5.4)	
Pack years of cigarette smoking[1]	16.8 (36.6)	3.3 (19.1)	<0.001
Body mass index, kg/m²	25.9 (5.6)	26.9 (4.8)	0.034
Hypertension[2]	87 (52.7)	318 (49.4)	0.443
Mean systolic blood pressure, mmHg	152.0 (23.0)	153.0 (28.0)	0.472
Mean diastolic blood pressure, mmHg	83.0 (15.0)	84.0 (14.0)	0.664
Diabetes[3]	27 (16.4)	80 (12.4)	0.182
Glucose in serum, mmol/l	5.5 (0.9)	5.5 (0.8)	0.178
Total cholesterol in serum, mmol/l	5.3 (1.4)	5.3 (1.5)	0.446
HDL-cholesterol in serum, mmol/l	1.4 (0.6)	1.4 (0.5)	0.200
Triglycerides in serum, mmol/l	1.2 (0.6)	1.2 (0.7)	0.152
Creatinin in serum, µmol/l	81.0 (27.5)	81.0 (25.0)	0.150
APOE ε3/ε3 genotype	89 (53.9)	365 (56.6)	0.724
Hematocrit, %	44.0 (4.0)	44.0 (5.0)	0.085
Antithrombotic use ever	93 (57.8)	325 (51.5)	0.156
Lipid reducing agent use ever	57 (34.5)	217 (33.6)	0.827
FEV_1, % predicted	81.2 (25.5)	111.5 (24.1)	<0.001
FEV_1/FVC, %	65.6 (7.6)	78.0 (6.4)	<0.001
$DL_{CO,c}$,% predicted	87.9 (26.0)	99.3 (22.4)	<0.001

Categorical variables are expressed as numbers (percentage). Values of continuous variables are expressed as median (IQR). [1]Smoking status and pack years were self-reported. [2]Hypertension was defined as antihypertensive medication use and/or an average systolic blood pressure of ≥ 160 mmHg and/or an average diastolic blood pressure of ≥ 100 mmHg. [3]Diabetes mellitus was defined as blood glucose-lowering medication use and/or a non-fasting serum glucose level of ≥11.1 mmol/L and/or fasting serum glucose levels ≥7 mmol/L. Abbreviations: COPD=Chronic Obstructive Pulmonary Disease; $DL_{CO,c}$ = diffusing capacity of the lungs measured using carbon monoxide and corrected for the hemoglobin concentration; FEV_1= forced expiratory volume in one second; FEV_1/FVC= proportion of the forced vital capacity exhaled in the first second; HDL= High-Density Lipoprotein

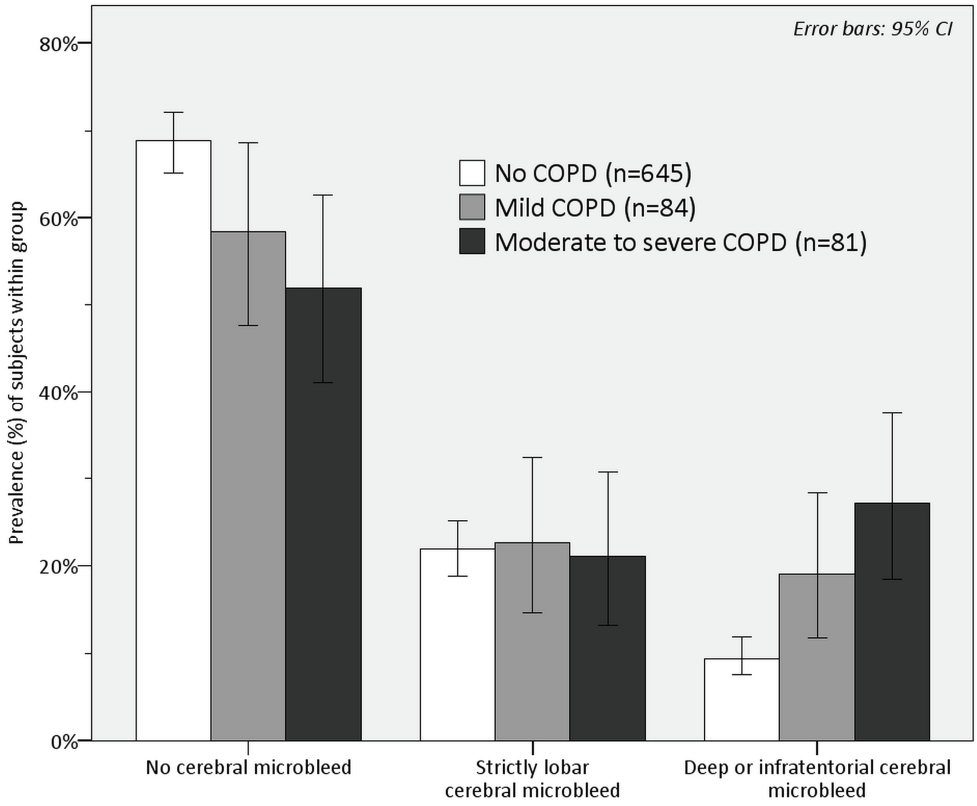


Figure 2: Prevalence (%) of subjects without a cerebral microbleed, with strictly lobar cerebral microbleed(s) and with deep or infratentorial cerebral microbleed(s) subdivided by no COPD, mild COPD or moderate to severe COPD.

In contrast, COPD was significantly associated with deep or infratentorial microbleeds, independent of age, sex, smoking status, and pack years.*(Table 2)* The odds ratio was highest and comparable for COPD subjects with severe airflow limitation, COPD subjects with frequent exacerbations, and COPD subjects belonging to GOLD group D.*(Table 2)* Since the airflow limitation in COPD is caused by a combination of small airways disease (bronchiolitis) and loss of elastic recoil due to parenchymal destruction (emphysema), we evaluated continuous lung function parameters. *Table 2* shows that per 10% predicted increase in FEV_1, the prevalence of deep or infratentorial microbleeds decreased by 17%. Furthermore, per percentage increase in FEV_1/FVC and diffusing capacity, the prevalence of deep or infratentorial microbleeds decreased by 5% and 2% respectively. Regarding the effect of COPD independent of smoking effects, the prevalence of deep or infratentorial cerebral microbleeds was significantly higher in (ever) smoking COPD subjects (32.7%; 95% CI: 24.4 to 42.2%) compared to (ever) smoking subjects without COPD (10.0%; 95% CI: 7.1 to 13.9%), even after controlling for age, sex, and pack years ($p<0.001$).

Table 2: COPD and the risk on cerebral microbleeds in deep or infratentorial locations (n=633).

	Model 1			Model 2		
Categorical, all versus no COPD	**OR**	**95% CI**	**P-value**	**OR**	**95% CI**	**P-value**
COPD *(n=129)*	2.7	1.68-4.36	<0.001	3.3	1.97-5.53	<0.001
COPD, *mild (n=65)*	2.2	1.17-4.13	0.015	2.6	1.34-5.00	0.005
COPD, *moderate (n=57)*	3.2	1.71-5.96	<0.001	4.1	2.11-8.00	<0.001
COPD, *severe (n=7)*	4.4	0.94-20.30	0.060	6.8	1.31-34.87	0.023
COPD, *group A (n=61)*	1.8	0.91-3.54	0.090	2.1	1.05-4.31	0.036
COPD, *group B (n=45)*	3.6	1.83-7.11	<0.001	4.4	2.19-9.02	<0.001
COPD, *group C (n=6)*	2.9	0.52-16.66	0.224	3.9	0.66-23.48	0.134
COPD, *group D (n=17)*	4.4	1.59-12.08	0.004	6.6	2.20-20.00	0.001
COPD, *dyspnea score <2 (n=67)*	1.9	0.99-3.60	0.053	2.2	1.15-4.39	0.018
COPD, *dyspnea score ≥2 (n=62)*	3.8	2.10-6.88	<0.001	4.9	2.58-9.28	<0.001
COPD, *exacerbations <2 (n=112)*	2.5	1.51-4.15	<0.001	3.0	1.77-5.19	<0.001
COPD, *exacerbations ≥2 (n=17)*	4.3	1.57-12.01	0.005	6.2	2.09-18.10	0.001
COPD, *no chronic bronchitis (n=109)*	2.7	1.66-4.53	<0.001	3.3	1.93-5.60	<0.001
COPD, *chronic bronchitis (n=18)*	3.0	1.05-8.32	0.040	4.3	1.39-13.40	0.012
Continuous, lung function	**OR**	**95% CI**	**P-value**	**OR**	**95% CI**	**P-value**
FEV_1 (per 10% predicted increase)	0.85	0.77-0.94	0.002	0.83	0.74-0.92	0.001
FEV_1/FVC (per 1 % increase)	0.95	0.93-0.98	<0.001	0.95	0.92-0.97	<0.001
$DL_{CO,c}$ (per 1% predicted increase)	0.98	0.97-0.99	0.005	0.98	0.96-0.99	0.003

Model 1: age and sex adjusted.

Model 2: adjusted for age, sex, smoking status, and pack years.

COPD was defined as $FEV_1/FVC < 70\%$ and categorized according to the modified Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification of airflow limitation 2007[24] into mild COPD (GOLD1; $FEV_1 \geq 80\%$pred), moderate COPD (GOLD2; $50\% \leq FEV_1 < 80\%$pred) & severe COPD (GOLD3; FEV1<50%pred) and according to the updated GOLD group categorization 2013[1] A (low risk, less symptoms), B (low risk, more symptoms), C (high risk, less symptoms) and D (high risk, more symptoms).

Dyspnea score was based on 5 dyspnea-questions and scored from 0 (never dyspneic) to 5 (even dyspneic at rest) Exacerbations were defined as the total number of moderate and severe exacerbations in the year prior to the MRI examination.

Chronic bronchitis was defined as the self-reported presence of cough and sputum for at least 3 months in each of two consecutive years (http://www.goldcopd.org).

Abbreviations: OR= Odds Ratio; CI= Confidence Interval; COPD= Chronic Obstructive Pulmonary Disease; FEV_1= forced expiratory volume in one second; FEV_1/FVC = proportion of the forced vital capacity exhaled in the first second; $DL_{CO,c}$ = diffusing capacity of the lungs measured using carbon monoxide and corrected for the hemoglobin concentration

Figure 3 demonstrates the prevalence (%) of subjects without a cerebral microbleed, with strictly lobar cerebral microbleed(s) and with deep or infratentorial cerebral microbleed(s) subdivided by non-smoking control, (ever) smoking control or COPD. A sensitivity analysis excluding the participants with a history of stroke to assess the confounding effect of previously existent cerebrovascular disease, did not change the point estimate of the risk of COPD on cerebral microbleeds nor on deep or infratentorial microbleeds. In 39 well-defined elderly asthmatics and the 645 controls, no significant associations were noted between asthma and (all) cerebral microbleeds, deep or infratentorial microbleeds, or strictly lobar microbleeds (data not shown). In an age and sex-adjusted model of 45 subjects with a spirometry suggestive of restrictive respiratory disease compared to the 645 controls, a restrictive spirometric pattern was significantly associated with (all) cerebral microbleeds (OR 2.6, 95% CI 1.4-4.9 , p=0.002), deep or infratentorial microbleeds (OR 3.8, 95% CI 1.7-8.4, p=0.001) and strictly lobar microbleeds (OR 2.1, 95% CI: 1.0-4.3, p=0.041). The associations with cerebral microbleeds (OR 2.9, 95% CI 1.5-5.6, p=0.002) and deep or infratentorial microbleeds (OR 6.1, 95% CI 2.5-15.1, p<0.001) remained significant after adjustment for age, sex, BMI, smoking status, hematocrit, HDL cholesterol, triglycerides, glucose and serum creatinin (and borderline significant for strictly lobar microbleeds (OR 2.1, 95% CI 1.0-4.6, p=0.064)).

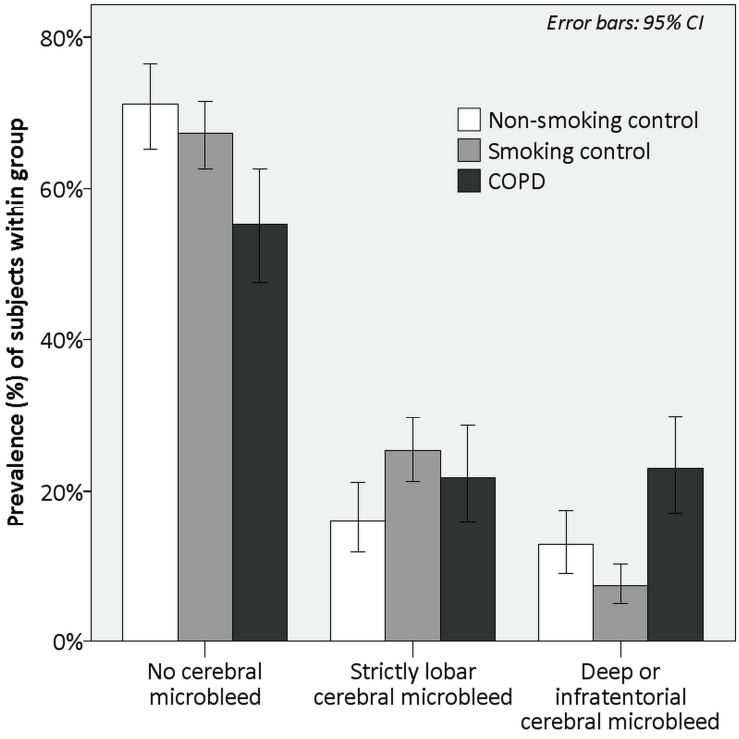


Figure 3: Prevalence (%) of subjects without a cerebral microbleed, with strictly lobar cerebral microbleed(s) and with deep or infratentorial cerebral microbleed(s) subdivided by non-smoking control, (ever) smoking control or COPD.

Longitudinal analyses of COPD and incident cerebral microbleeds

In order to investigate causality, longitudinal analyses were performed in 553 participants of the Rotterdam Study who had two MRI brain scans and no cerebral microbleed at the time of the first MRI scan.*(Figure 4)* The median time interval between the two MRI scans was 3.42 years (IQR: 79.5 days) and was not significantly different between subjects with or without COPD (p=0.663). *Table 3* in the Online Data Supplement shows the baseline characteristics of the longitudinal study population. COPD subjects were more often male, diabetic, and current smokers. During follow-up, 54 subjects without cerebral microbleeds at the time of the first MRI scan developed a cerebral microbleed, 18 of them at a deep or infratentorial location.*(Figure 4)*

Of the 46 COPD subjects without cerebral microbleeds at the time of the first MRI scan, 5 (10.9%) subjects developed a deep or infratentorial microbleed, compared to 13 (2.6%) of the 507 subjects without COPD. Adjusted for age, sex, and pack years, COPD was associated with a significantly increased risk of developing a deep or infratentorial microbleed (OR 7.1, 95%CI: 2.1-24.5; p=0.002). No significant association was noted for COPD and incident strictly lobar cerebral microbleeds.

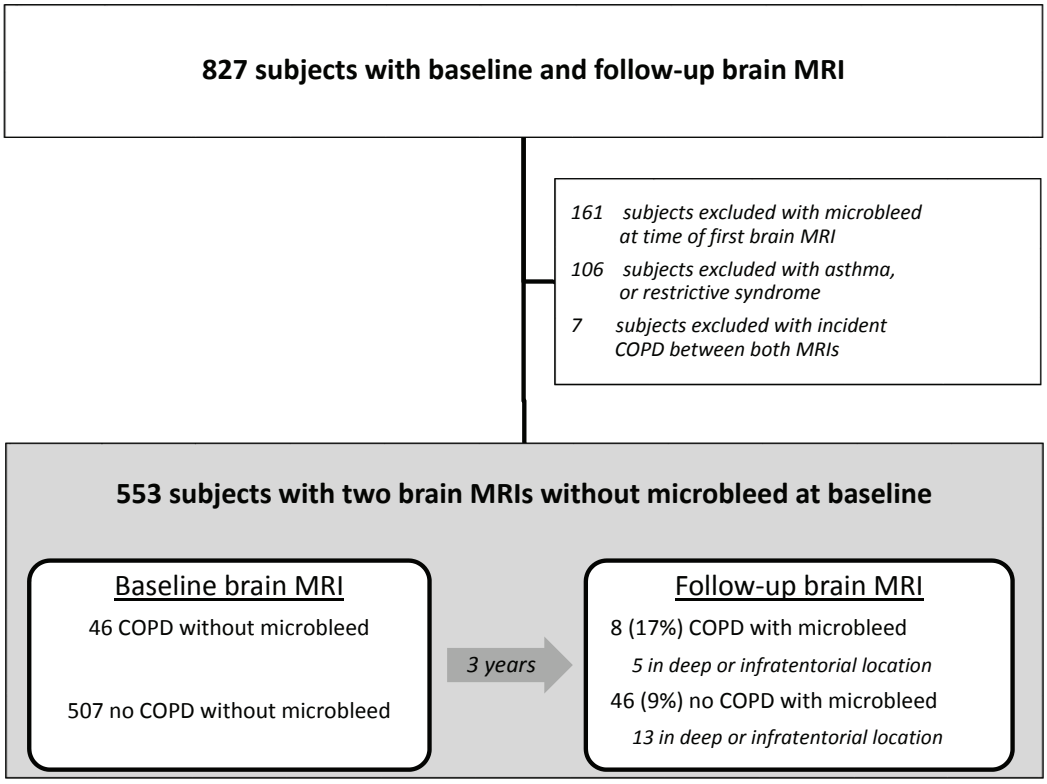


Figure 4: Study profile of longitudinal analysis.

Table 3: Baseline characteristics of the longitudinal study population (n=553).

Covariable measured at baseline (=first MRI)	**COPD (n=46)**	**No COPD (n= 507)**	**P-value**
Age, years	66 (9)	66 (6)	0.122
Males	30 (65.2)	248 (48.9)	0.034
Smoking status[1]			<0.001
Never smoker	5 (11.1)	181 (36.1)	
Former smoker	23 (51.1)	276 (55.1)	
Current smoker	17 (37.8)	44 (8.8)	
Pack years cigarette smoking[1]	28.1 (33.7)	2.5 (17.3)	<0.001
Body mass index , kg/m²	26.3 (3.6)	27.0 (4.8)	0.529
Mean systolic blood pressure , mmHg	147.0 (29.5)	141.5 (22.0)	0.171
Mean diastolic blood pressure , mmHg	84.0 (16.0)	81.5 (12.1)	0.315
Diabetes[2]	8 (17.8)	38 (7.6)	0.019
Glucose in serum, mmol/l	5.4 (1.5)	5.4 (0.7)	0.252
Total cholesterol in serum, mmol/l	5.5 (1.2)	5.7 (1.3)	0.119
HDL-cholesterol in serum, mmol/l	1.3 (0.5)	1.4 (0.5)	0.219
Antihypertensive use	19 (41.3)	177 (34.9)	0.385
Antithrombotic use	10 (21.7)	82 (16.2)	0.332
Lipid reducing agent use	7 (15.2)	100 (19.7)	0.459

Categorical variables are expressed as numbers (percentage). Values of continuous variables are expressed as median (IQR). [1]Smoking status and pack years were self-reported. [2]Diabetes mellitus was defined as blood glucose-lowering medication use and/or a non-fasting serum glucose level of ≥11.1 mmol/L and/or fasting serum glucose levels ≥7 mmol/L.

Abbreviations: COPD= Chronic Obstructive Pulmonary Disease; HDL= High-Density Lipoprotein

DISCUSSION

This large population based study in elderly demonstrates that COPD is associated with a higher prevalence of cerebral microbleeds, a marker of cerebral small-vessel disease determined by MRI.[202] These findings tend to be driven by a greater occurrence of microbleeds in deep or infratentorial locations in subjects with COPD. Furthermore, follow-up analyses within subjects without a cerebral microbleed at baseline, demonstrated that COPD is an independent risk factor to develop deep or infratentorial cerebral microbleeds. Our results are in line with two previous cross-sectional studies which showed that patients with COPD had a significantly increased volume of cerebral white matter lesions, which is another marker of cerebral small vessel disease, and known to be associated with microbleeds in a deep or infratentorial region.[200, 201]

The location of cerebral microbleeds might give more insight into the underlying mechanism of the association between COPD and cerebral microbleeds. In our population, the association of cerebral microbleeds with COPD was especially strong for microbleeds in deep or infratentorial locations, which are thought to occur by arteriolosclerosis on the basis of hypertensive vasculopathy and lipohyalinosis.[202] Recently, we have demonstrated that COPD subjects had a significant increased prevalence of atherosclerotic macroangiopathy as evidenced by the increased prevalence of carotid artery wall thickening in subjects with COPD.[232] Therefore, carotid artery wall thickening was taken into account as potential confounder in the present study. The current results suggest that COPD might affect large and small blood vessels simultaneously. The systemic inflammation present in a subset of COPD patients, as well as the hypoxia due to progressive airflow limitation and emphysema, might contribute to vessel wall changes resulting in stiffening of arteries and arterioles.[232, 262, 263] Although severity of airflow limitation may not entirely reflect disease activity, our results suggest that cerebral small vessel disease is more present in COPD patients with more severe airflow limitation.[351] In addition, the prevalence of deep or infratentorial microbleeds was more pronounced in COPD subjects with respiratory symptoms or frequent exacerbations. Interestingly, the effect size of GOLD group B did not seem to be lower than group C. Previously, a higher mortality risk for group B than C was established.[26, 27] The high percentage of subjects in group A and the very small number in group C are in line with other general population studies.[26-28]

In order to investigate the potential impact of emphysema on the prevalence of cerebral microbleeds, we examined the influence of the diffusing capacity, a measure of the rate of CO uptake by the lungs.[23] The diffusing capacity was significantly associated with the prevalence of deep or infratentorial microbleeds.

The impact of smoking is of particular importance since most COPD patients are current or former smokers, and smoking was previously identified as a risk factor for the presence of cerebral microbleeds.[201] Therefore, we compared the prevalence of microbleeds between

COPD subjects who had ever smoked and smoking subjects without airflow limitation. We found that smoking COPD subjects had a significantly higher prevalence of deep or infratentorial microbleeds than smoking subjects without COPD, suggesting that the association between COPD and deep or infratentorial microbleeds could not be explained by smoking solely. These results are in line with previous findings that oxidative stress persists in patients with COPD despite smoking cessation, and has a crucial role in the perpetuation of the inflammation.[13] Previously, the association between impaired lung function and cerebral small vessel disease (white matter lesions) on the one hand and macrovascular disease (carotid intima-media thickness, arterial stiffness) on the other hand, was found to be independent of smoking.[200, 231-233] Although smoking tends to increase the prevalence of cerebral microbleeds within COPD subjects of our study, our results suggest that an interplay with, or a certain susceptibility for COPD seems necessary before the harmful effect of smoking on deep or infratentorial microbleeds becomes apparent. Potentially, there might be a phenotype more sensitive to smoking, simultaneously developing cardiovascular, pulmonary and cerebral abnormalities. Further research is warranted to explore whether a general phenotype of 'systemic COPD' exists and might benefit from more tailored treatment options.[368] Although the reported longitudinal association is more compatible with a causative role for COPD in the development of cerebral small vessel disease, we cannot exclude that pulmonary manifestations present themselves earlier than cerebrovascular abnormalities.

Aging is another important determinant since cerebral microbleeds gradually increase with age.[201] COPD subjects within the cross-sectional study population were slightly older and therefore all analyses were adjusted for age. However, it is unlikely that the small difference in age could explain the much higher prevalence of cerebral microbleeds compared to the controls, or compared to the age-specific prevalence of cerebral microbleeds described by Poels *et al.*[201]

Strengths of this study are the high quality information derived from state of the art diagnostic imaging techniques which allowed highly sensitive detection of cerebral microbleeds, and the prospective data collection. The population based setting and large sample size of our cohort allowed us to examine COPD patients with a range of disease severity. Furthermore, we made a distinction between different locations of microbleeds in the brain, enabling us to separately assess the prevalence of microbleeds in deep or infratentorial regions versus strictly lobar regions, which embodies a different etiology.[202]

A first potential limitation of this observational study is the cross-sectional design of our main analysis. However, the gradual increase in prevalence of deep or infratentorial microbleeds according to severity of airflow limitation, suggests a potential causal mechanism between COPD and cerebral microbleeds. Therefore, we further examined the association longitudinally involving 553 participants of the Rotterdam Study with two MRI

scans of the brain and without cerebral microbleeds at the time of the first scan. These results showed that COPD is significantly associated with an increased risk of developing deep or infratentorial microbleeds in the subsequent three years, which further substantiates the plausibility of a causal association between COPD and microbleeds in deep and infratentorial locations. A second consideration is that although COPD is primarily a lung disease, the disease is very heterogeneous and associated with multiple comorbid conditions. The specific role of COPD as a risk factor for cerebral small-vessel disease in patients with multiple comorbidities could therefore be difficult to ascertain. However, the associations between COPD and cerebral microbleeds in our population were independent of known other physiological and cardiovascular risk factors. Finally, we did not perform computed tomography (CT) of the lungs in our population to corroborate emphysema. Although diffusing capacity correlates fairly with lung CT density and loss of alveolar membrane surface area (in emphysema) is one of the primary causes of a low DL_{CO}, it is not the gold standard to measure emphysema.[369]

In conclusion, the results of this study are compatible with an increased risk of COPD on the development of cerebral microbleeds in deep or infratentorial locations. Given the importance of cognitive and functional consequences, our results might lead to a better recognition of vulnerable patient groups, and enhance research into necessary preventive strategies.

ABSTRACT

Background

Worldwide, chronic obstructive pulmonary disease (COPD) and stroke are leading causes of death. Moreover, increasing evidence suggests an association between both diseases, potentially caused by an increased atherosclerosis risk in patients with COPD.

Objectives

To examine the associations between COPD and the different subtypes of stroke in the general population.

Methods

Within the prospective population-based Rotterdam Study, we followed 13 115 participants without history of stroke or asthma for occurrence of stroke. Follow-up started in 1990-2008 and finished in 2012. COPD was related to stroke using a time-dependent Cox proportional hazard model adjusted for age, sex and smoking.

Results

1250 of the 13115 participants incurred a stroke, of whom 701 an ischemic stroke. Adjusted for age and sex, COPD was significantly associated with all stroke (HR 1.22; 95%CI 1.03-1.46), ischemic stroke (HR 1.34; 1.07-1.67), and hemorrhagic stroke (HR 1.79; 1.07-2.99). Additionally adjusting for smoking attenuated the effect sizes (HR 1.12 (0.93-1.33) for all stroke, HR 1.19 (0.93-1.49) for ischemic stroke, and HR 1.63 (0.97-2.76) for hemorrhagic stroke).

Conclusions

COPD is associated with incident stroke, both ischemic and hemorrhagic. Associations were mainly, but not completely driven by smoking, suggesting that clinicians should be aware of the higher risk of both ischemic and hemorrhagic stroke in persons with COPD.

11. COPD and stroke

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is primarily characterized by persistent airflow limitation that is associated with an abnormal inflammatory response of the lungs to noxious particles or gases and leads to many extrapulmonary manifestations.[13] A stroke is characterized as a neurological deficit lasting 24 hours or leading to death, due to an acute focal injury of the central nervous system by a vascular cause.[370] This can be either an infarction or intracerebral hemorrhage. COPD and stroke are both leading causes of morbidity and mortality worldwide [2, 371] and evidence for an association between both diseases is increasing.[35] This association could be causal, since some studies showed that the risk of stroke correlates with the degree of airflow limitation.[183, 188] Especially during COPD exacerbations, pulmonary inflammation can lead to systemic inflammation[30], which plays a role in the forming of instable atherosclerotic plaques.[372] Recent studies showed that in COPD patients carotid arterial plaque burden is increased and more prone to rupture, leading to an increased risk of stroke.[191, 192, 232] However, any associations between COPD and stroke might also emerge due to shared etiology. COPD and stroke are both associated with cardiovascular risk factors as diabetes, hypertension, and particularly smoking.[232, 373, 374] Whether associations are independent from these cardiovascular risk factors is still unclear. Some previous population-based found associations between COPD and stroke[131, 190, 375-377], but only few had information about smoking.[190, 375, 377] Furthermore, previous studies only examined associations with all subtypes of stroke combined. However, if associations are due to a higher risk of atherosclerosis, associations will be mainly present for ischemic stroke. Still, we previously showed an increased risk of microbleeds in persons with COPD, suggesting that COPD might also be associated with intracerebral hemorrhage.[141]

Therefore, the aim of our prospective population-based study was to examine the associations between COPD and stroke, taking into account the different subtypes of stroke. Furthermore, we investigated the role of cardiovascular risk factors on these associations.

METHODS

Setting and study population

This study was conducted within the population-based Rotterdam Study, which has as aim to assess incidence and risk factors of chronic diseases in the elderly. Initially, from 1990 onwards 7983 persons aged 55 years and older and living in the Ommoord district in the city of Rotterdam were included (Rotterdam Study I). The cohort was extended in 2000 with 3011 participants who moved into the study district or had become 55 years or since the start of the study (Rotterdam Study II). In 2006, the study was further extended with 3932 persons that were aged 45-54 years (Rotterdam Study III). Details regarding the objective and design of the Rotterdam Study have been described elsewhere.[20]

The study population consisted of participants who gave informed consent for follow-up and had no history of stroke at baseline. Patients with physician diagnosed asthma or asthma-

COPD overlap syndrome (ACOS) were excluded. The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. A written informed consent was obtained from all participants.

Assessment of COPD

COPD was diagnosed by an obstructive spirometry (proportion of the forced vital capacity exhaled in the first second (FEV_1/FVC) < 0.7) during the research centre visit or by a pulmonologist or general practitioner.[5] The incident COPD date was defined as the date of COPD diagnosis in the medical records, the date of a first COPD medication prescription or the date of obstructive lung function examination, whichever came first. Medication use was obtained through automated linkage with pharmacy filled prescription data.[378]

Assessment of stroke

Stroke was defined according to the World Health Organization criteria as syndrome of rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or leading to death, with no apparent cause other than of vascular origin.[379] Prevalent stroke at baseline was assessed using the home interview and verified by reviewing medical records. From baseline onwards, all participants were continuously followed-up for occurrence of stroke through automatic linkage of general practitioners' medical records with the study database. Additionally, nursing home physicians' medical records and medical records from general practitioners of participants who moved out of the Ommoord district were inspected on a regular basis. Research physicians reviewed potential strokes using hospital discharge letters and information from general practitioners and an experienced neurologist verified the stroke diagnoses.[366] Strokes were subclassified in ischemic or hemorrhagic based on neuroimaging reports. If no neuroimaging was performed, strokes were classified as unspecified. Strokes were further subclassified in cortical or lacunar, based on clinical symptoms. Furthermore, strokes were subclassified according to TOAST criteria.[380] The follow-up was complete until January 1st, 2012 for 96.3% of potential person-years.

Covariates

Covariates were measured at baseline of each cohort. Smoking status and medication use were assessed during a home interview. Smoking was categorized into current, past, and never smoking. Additionally, cardiovascular risk factors were measured during a visit at the study center.[381] Blood pressure was measured two times in sitting position on the right arm with a random-zero sphygmomanometer. The average of the two consecutive measurements was used in the analyses. Total cholesterol, high-density lipoprotein (HDL) cholesterol, and C-reactive protein (CRP) were measured in serum. Diabetes mellitus was defined as having an overnight fasting glucose level 7.0 mmol/L or higher, a non-fasting glucose level of 11.0 mmol/L or higher, or using antidiabetic medication.

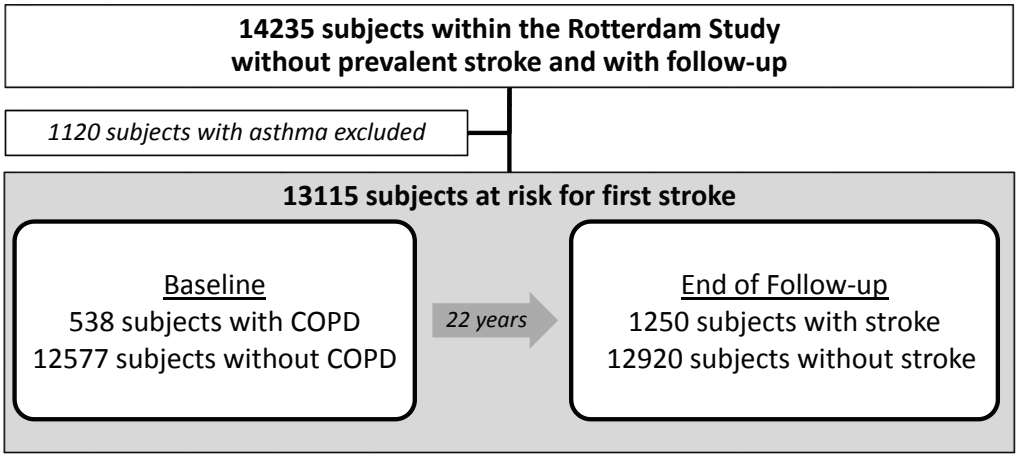
Statistical analyses

Analysis of covariance was used to study differences in baseline characteristics between subjects with and without COPD and between persons with prevalent or incident COPD, adjusted for age and sex. Associations between COPD and stroke were evaluated using Cox proportional hazards regression. COPD was added as time-varying covariate in the analysis, which captured persons that developed COPD during follow-up. Participants were censored at date of stroke, date of death, last date of follow-up, or January 1st, 2012, whichever came first. Furthermore, we examined associations of COPD with different subclassifications of stroke. All models were adjusted for age and sex, and additionally for potential confounders, such as smoking, systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication, total cholesterol, HDL cholesterol, lipid-lowering medication, diabetes mellitus, and BMI. Missing data on covariates (for all covariates 14.1% or less) were imputed based on the other covariates using multiple imputation with 5 imputation sets. All analyses were done using IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY).

RESULTS

Participants that did not give informed consent for collection of follow-up data (n=238), had history of stroke at baseline (n=453), or had history of asthma or ACOS at baseline (n=1120) were excluded. Consequently, 13 115 participants were eligible for analysis.(*Figure 1*) During 9.6 (±6.0 SD) years of follow-up, 1250 subjects suffered a stroke, of whom 701 an ischemic stroke, 107 a hemorrhagic stroke, and 442 an unspecified stroke.

Figure 1: Flow chart of the study population.



The baseline characteristics of the study population are presented in *Table 1*. 538 participants had COPD at baseline and 1028 participants developed COPD during follow-up. The mean age (±SD) was 67.0 years (±9.3) for persons with COPD at baseline, 65.2 (±7.4) for persons that developed COPD during follow-up, and 65.7 (±10.3) for persons without COPD. Persons with COPD were more often male and more (current) smokers.*(Table 1)*

Table 1: Baseline characteristics of the study population (n=13 115).

	Prevalent COPD N=538	***Incident COPD N=1028***	***No COPD N=11549***
Age, years	67.0 (9.3)*†	65.2 (7.4)	65.8 (10.6)
Female	250 (46.5%)*	449 (43.7%)*	6971 (60.4%)
Smoking			
Never	80 (15.0%)*	151 (14.8%)*	4052 (35.9%)
Past	240 (44.9%)†	410 (40.2%)*	4797 (42.5%)
Current	214 (40.1%)*	459 (45.0%)*	2449 (21.7%)
Systolic blood pressure, mmHg	139 (21)	137 (21)	138 (22)
Diastolic blood pressure, mmHg	77 (12)†	76 (12)*	77 (12)
Use of blood pressure-lowering medication	182 (34.0%)	288 (28.1%)	2436 (29.8%)
Total cholesterol, mmol/L	6.1 (1.2)†	6.3 (1.2)*	6.2 (1.2)
HDL-cholesterol, mmol/L	1.43 (0.44)*†	1.34 (0.36)	1.37 (0.39)
Use of lipid-lowering medication	62 (11.6%)*†	74 (7.2%)*	1047 (9.1%)
Diabetes mellitus	55 (11.9%)†	67 (7.1%)*	922 (9.1%)
Body mass index	26.4 (4.1)*	26.0 (3.8)*	26.9 (4.0)
hsCRP, mg/L	3.8 (5.7)*	3.1 (5.1)	2.8 (6.0)

Data are presented as mean (standard deviations) or counts (percentages).

* Significantly different between persons with and without COPD, after sex and age adjustment – if applicable.

† Significantly different between persons with prevalent and incident COPD at baseline, after sex and age adjustment – if applicable.

Abbreviations: COPD= Chronic Obstructive Pulmonary Disease; HDL= High-Density Lipoprotein; hsCRP = high-sensitivity C-reactive protein; N= number of persons included in study;

In *Table 2* we show associations of COPD and the risk of stroke. When we adjusted for age and sex, we found a significant association of COPD with all stroke (HR 1.22; 95%CI 1.03 – 1.46), ischemic stroke (HR 1.34; 1.07 – 1.67), and hemorrhagic stroke (HR 1.79; 1.07 – 2.99). However, additionally adjusting for smoking attenuated the associations. Further adjusting for systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication, total cholesterol, HDL cholesterol, lipid-lowering medication, diabetes mellitus and BMI hardly changed the estimates.*(Table 2)*

Associations between COPD and ischemic stroke were mainly present for persons with a cortical stroke (HR adjusted for age, sex, and smoking 1.40; 1.00 – 1.96) and persons with a stroke based on large artery atherosclerosis (HR adjusted for age, sex, and smoking 1.94; 1.11-3.38).

Table 2: COPD and the hazard on stroke.

	All stroke n/N 1250/13115	**Ischemic stroke n/N 701/13115**	**Hemorrhagic stroke n/N 107/13115**
Model 1	1.22 (1.03; 1.46)	1.34 (1.07; 1.67)	1.79 (1.07; 2.99)
Model 2	1.12 (0.93; 1.33)	1.19 (0.95; 1.49)	1.63 (0.97; 2.76)
Model 3	1.12 (0.93; 1.33)	1.19 (0.95; 1.49)	1.57 (0.93; 2.65)

Values are hazard ratios with 95% confidence intervals.
Model 1: age and sex adjusted.
Model 2: adjusted for age, sex and smoking.
Model 3: adjusted for age, sex, systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication, total cholesterol, HDL cholesterol, lipid-lowering medication, diabetes mellitus, BMI, and smoking.

DISCUSSION

In this large population-based cohort study, we found that COPD was associated with a higher risk of stroke, both ischemic stroke and hemorrhagic stroke. Associations between COPD and stroke attenuated after adjusting for smoking.

Other studies found age and sex adjusted odds ratios for the association of COPD with stroke varying between 1.24 and 1.51, which is comparable to the HR of 1.22 (1.03 – 1.46) that we found.[131, 190, 376, 377] One study found a larger effect size, with a HR of 3.34 (3.21 – 3.48), but this was driven by the younger age groups until 54 years.[375] We found associations for both ischemic and hemorrhagic stroke. None of the previous studies examined associations with ischemic or hemorrhagic stroke separately.

The most likely explanation for the higher risk of ischemic and hemorrhagic stroke in subjects with COPD is the shared risk factor smoking. Associations attenuated after adjusting for smoking. In a previous nested case-control study, a similar attenuation in effect on all stroke was found after adjusting for smoking.[190] Two other studies that also adjusted for smoking found a stronger and significant effect, but one only in younger age groups [375] and the other only in the elderly.[377] However, not all of our findings can be explained by smoking. Associations with hemorrhagic stroke were still borderline significant after adjusting for smoking and for ischemic stroke we found larger associations in persons with cortical stroke or strokes due to large artery atherosclerosis, which suggests that part of the association is influenced by effects of COPD itself. For instance due to the systemic inflammation occurring in COPD, especially during exacerbations. Inflammation in the systemic circulation leads to forming of instable atherosclerotic plaques and a pro-thrombotic state, which could lead to ischemic stroke.[372, 382, 383] Another causal mechanism might be hypoxia. Previous studies found an increased risk of stroke with decreasing lung function.[182, 183, 188] Hypoxia could lead to endothelial dysfunction which might predispose to stroke.[384, 385]

The strengths of our study are the longitudinal design with long-term follow-up, the population-based setting, and the thorough collection of COPD, exacerbations and stroke information during follow-up. This study also has limitations. We adjusted for baseline covariates, whereas the status of those variables might change during follow-up. Another limitation is that not all strokes could be classified into ischemic or hemorrhagic stroke due to lack of imaging data. Furthermore, we could not subclassify all ischemic strokes into lacunar or cortical and could not subclassify all ischemic strokes according to the TOAST criteria, because we were limited by the information from medical records.

In conclusion, we found that COPD was associated with stroke, both ischemic and hemorrhagic. Associations were mainly, but not completely driven by smoking, suggesting that in persons with COPD clinicians should be aware of the higher risk of both ischemic and hemorrhagic stroke.

PART IV: COPD AND FRAILTY

ABSTRACT

Objectives

To investigate the prevalence of frailty in a Dutch elderly population and to identify adverse health outcomes associated with the frailty phenotype independent of the comorbidities.

Methods

Cross-sectional and longitudinal analyses within the Rotterdam Study (the Netherlands), a prospective population-based cohort study in persons aged ≥55 years. Frailty was defined as meeting three or more of five established criteria for frailty, evaluating nutritional status, physical activity, mobility, grip strength and exhaustion. Intermediate frailty was defined as meeting one or two frailty criteria. Comorbidities were objectively measured. Health outcomes were assessed by means of questionnaires, physical examinations and continuous follow-up through general practitioners and municipal health authorities for mortality.

Results

Of 2833 participants (median age 74.0 years, IQR 9) with sufficiently evaluated frailty criteria, 163 (5.8%) participants were frail and 1454 (51.3%) intermediate frail. Frail elderly were more likely to be older and female, to have an impaired quality of life and to have fallen or to have been hospitalized. 108 (72.0%) frail participants had ≥2 comorbidities, compared to 777 (54.4%) intermediate frail and 522 (44.8%) non-frail participants. Adjusted for age, sex and comorbidities, frail elderly had a significantly increased risk of dying within 3 years (HR 3.4; 95% CI 1.9 to 6.4), compared to the non-frail elderly.

Conclusions

This study in a general Dutch population of community-dwelling elderly able to perform the frailty tests, demonstrates that frailty is common and that frail elderly are at increased risk of death independent of comorbidities.

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12. Frailty in the elderly

INTRODUCTION

Elderly people of the same *chronological age* demonstrate that there is a large heterogeneity in terms of *biological age.*[58, 386] Some are still fit and energetic while a relatively large number of elderly people has an accelerated decline in well-being and resilience.[37] Given the expanding elderly population and the major impact on health and social care, the identification of this frail group of elderly, which could benefit from more adequate interventions, becomes of increasing interest.[37] Frailty is defined as a biological syndrome in which a progressive, cumulative decline in the reserve capacity of multiple physiological systems elicits an abnormal vulnerability to common stressors.[38] In short, frailty could be defined as the disability to compensate function loss. Increasing evidence suggests that frail elderly are at an increased risk of adverse health outcomes (disability, falls, hospitalizations, institutionalizations and death).[38-42] Conceptually, it is important to distinguish "*frailty*" from "*disability*" and "*comorbidity*" and therefore, not to use an instrument integrating disability or comorbidity items to measure frailty.[57] In the Cardiovascular Health Study, more than a quarter of the elderly categorized as frail according to the Fried frailty criteria were not disabled and had no comorbidity.[38] However, these three different entities are related.[37] Those who are frail have a higher prevalence of concomitant chronic diseases and of disability than those who are non-frail. Moreover, comorbidity is a risk factor for frailty, whereas disability is an adverse outcome of frailty and disability may aggravate frailty.[56] Because of the complex relationship between *comorbidity* and *frailty*, it is not always clear whether *frailty* is a predictor of *mortality* independent of *comorbidities*. To our knowledge, the very few studies who investigated the association between frailty and mortality only adjusted for some selected, mainly self-reported, chronic diseases.[38, 40-42, 387]

An unambiguous definition of frailty is of great importance for clinicians to identify those at an increased risk of adverse health outcomes, but also for policy makers to make cost-effective decisions in health care.[58] Since there is no distinct uniform definition of frailty, reported prevalences vary greatly from 5% to 58%.[388] Generally, two different definitions of frailty are most commonly used:[58] a broader definition of frailty taking into account more social and psychological aspects;[39] and one based on predominantly physical criteria.[38] Within the latter definition based on physical aspects, frailty is defined as meeting three or more of five established criteria for frailty, namely evaluating: i) nutritional status (unintentional weight loss), ii) physical activity, iii) mobility (slow walking speed), iv) weakness (reduced grip strength), and v) exhaustion (self-reported).[38] Elderly individuals with one or two criteria are defined as intermediate frail or pre-frail and have been found to be at increased risk of becoming frail.[38] The physical frailty definition by Fried *et al.* is associated with a lower prevalence and less variable prevalence rates.[38] Although it is suggested that frailty is more than a decline in physical functions, the model based on physical aspects is more practical for fundamental research since the criteria are limited in

number, has clear cut-offs between frail versus non frail and constitutes a more objective measure.[58, 388] Moreover, the frailty phenotype by Fried *et al.* is the most widely used instrument in frailty research, allowing comparisons with other studies, and has been most extensively tested for its validity, including the external validity of adverse health outcomes.[38, 57]

The aim was to investigate the prevalence of physically frail elderly in a Dutch population-based cohort study and the impact on adverse health outcome including all-cause mortality independent of comorbidity.

METHODS

Study population and design

This study was embedded within cohort I and II (RSI and RSII) from the Rotterdam Study, a prospective population-based cohort study ongoing since 1990.[108] The rationale and design of the Rotterdam Study have been extensively described.[20, 21, 55, 85, 108, 389] In short, all inhabitants aged 55 years and older of one district of Rotterdam (Ommoord) were invited to the original cohort (RSI). Names and addresses were drawn from the municipal register which is reliable, complete and up to date.[21] 7 983 subjects participated (78% of 10,215 invitees). 3 011 participants (out of 4 472 invitees) who had become 55 years of age or moved into the study district since the start of the study were added as RSII in 1999. Almost all participants are from Caucasian descent. All participants are invited every 3 to 5 years to the research center for follow-up examinations, including physical examination and blood sampling. Moreover, the participants are continuously monitored for clinically meaningful outcomes. The assessment of the study participants' characteristics and frailty criteria used in this study took place from March 2009 until March 2012 among 4027 participants. The follow-up period started at the time of the frailty-assessment until death or end of the study period (January 1st, 2013). This study was approved by the medical ethics committee of the Erasmus Medical Center, Rotterdam, and the review board of The Netherlands Ministry of Health, Welfare and Sports. Participants gave written informed consent.

Definition of frailty

Frailty was determined using the physical definition of frailty, which has been developed in the population-based Cardiovascular Health Study and is since then the most widely used and validated instrument in frailty research.[38, 57, 390] Frailty was defined as meeting three or more of the five established frailty criteria. Grip strength was assessed using a handgrip dynamometer and the grip strength was defined as the highest value (kg) of three trials performed in the non-dominant hand.[108] Weakness was defined as having a grip strength for men ≤29 kg (if body mass index (BMI)≤24, or ≤30 kg if BMI≤24.1-28, or ≤32 kg if BMI>28) and for women ≤17 kg (if BMI≤23, or ≤17.3 kg if BMI≤23.1-26, or ≤18 kg if BMI≤26.1-29, or ≤21 kg if BMI>29).[38] Weight loss was defined as losing more than 5% body weight compared to the previous examination (six years earlier). Exhaustion was defined as answering "frequently"

or “mostly” to one of the following two statements from the Center for Epidemiological Studies Depression (CES-D) scale: (a) I felt that everything I did was an effort; (b) I could not get going.[391] Physical activity was determined from an extensive questionnaire about leisure time and sports; the physical activities were weighted by their intensity and all awarded kilocalories per week were summed.[38, 392] Low physical activity was defined as expending less than 383 kcal per week for men and less than 270 kcal per week for women.[38] Finally, slowness was defined as walking at a velocity of less than 0.76 m/s if height was more than 173 cm for men or more than 159 cm for women (otherwise less than 0.65 m/s).[38] Assessment of gait was performed in all participants using the GAITRite walkway (CIR Systems, Inc., Sparta, New Jersey). Only participants who had a sufficient number of criteria to confirm or to exclude frailty were included (e.g. at least three concordant positive or negative criteria evaluated). Participants with one or two criteria were defined as intermediate frail or pre-frail.[38]

Characteristics, comorbidities and adverse health outcomes

Standardized questionnaires including information on smoking status, pack years, falls and hospitalizations, were completed at the time of the frailty-assessment in the research center. Participants were asked “Did you fall in the past 12 months?” and “Have you been hospitalized in the past 12 months?”. Mortality information was obtained from the municipal health authorities in Rotterdam in addition to computerized reports from the general practitioners.[108] Smoking status was distinguished into never, former, and current smoking. Cigarette pack years were computed as duration of smoking (years) multiplied by the number of smoked cigarettes, divided by 20. The BMI was calculated as weight divided by height squared.

Diabetes mellitus was defined as the use of blood glucose-lowering medication based on automated pharmacy records.[393] Chronic obstructive pulmonary disease (COPD) was assessed by spirometry, performed at the research center. The participants were classified as having COPD when the ratio of forced expiratory volume in 1 second (FEV_1) to forced vital capacity (FVC) was less than 70%, in the absence of asthma.[232] Hypertension was defined as the use of antihypertensive medication during follow-up, and/or a systolic brachial blood pressure of ≥160 mmHg, and/or a diastolic brachial blood pressure of ≥100 mmHg (Grade 2 according to European Society of Cardiology criteria).[343] Coronary revascularization was defined as coronary artery bypass grafting and percutaneous coronary intervention. Myocardial infarction, heart failure, stroke and cancer were clinically validated. Osteoporosis was defined using femoral neck bone mineral density measured by Dual-energy X-ray absorptiometry applying World Health Organization (WHO) criteria.[394] Kidney disease was defined according to the National Kidney Foundation guidelines as having a glomerular filtration rate (GFR) <60 mL/min/1.73m or as having markers of kidney damage (i.e. a urine albumin – creatinine ratio greater than 17 mg/g in men and greater than 25 mg/g in women) [395]. The GFR was calculated using the Modification of Diet in renal Disease Study Equation of Levy *et al.*[396] Anemia was defined according to the WHO guidelines <120 g/L for women and

<130 g/L for men.[397] The comorbidity count which distinguishes between none, one and two or more comorbidities, was calculated for all participants of whom we had complete information on all ten diseases. Blood samples for determination of levels of serum glucose, creatinin, hemoglobin (Hb), cholesterol, and white blood cell (WBC) count were obtained at the research center.

Statistical analyses

For the baseline characteristics, logistic regression models, adjusted for age and sex, were performed. Cox proportional hazard ratios were calculated to evaluate the risk of mortality for frail elderly. The following variables were considered as potential confounders: age, sex, body mass index, smoking status, pack years and the comorbidity-index. Models were adjusted for co-variables that changed the point estimate by more than 5%. Kaplan-Meier survival analysis was used to illustrate the percentage of participants who died during follow-up. The Log rank test was used to determine whether the difference in survival between frail elderly and intermediate and non-frail elderly was significant. Stratification for sex was performed and individual Kaplan-Meier curves were depicted. Proportionality of hazards was checked for the Cox-regression models. The influence of the use of age as time scale instead of the use of the standard survival analysis corrected for age, was evaluated using R Statistical Software (Foundation for Statistical Computing, Vienna, Austria). All other statistical analyses were performed using IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp, Armonk, NY). P-values below the conventional level of significance ($p<0.05$) were considered as statistically significant.

RESULTS

Characteristics of the study population

Within the Rotterdam Study, the frailty status could be determined in 2833 (71.6%) of the 4027 participants who recently visited the research center. Of these, 1585 participants (55.9% of the study population) were women and the median age was 74 years (IQR 9). The prevalence of the individual frailty criteria was: 1) 1113 (40.3%) out of 2765 participants had reduced grip strength, 2) 534 (19.4%) out of 2753 evaluated participants had ≥5% weight loss over the past six years, 3) 61 (3.6%) out of 1688 participants exhibited a slow walking speed, 4) 118 (4.4%) out of 2695 participants had a low physical activity, and 5) 366 (13.0%) out of 2816 participants reported exhaustion (*Figure 1*). No walking speed measurement was available in 1145 (40.4%) participants due to a postponed start period of the gait assessment. Three or more of the five frailty criteria were present in 163 participants, implicating that the prevalence of frailty in the Rotterdam Study is 5.8% (95% CI: 5.0 to 6.7%; 4.1% in males, 7.1% in females). 1216 (42.9%) and 1454 (51.3%) participants were non-frail and intermediate frail, respectively.

Frail participants were older and more frequently female compared to participants with less than three frailty criteria (*Table 1*). In addition, adjusted for age and sex, frail participants had a significantly impaired quality of life (QoL) and were more frequently current smokers with a higher number of pack years.

Compared to the participants in whom the frailty status could be determined (n=2833), the participants with incomplete information (n=1194) were older and more frequently female, had a worse quality of life, higher mortality and more comorbidities (*Table 2*). In a sensitivity analysis using multiple imputation, the prevalence of frailty was not significantly higher in the participants with incomplete information (6.9% compared to 5.8% in the participants where the frailty status could be determined; p=0.177).

Table 1: Baseline characteristics.

	Non-frail	Intermediate Frail	Frail	P-value
Number	1216 (42.9%)	1454 (51.3%)	163 (5.8%)	
Age (years)	73 (7)	75 (9)	81 (8)	<0.001
Female	623 (51.2%)	850 (58.5%)	112 (68.7%)	0.001
QoL	80 (15)	80 (15)	70 (20)	<0.001
BMI	26.6 (4.5)	27.4 (5.3)	27.0 (6.6)	0.811
Pack years	5.4 (23.1)	5.0 (23.0)	6.5 (35.3)	<0.001
Smoking status				
Never	393 (32.3%)	497 (34.2%)	60 (36.8%)	
Former	715 (58.8%)	832 (57.2%)	84 (51.5%)	0.913
Current	108 (8.9%)	125 (8.6%)	19 (11.7%)	0.035
Lab results				
Hb (mmol/l)	8.8 (0.9)	8.7 (0.9)	8.4 (0.9)	<0.001
WBC (count/mm^3)	6,700 (1,900)	6,900 (2,200)	7,200 (2,600)	0.007
Cholesterol (mmol/l)	5.4 (1.5)	5.3 (1.5)	5.0 (1.5)	<0.001
Glucose (mmol/l)	5.5 (0.9)	5.6 (1.0)	5.5 (1.1)	0.881
Creatinin (µmol/l)	79 (22.0)	78 (24.8)	78 (26.0)	0.288
Comorbidity				
Coronary disease	29 (2.4%)	75 (5.2%)	5 (3.1%)	0.280
Heart failure	21 (1.7%)	60 (4.1%)	18 (11.0%)	0.006
Hypertension	865 (71.1%)	1124 (77.3%)	140 (85.9%)	0.034
Stroke	10 (0.8%)	13 (0.9%)	4 (2.5%)	0.001
Diabetes	72 (5.9%)	122 (8.4%)	17 (10.4%)	0.095
Osteoporosis	121 (10.2%)	181 (12.4%)	40 (25.0%)	0.001
Cancer	159 (13.1%)	206 (14.2%)	29 (17.8%)	0.160
COPD	172 (14.1%)	238 (16.4%)	47 (28.8%)	<0.001
Anemia	45 (3.8%)	127 (8.9%)	30 (19.9%)	<0.001
Kidney disease	306 (25.5%)	470 (32.6%)	66 (43.1%)	0.380
Comorbidity count				
0	173 (14.8%)	151 (10.6%)	7 (4.7%)	
1	471 (40.4%)	501 (35.1%)	35 (23.3%)	0.601
≥2	522 (44.8%)	777 (54.4%)	108 (72.0%)	0.057
Falls in preceding year	180 (21.2%)	298 (26.8%)	53 (39.8%)	0.001
Hospitalization in preceding year	60 (10.6%)	144 (17.4%)	27 (24.5%)	0.006

Categorical variables are expressed as numbers (percentages). Values of continuous variables are expressed as median (interquartile range (IQR)). P-values comparing the frail group against the non-frail and intermediate frail group combined, are age and sex adjusted (age and sex adjusted for one another). Comorbidity count determined in participants without missing comorbidities (n=2745; coronary disease was missing in 6 participants, heart failure in 12, osteoporosis in 29, anemia in 58 and kidney disease in 37 participants). Abbreviation: QoL = Quality of Life, BMI = Body Mass Index, Hb = Hemoglobin, WBC = White Blood Cells, COPD = Chronic Obstructive Pulmonary Disease

Table 2: Baseline characteristics of the included (Frailty status determined) and excluded group (No frailty status determined).

	Frailty status determined	No frailty status determined	P-value
Number	2833 (70.4%)	1194 (29.6%)	
Age (years)	74 (9)	80 (11)	<0.001
Female	1585 (55.9%)	796 (66.7%)	<0.001
QoL	80 (15)	70 (15)	<0.001
BMI	27.0 (5.0)	27.4 (5.9)	0.103
Pack years	5.2 (23.6)	4.5 (24.5)	0.222
Smoking status			
Never	950 (33.5%)	444 (37.2%)	
Former	1631 (57.6%)	600 (50.3%)	
Current	252 (8.9%)	150 (12.6%)	<0.001
Lab results			
Hb (mmol/l)	8.7 (1.0)	8.5 (1.1)	<0.001
WBC (count/mm^3)	6,800 (2,000)	7,300 (2,300)	<0.001
Cholesterol (mmol/l)	5.4 (1.5)	5.3 (1.5)	0.109
Glucose (mmol/l)	5.5 (0.9)	5.6 (1.2)	0.116
Creatinin (µmol/l)	78 (23.0)	77 (26.0)	0.404
Comorbidity			
Coronary disease	109 (3.9%)	67 (5.7%)	0.010
Heart failure	99 (3.5%)	124 (10.5%)	<0.001
Hypertension	2129 (75.2%)	841 (71.3%)	0.012
Stroke	27 (1.0%)	20 (1.7%)	0.051
Diabetes	302 (12.4%)	64 (18.6%)	0.001
Osteoporosis	342 (12.2%)	65 (13.9%)	0.297
Cancer	433 (17.4%)	68 (19.0%)	0.464
COPD	457 (16.1%)	200 (16.8%)	0.627
Anemia	202 (7.3%)	59 (12.6%)	<0.001
Kidney disease	841 (30.1%)	201 (41.6%)	<0.001
Comorbidity count			
0	287 (11.8%)	22 (6.5%)	
1	806 (33.2%)	90 (26.5%)	
≥2	1337 (55.0%)	227 (67.0%)	<0.001
Falls in preceding year	531 (25.3%)	294 (33.6%)	<0.001
Hospitalization in preceding year	231 (15.4%)	155 (19.8%)	0.008
Frailty (imputed)	163 (5.8%)	82 (6.9%)	0.177
Deaths	118 (4.2%)	198 (16.6%)	<0.001

Categorical variables are expressed as numbers (percentages). Values of continuous variables are expressed as median (interquartile range (IQR)). Abbreviation: QoL = Quality of Life, BMI = Body Mass Index, Hb = Hemoglobin, WBC = White Blood Cells, COPD = Chronic Obstructive Pulmonary Disease

Frailty and comorbidities

72.0% of those who were frail had two or more comorbidities compared to 44.8% of the non-frail participants (*Table 1*). Frail participants had a significantly higher prevalence of heart failure, hypertension, stroke, osteoporosis, COPD and anemia independent of age and sex than those who were non-frail and intermediate frail (*Table 1*). Furthermore, frail participants had lower blood levels of hemoglobin and cholesterol, whereas they had an increased white blood cell count, even when adjusted for age, sex, BMI, smoking status, pack years and the comorbidity count (p=0.001, p=0.003 and p=0.040 respectively). There were no remarkable differences regarding BMI, blood glucose or creatinin levels.

Frailty and adverse health outcomes

More than one third of the frail participants had fallen in the preceding year and one quarter had been hospitalized in the previous year, compared to approximately one fifth and one tenth of the non-frail participants respectively (*Table 1, Figure 2*). In *Figure 2A* the percentage of participants who fell is depicted per frailty category; *Figure 2B* shows the percentage of participants who were hospitalized.

During the more than three years of follow-up between March 2009 and January 2013 (average follow-up 805 days, SD 310 days), 118 (4.2%) of the 2831 participants with follow-up died. 26 (16.0%) among the frail, 63 (4.3%) among the intermediate frail and 29 (2.4%) among the non-frail. Survival was significantly worse for frail elderly compared to the other two groups (Log rank p<0.001; *Figure 3A*). The Kaplan-Meier curves stratified by sex suggest a different survival pattern for men (immediate decline) compared to women (steady progressive decline) *(Figure 3B,C).*

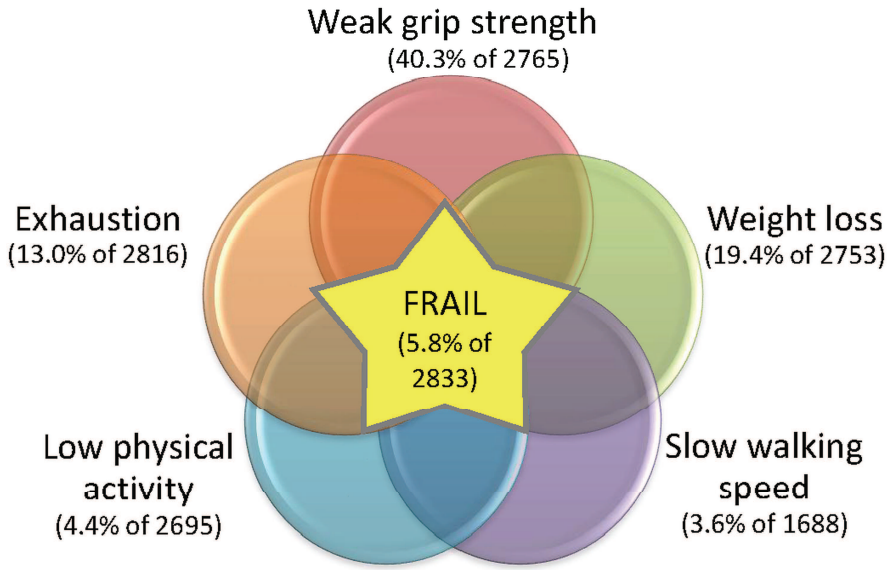
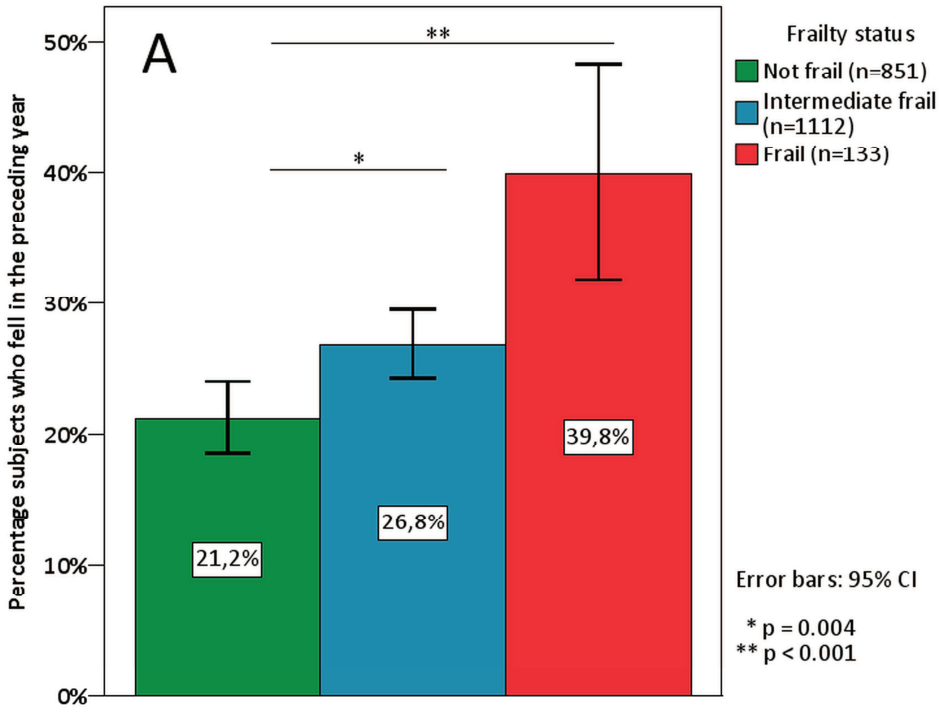


Figure 1: The frailty flower.



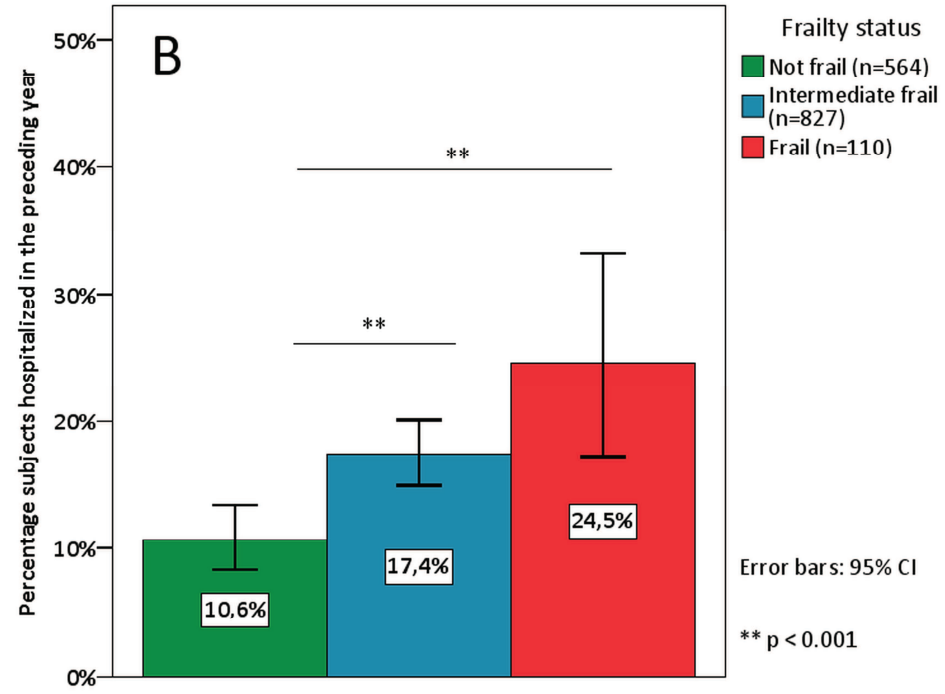
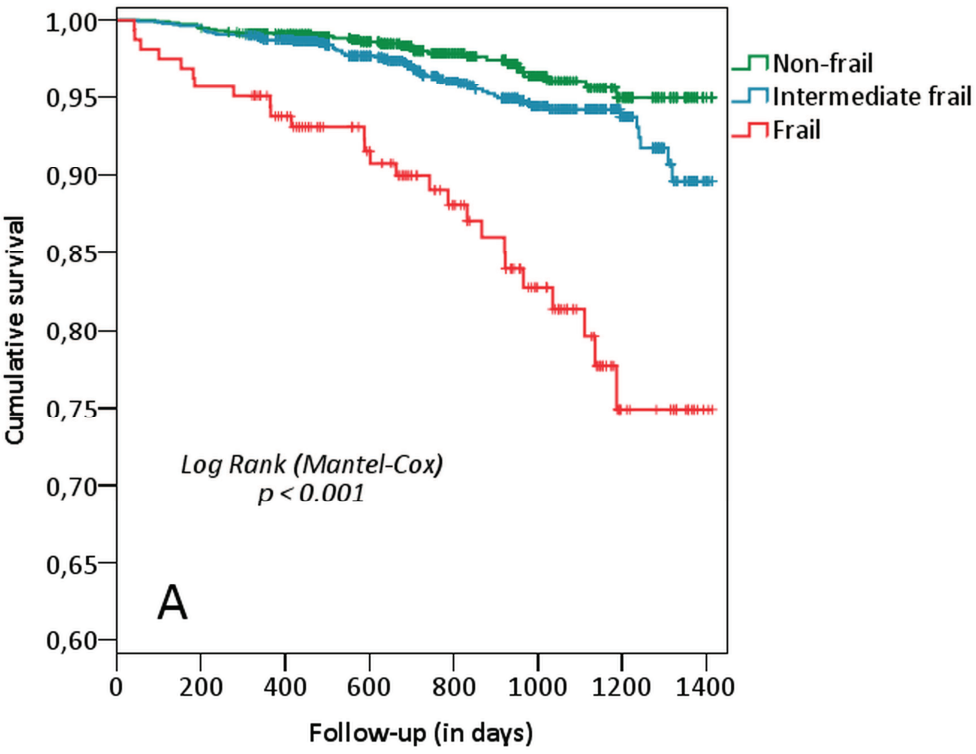


Figure 2: Falls **(A)** and hospitalizations **(B)** in the preceding year according to frailty status.



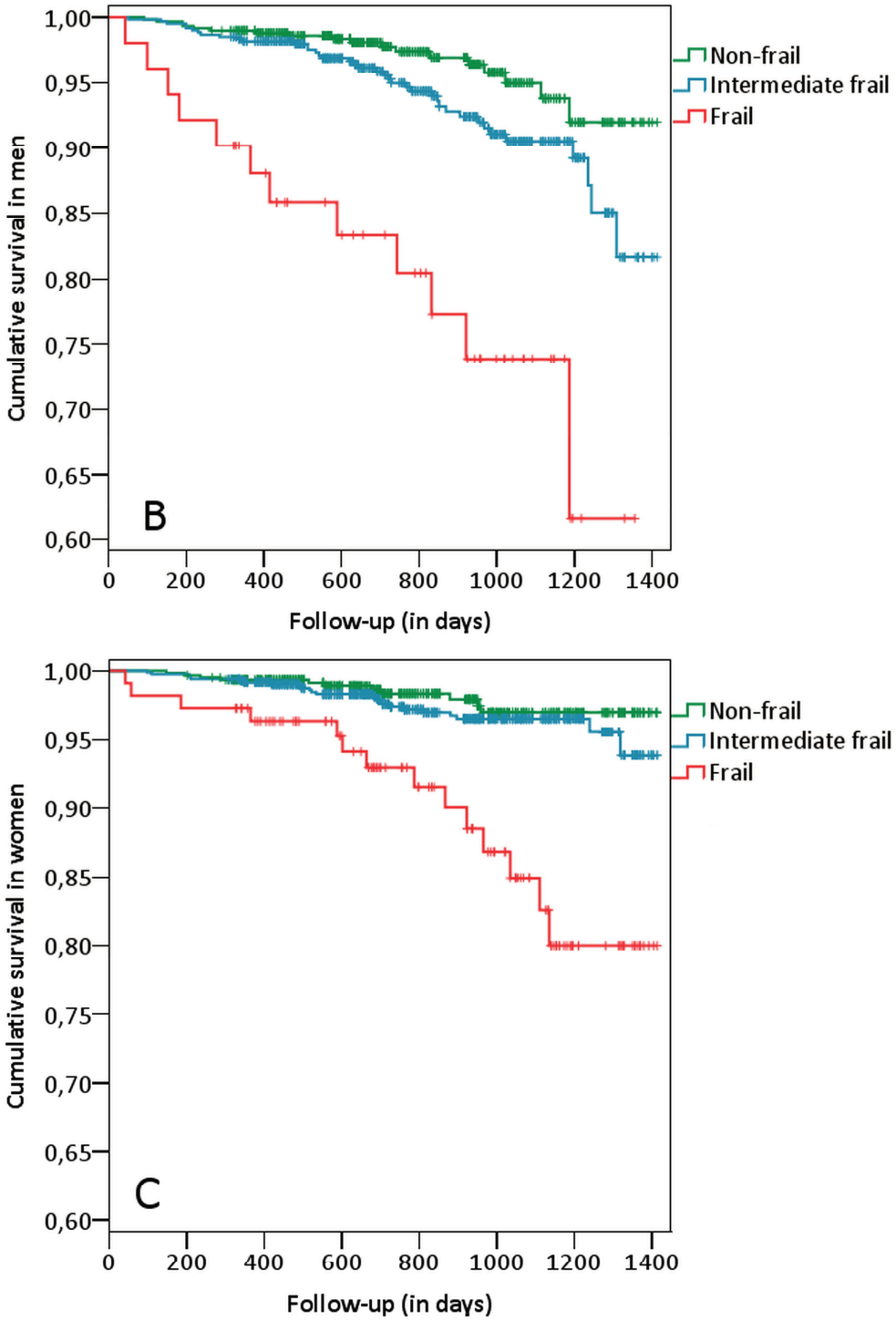


Figure 3: Kaplan-Meier survival curves overall **(A)**, in men **(B)** and in women **(C)**.

When adjusting for age, sex and the comorbidity count in the Cox hazard proportion model, frail elderly had a more than threefold increased risk of mortality (HR 3.43, 95% CI 1.85 to 6.36; p<0.001, *Table 3*). Importantly, the addition of all enumerated potential confounders and the ten diseases of the comorbidity count to the model, did not change the independency of the frailty effect on mortality (p=0.002). A trend towards an increased mortality risk could be noticed in the (male) pre-frail group (intermediate frail elderly); however, this was no longer significant after adjustment (*Table 3*). The use of age as time scale did not substantially influence the frailty point estimate (data not shown). Stratified by sex and adjusted for age and comorbidity, frail men had a 4.3 fold increased risk of mortality and frail women a 2.5 fold increased risk of mortality (*Table 3*). However, confidence intervals largely overlapped and the interaction term between frailty and sex was non-significant.

Table 3: Cox proportional hazard model of frailty on the risk of mortality.

		Mortality							
		Crude model (n=2831)				Adjusted model (n=2745)			
		HR	95% CI		P-value	HR	95% CI		P-value
			Lower	Upper			Lower	Upper	
All	Non-frail	*Reference*				*Reference*			
	Intermediate frail	1.63	1.05	2.54	0.029	1.31	0.81	2.10	0.267
	Frail	5.77	3.39	9.80	<0.001	3.43	1.85	6.36	<0.001
Males	Non-frail	*Reference*				*Reference*			
	Intermediate frail	1.93	1.10	3.37	0.022	1.50	0.81	2.75	0.196
	Frail	7.25	3.49	15.08	<0.001	4.25	1.84	9.78	0.001
Females	Non-frail	*Reference*				*Reference*			
	Intermediate frail	1.44	0.70	2.94	0.318	1.04	0.49	2.22	0.919
	Frail	6.08	2.76	13.40	<0.001	2.49	1.00	6.22	0.051

Adjusted model: adjusted for age, sex and comorbidity count.
Abbreviations: HR = Hazard Ratio; CI = Confidence Interval.

DISCUSSION

Overall, we observed a prevalence of frailty of almost 6 percent within the Rotterdam Study. Compared to the non-frail, frail participants were more likely to be older and female. Frail elderly people had more falls and hospitalizations in the previous year and had an increased risk of mortality independent of age, sex and comorbidity.

Our observed frailty prevalence is in line with the 6.9% prevalence estimate that Fried *et al.* found in the Cardiovascular Health Study.[38] In addition, a review by Collard *et al.* reported an overall prevalence of frailty of 9.9% in studies using physical frailty definitions.[58] The results in our study confirm the consistency of the Fried frailty criteria. In the Cardiovascular Health Study, 68.0% of the frail elderly had 2 comorbidities or more and 7.3% had none of 9 co-morbidities.[38] Consistently, we found that 72.0% of the frailty group had two or more, and 4.7% had none of the co-morbidities, respectively. This indicates that frailty is not identical to comorbidity. It is more likely that these entities frequently coexist, and that comorbidity only partly explains the presence of frailty.[56]

Notably, the body mass index did not differ significantly between frailty groups, which is similar to previous findings.[387, 398] Although frail people in our study had a significantly lower weight, they were also significantly shorter. As the BMI is a ratio, both parameters are important. Furthermore, not only weight loss, but also 'sarcopenic obesity' might be a risk factor of frailty.[399] Sarcopenia is a syndrome characterized by a progressive and generalized loss of skeletal muscle mass and muscle function (strength) with an increased risk of adverse health outcomes. 'Sarcopenic obesity' is the condition where lean body mass is lost, while fat mass may be preserved or even increased.[400] Thus not only loss of body weight, through loss of muscle tissue, but also intramuscular and visceral fat accumulation, are important for developing muscle weakness.[400]
Interestingly, in our study we found an increased WBC count and low hemoglobin levels to be associated with frailty. Chaves *et al.* found that the risk of being frail in community-dwelling older women progressively declined with increasing hemoglobin levels up to what is currently considered mid-normal levels (13.5 g/dL).[401] In the Women's Health and aging Study, higher WBC counts and IL6 levels were independently associated with prevalent frailty.[51] Possibly, IL6 contributes to unexplained anemia in elderly people by inhibiting erythropoietin production or by interaction with the erythropoietin receptor.[47] This supports the assumption of an association between frailty and a state of chronic systemic inflammation.[37]

In the Rotterdam Study, frail elderly had an increased risk of death compared to non-frail participants, independent of age, sex and co-morbid disease. Fried and others have also found an increased risk of mortality in the frail group, although the mean follow-up period in these studies ranges from 3 to 9.7 years.[38, 42, 387] Puts *et al.* stated that men appeared to die more suddenly, while women had a more steady progressive decline.[40] Although our survival

pattern for men compared to women seemed to confirm this statement, the mortality hazards were not significantly influenced by time in the Cox regression model. In our study, the short-term mortality risk was not significantly increased for the intermediate frail group independently of age, sex and comorbidity. Fried *et al.* found that intermediately frail elderly were at an increased risk of becoming frail.[38] This is in accordance with the concept that the frailty syndrome is a dynamic process, where transitions to states of greater frailty are more common, than transitions to states of lower frailty.[402] Thus, intermediate frailty could be considered as a state where the process of decline in multiple physiologic systems has already been set in motion, but reserves are still sufficient to withstand most stressors. This stage would therefore be suited for secondary prevention, including chronic disease management and geriatric assessment, fall prevention, exercise and nutritional modifications.[388, 403] Frailty, on the other hand, is often considered to be irreversible and tends to progress to an end-stage condition.[38, 404] This is shown in the study of Gill *et al.* where, over a 54 months period, the probability of transition from being frail to non-frail was very low (rates 0% to 0.9%).[402] Moreover, in an interventional trial the benefit of the intervention (physical training) was observed only among the participants with moderate frailty, but not among those with more severe frailty. Note that in this trial moderately frail elderly were defined as having only 1 physical criterion, and the severe frail as having 2.[405]

The strengths of our study are the high quality and detail of medical information, the prospective mortality data collection, objective measurement of comorbidities and the general population-based setting of the Rotterdam Study. A potential limitation is that measurement of walking speed was only recently introduced. Because solely participants with at least three concordant positive or negative criteria were included in the study (e.g. the result of the missing gait assessment no longer mattered to conclude frailty), it is unlikely that this alone has caused misclassification of frail participants within the study. However, the percentage of intermediate frail participants within the study might have been underestimated. Second, falls and hospitalizations were assessed retrospectively. The recall of any fall in the previous year is relatively specific (91–95%) but somewhat less sensitive (80–89%) than intensive prospective data collection and individuals with poorer cognitive function may be less likely to recall falling (or hospitalization) in the previous 12 months.[406] Thirdly, weight loss was not self-reported but directly measured without asking whether any weight loss was intentional. Although we believe that the objective weight measurement is a strength, we cannot exclude the possibility that the actual prevalence might be lower due to intentional weight loss. In contrast, it is important to note that the true prevalence of frailty might be higher than measured if frail people do not feel fit enough to perform physical tests or complete questionnaires. The comparison of participants in whom the frailty status could be determined with those participants with incomplete information, seemed to point in the direction that the more vulnerable people are less able to perform the physical frailty tests.

Consequently, the impact of frailty on mortality may be even larger if this phenomenon is limited to the most vulnerable frail people. In conclusion, frailty is common in those participants of the Rotterdam Study able to perform the frailty tests, and more prevalent in females than males. Frail elderly are at an increased risk of dying within three years independent of comorbidity. We suggest that clinicians should focus on intermediate frail elderly to implement preventive measures.

ABSTRACT

Background

With the longevity of our population, the prevalence of chronic illnesses such as Chronic Obstructive Pulmonary Disease (COPD) and geriatric syndromes such as frailty increases.

Objectives

To investigate whether COPD is an independent risk factor for frailty.

Methods

This study was part of the Rotterdam Study, a prospective population-based cohort study performed in subjects aged ≥ 55 years. Diagnosis of COPD was confirmed by spirometry. Frailty was defined as meeting 3 or more of five established criteria for frailty, evaluating nutritional status, physical activity, mobility, strength and energy.

Results

Of the 2142 subjects with frailty and lung function assessments, 100 subjects were frail including 41 of the 402 (10.2%) subjects with COPD compared to 59 of the 1740 (3.4%) subjects without COPD. The prevalence of frailty was significantly increased in subjects with COPD, especially in those with severe airflow limitation, dyspnea and frequent exacerbations. Subjects with mild airflow limitation were more frequently pre-frail. Independent of age, sex, smoking, cumulative use of systemic corticosteroids and other confounders, subjects with COPD had a more than twofold increased prevalence of frailty (OR 2.2, 95% CI 1.34-3.54, p=0.002).

Conclusions

This population-based cohort study in elderly demonstrates that COPD is associated with frailty, independent of common risk factors and comorbidities.

13. COPD, comorbidities and frailty

INTRODUCTION

With the aging of our population and the reduction in early deaths from acute illnesses and infections, more people are living longer but frequently suffer from chronic illnesses such as Chronic Obstructive Pulmonary Disease (COPD). COPD is characterized by various systemic manifestations in addition to the progressive chronic airflow limitation.[24] The most prevalent known comorbidities are cardiovascular disease, metabolic syndrome, osteoporosis, depression, and lung cancer.[24] Other systemic manifestations of COPD include skeletal muscle wasting, cachexia and normocytic anemia.[373, 407] These comorbid conditions have a clear impact on the clinical presentation and prognosis of patients with COPD and the identification and treatment of comorbid diseases are key elements in COPD management.[1]

In contrast to organ-specific diagnoses and treatment, frailty points more to a holistic viewpoint of the elder patient and their predicament.[37] The definition of frailty describes a biological syndrome in which a progressive, cumulative decline in the reserve capacity of multiple physiological systems elicits an abnormal vulnerability to common stressors.[38] Frailty is a distinct syndrome associated with functional decline, loss of independence, and mortality, and is characterized by the disability to compensate function loss.[38] Frailty is defined as meeting 3 or more of five established criteria for frailty, evaluating nutritional status, physical activity, mobility, strength and energy.[38] Individuals with one or two criteria are defined as intermediate frail or pre-frail and have been found to be at increased risk of becoming frail.[38] Previously, we have demonstrated that frailty is common in the general elderly population.[408] Moreover, we demonstrated that frail elderly subjects had more falls and hospitalizations and an increased risk of mortality independent of age, sex and comorbidity.[408] Distinction of frail elderly people from those who are not frail is essential in an aging population to better outweigh benefits and risks of invasive procedures or potentially harmful medication in vulnerable elderly.[37]

The demographic change is a great challenge for social welfare systems and healthy aging is becoming increasingly important. Both COPD and frailty are prevalent syndromes in the elderly, associated with substantial morbidity, disability and mortality. Although smoking and aging are the main risk factors for COPD, and have been associated with an increased risk of frailty, the link between COPD and frailty remains insufficiently investigated. Frailty might identify those COPD subjects at high risk of adverse health events including mortality. Moreover, frailty -especially the intermediate stage- might be more reversible than COPD itself. Therefore, the identification and risk stratification of frailty in patients with COPD might be the first step towards improved COPD management and prolonged healthy aging in general.[409] Therefore, we have investigated the interrelationship between COPD and frailty in a large population-based cohort study.

METHODS

Study population and design

This study was embedded within the Rotterdam Study, a population-based cohort study in the city of Rotterdam aimed at assessing the occurrence of, and risk factors for chronic diseases in the elderly.[108] The study started in 1990 and all participants are invited every 3 to 5 years to the research centre for follow-up examinations, including physical examination, blood sampling and spirometry. In addition, information is obtained by questionnaires on health status, medical history, smoking, socio-economic status, drug use, dietary habits, physical activity, exhaustion and respiratory symptoms. The participants are moreover continuously monitored for the onset of major events which occur during follow-up. This current study was embedded within cohort I and II (RSI and RSII) from the Rotterdam Study. The Rotterdam Study was approved by the medical ethics committee of the Erasmus Medical Center, Rotterdam, and the review board of The Netherlands Ministry of Health, Welfare and Sports. All participants gave written informed consent.

Frailty assessment

The definition and assessment of frailty within the Rotterdam Study have been extensively described.[408] In short, frailty was determined using the physical definition of frailty by Fried *et al.* which has been the most widely used and validated instrument in frailty research.[38, 57, 390] Frailty was defined as meeting three or more of the five established frailty criteria evaluating nutritional status (weight loss), physical activity (low physical activity), walking speed (slow gait velocity), weakness (reduced grip strength), and self-reported exhaustion.[38] First, weakness was defined as having a grip strength for men ≤29 kg (if body mass index (BMI)≤24, or ≤30 kg if BMI≤24.1-28, or ≤32 kg if BMI>28) and for women ≤17 kg (if BMI≤23, or ≤17.3 kg if BMI≤23.1-26, or ≤18 kg if BMI≤26.1-29, or ≤21 kg if BMI>29).[38] Weight loss was defined as losing more than 5% body weight compared to the previous examination. Exhaustion was defined as answering “frequently” or “mostly” to one of the following two statements from the Center for Epidemiological Studies Depression (CES-D) scale: (a) I felt that everything I did was an effort; (b) I could not get going.[391] Low physical activity was defined as expending less than 383 kcal per week for men and less than 270 kcal per week for women.[38] Finally, slowness was defined as walking at a velocity of less than 0.76 m/s if height was more than 173 cm for men or more than 159 cm for women (otherwise less than 0.65 m/s).[38] Only participants who had a sufficient number of criteria to confirm or to exclude frailty were included (e.g. at least three concordant positive or negative criteria evaluated). Participants with one or two criteria were defined as intermediate or pre-frail.[38]

COPD assessment and classification

COPD cases and controls were nested in all participants of the source population with an interpretable lung function measurement at the last research centre visit of whom sufficient criteria were examined to confirm or to exclude frailty. The diagnosis of COPD was based on a spirometry examination conducted between March 2009 and March 2012. No reversibility

tests were conducted. However, asthma patients and participants with a spirometry report suggestive of restrictive respiratory disease [FEV1/FVC ≥ 0.7 and forced expiratory vital capacity (FVC) and/or FEV_1 < 80% predicted] were excluded. Spirometry was performed using a Master Screen® PFT Pro (Care Fusion, the Netherlands) by trained paramedical personnel according to the ATS/ERS guidelines.[22] Spirometry results which did not meet ATS/ERS criteria for acceptability and reproducibility were classified as not interpretable. COPD was defined by an obstructive spirometry [proportion of the forced vital capacity exhaled in the first second (FEV_1/FVC) < 0.7] and classified as mild, moderate or severe COPD by forced expiratory volume in one second (FEV_1)% predicted of ≥ 80%, 50-80% and < 50% respectively.

In addition, COPD subjects were classified according to the recent GOLD update (2011) which includes the evaluation of symptoms and the history of exacerbations next to the severity of airflow limitation. GOLD Group A consists of patients with a low risk and less symptoms (i.e. mild or moderate airflow limitation), (n)one exacerbation a year and a dyspnea score of 0 or 1. Group B includes patients with a low risk but with more symptoms (dyspnea score ≥ 2). Group C gathers patients with high risk (severe or very severe airflow limitation and/or ≥ 2 exacerbations a year) but less symptoms. Finally, group D consists of patients with a high risk and more symptoms. The dyspnea score was based on the following five dyspnea-questions: 1. Are you troubled by shortness of breath when climbing stairs? (i.e. at a normal speed) 2. Are you troubled by shortness of breath when walking on level ground? 3. Do you have shortness of breath? (i.e. during normal/daily life activities) 4. Are you troubled by shortness of breath when lying down, while this improves when you sit up or when you sleep on more pillows? 5. Are you short of breath at rest? Based on these five dyspnea questions, a dyspnea score was added from 0 (all questions negative, never dyspneic) to 5 (all positive, even dyspneic at rest).[232]

Comorbidity assessment

Nine comorbidities were chosen and assessed as prescribed previously.(Ref) Hypertension was defined as the use of antihypertensive medication during follow-up, and/or a systolic brachial blood pressure of ≥160 mmHg, and/or a diastolic brachial blood pressure of ≥100 mmHg (Grade 2 according to European Society of Cardiology criteria).[343] Coronary revascularization was defined as coronary artery bypass grafting and percutaneous coronary intervention. Myocardial infarction, heart failure, stroke and cancer were clinically validated. Diabetes mellitus was defined as the use of blood glucose-lowering medication based on automated pharmacy records.[393] Osteoporosis was defined using femoral neck bone mineral density measured by Dual-energy X-ray absorptiometry applying World Health Organization (WHO) criteria.[394] Kidney disease was defined according to the National Kidney Foundation guidelines as having a glomerular filtration rate (GFR) <60 mL/min/1.73m or as having markers of kidney damage (i.e. a urine albumin – creatinine ratio greater than 17 mg/g in men and greater than 25 mg/g in women) [395]. The GFR was calculated using the Modification

of Diet in renal Disease Study Equation of Levy *et al.*[396] Anemia was defined according to the WHO guidelines <120 g/L for women and <130 g/L for men.[397] The comorbidity count which distinguishes between none, one and two or more comorbidities, was calculated for all participants of whom we had complete information on all nine diseases. Blood samples for determination of levels of serum glucose, creatinin, hemoglobin (Hb), cholesterol, and white blood cell (WBC) count were obtained at the research center. Medication use was assessed through automated linkage with the serving pharmacies of filled prescriptions.

Statistical analyses

Differences between subjects with and without COPD were studied using Mann-Whitney U test for continuous variables and Chi-Square test for categorical variables. Logistic regression models were performed to assess the effect of COPD on frailty. Following variables were considered as potential confounders: age, sex, body mass index, smoking status, pack-years, and the comorbidity count. All models were adjusted for covariates that changed the point estimate by more than 5%. Finally, a stepwise regression with backward elimination (probability for stepwise entry was 0.05 and for removal 0.10) was performed including all comorbidities, lab values and medication use. All statistical analyses were performed using SPSS, version 20.0 for Windows (IBM, North Castle, NY). P-values below the conventional level of significance ($p< 0.05$) were considered as statistically significant.

RESULTS

Study flow and baseline characteristics of the study population

Of the 2833 subjects with frailty assessment, 2489 had performed an interpretable lung function test.(*Figure 1*) After the exclusion of 229 subjects with asthma and 118 subjects with a spirometry suggestive for a restrictive syndrome, 2142 subjects were included in the analyses. 100 subjects were frail defined as having three or more of the following characteristics: weak grip strength, weight loss, exhaustion, physical inactivity or slow walking time. 1074 subjects were intermediate or pre-frail defined as having one or two frailty characteristics. 968 subjects were non-frail defined as having none of the frailty characteristics. COPD subjects were significantly older, more frequently male and (current) smokers, had smoked more pack-years and had a slightly lower BMI than participants without COPD.(*Table 1*) Subjects with COPD had higher white blood cell counts, whereas they had lower serum levels of cholesterol compared to subjects without COPD. Regarding comorbidities, subjects with COPD had more prevalent coronary disease, heart failure, stroke, osteoporosis and kidney disease.

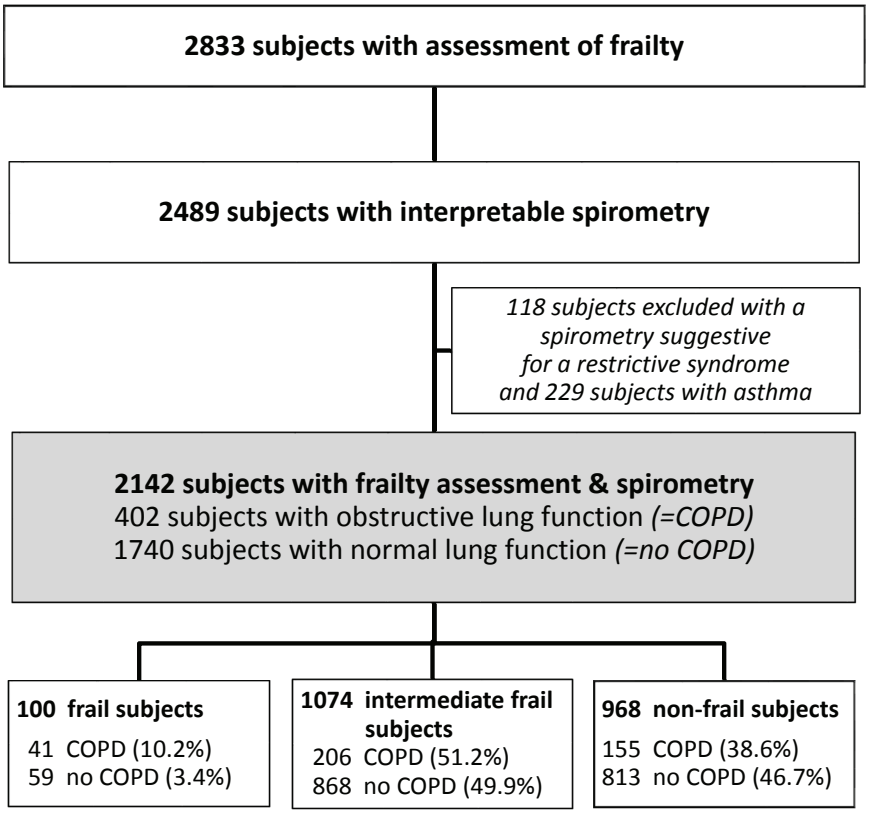


Figure 1: Study profile.

Table 1: Baseline characteristics.

	COPD (n=402)	No COPD (n=1740)	P-value
Age (years)	75 (9)	74 (8)	<0.001
Female	173 (43.0%)	990 (56.9%)	<0.001
BMI	26.0 (4.6)	27.0 (4.8)	<0.001
Pack years	22.0 (37.5)	3.0 (19.5)	<0.001
Smoking status			
Never	62 (15.4%)	646 (37.1%)	
Former	259 (64.4%)	985 (56.6%)	
Current	81 (20.1%)	109 (6.3%)	<0.001
FEV_1% predicted	79.6 (25.2)	89.4 (29.3)	<0.001
FEV_1/FVC	65.6 (7.8)	76.1 (8.1)	<0.001
Lab results			
Hemoglobin (mmol/l)	8.8 (1.1)	8.7 (1.0)	0.010
WBC (count/mm^3)	7,100 (2,300)	6,600 (1,900)	<0.001
Cholesterol (mmol/l)	5.2 (1.4)	5.4 (1.5)	<0.001
Glucose (mmol/l)	5.5 (0.9)	5.5 (0.9)	0.172
Creatinin (µmol/l)	81 (26.0)	78 (23.0)	0.019
Comorbidity			
Coronary disease	25 (6.2%)	56 (3.2%)	0.005
Heart failure	21 (5.2%)	37 (2.1%)	0.001
Hypertension	306 (76.1%)	1274 (73.2%)	0.233
Stroke	30 (7.5%)	74 (4.3%)	0.007
Diabetes	25 (6.2%)	122 (7.0%)	0.571
Osteoporosis	61 (15.2%)	190 (10.9%)	0.017
Cancer	58 (14.4%)	242 (13.9%)	0.787
Anemia	34 (8.5%)	109 (6.4%)	0.117
Kidney disease	137 (34.3%)	469 (27.1%)	0.004
Comorbidity count			
0	46 (11.6%)	264 (15.5%)	
1	138 (34.8%)	704 (41.3%)	
≥2	213 (53.7%)	736 (43.2%)	0.001

Categorical variables are expressed as numbers (percentages). Values of continuous variables are expressed as median (interquartile range (IQR)). Comorbidity count determined in participants without missing comorbidities (n=2101; coronary disease was missing in 4 participants, heart failure in 11, anemia in 28 and kidney disease in 12 participants).

Abbreviations: BMI = Body Mass Index, WBC = White Blood Cells, COPD = Chronic Obstructive Pulmonary Disease

COPD and frailty

Of the 2142 subjects included in the analyses, 402 subjects had an obstructive lung function and were categorized according to the modified Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification of airflow limitation into 200 (49.8%) mild, 174 (43.3%) moderate and 28 (7.0%) severe COPD.[24] 100 subjects were frail including 41 of the 402 (10.2%) subjects with COPD compared to 59 of the 1740 (3.4%) subjects without COPD. *Figure 2* represents the prevalence of frailty according to the COPD status and severity of airflow limitation. The prevalence of frailty shows to be highly associated with the severity of COPD according to the degree of airflow limitation (GOLD 2007). The prevalence of intermediate frailty was inversely associated with the severity of airflow limitation, while the prevalence of subjects without any of the frailty criteria did not change substantially over the COPD stages.

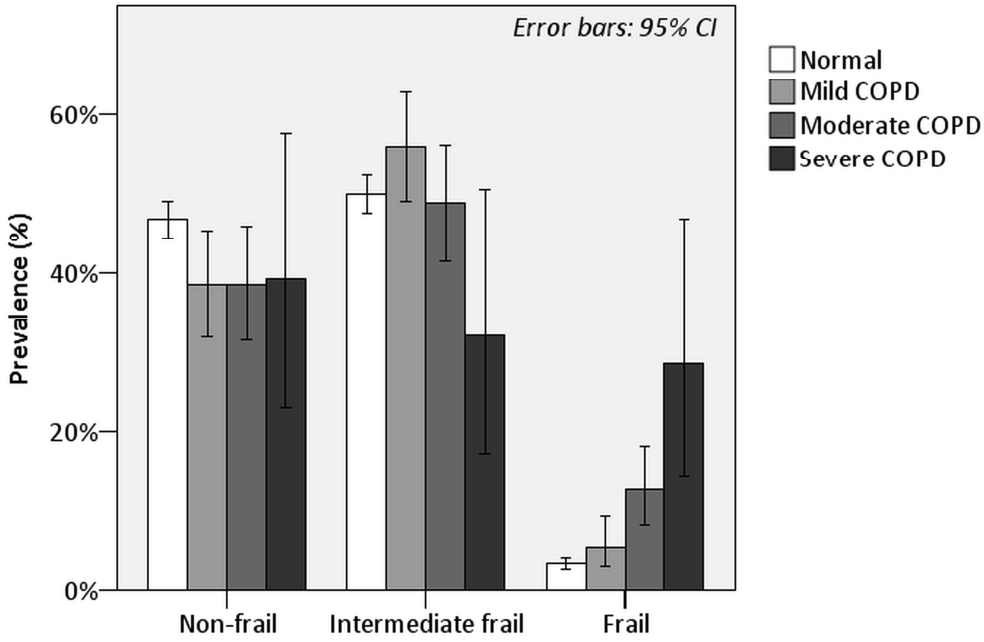


Figure 2: Prevalence of frailty according to severity of airflow limitation.
Abbreviations: CI = Confidence Interval, COPD = Chronic Obstructive Pulmonary Disease.

Table 2: COPD and the risk of frailty.

	Model 1 (n=2142)			Model 2 (n=2098)		
	OR	**95% CI**	**P-value**	**OR**	**95% CI**	**P-value**
COPD *(n=402)*	3.1	2.01-4.83	<0.001	2.4	1.52-3.86	<0.001
COPD, *mild (n=200)*	1.6	0.78-3.09	0.209	1.4	0.70-2.84	0.333
COPD, *moderate (n=174)*	4.0	2.28-6.86	<0.001	2.9	1.57-5.21	0.001
COPD, *severe (n=28)*	14.1	5.56-35.77	<0.001	10.0	3.84-26.30	<0.001
COPD, *exacerbations <2 (n=375)*	3.0	1.90-4.69	<0.001	2.3	1.41-3.71	0.001
COPD, *exacerbations ≥2 (n=27)*	5.1	1.62-16.23	0.005	4.4	1.41-14.04	0.011
COPD, *group A (n=199)*	1.8	0.91-3.47	0.090	1.5	0.74-3.00	0.264
COPD, *group B (n=152)*	3.9	2.20-6.86	<0.001	2.9	1.58-5.34	0.001
COPD, *group C (n=17)*	1.6	0.19-12.95	0.668	1.4	0.17-11.20	0.767
COPD, *group D (n=32)*	11.1	4.54-27.29	<0.001	8.3	3.25-20.96	<0.001

Model 1: age and sex adjusted.
Model 2: adjusted for age, sex, pack years and comorbidity count.
COPD was defined as $FEV_1/FVC < 70\%$ and categorized according to the modified Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification of airflow limitation 2007[24] into mild COPD (GOLD1; $FEV_1 \geq 80\%$pred), moderate COPD (GOLD2; $50\% \leq FEV_1 < 80\%$pred) & severe COPD (GOLD3; FEV1<50%pred) and according to the updated GOLD group categorization 2013[1] A (low risk, less symptoms), B (low risk, more symptoms), C (high risk, less symptoms) and D (high risk, more symptoms).
Abbreviations: OR= Odds Ratio; CI= Confidence Interval; COPD= Chronic Obstructive Pulmonary Disease

Table 2 demonstrates that the prevalence of frailty was significantly increased in subjects with COPD, even after adjustment for age, sex, pack years of smoking and the comorbidity count (OR 2.4, 95%CI 1.52-3.86, p<0.001). When classified according to severity of airflow limitation, severe COPD subjects had a tenfold increased risk of frailty compared to subjects with a normal lung function. Subjects with mild COPD were not significantly more frequently frail. However, they were more frequently pre-frail, defined as having one or two frailty characteristics (p=0.048, adjusted for age and sex). Since exacerbations and respiratory symptoms contribute to the morbidity and mortality in COPD, we categorized subjects with COPD according to the updated GOLD group categorization into 199 (49.8%) group A, 152 (38.0%) group B, 17 (4.3%) group C and 32 (8.0%) group D (GOLD 2011).[1] When classified according to the updated GOLD group categorization, the odds ratio for frailty was highest in COPD subjects belonging to GOLD group D. The frailty prevalence was not significantly higher for subjects belonging to GOLD group A and C, compared to subjects without COPD. Finally, frequent COPD exacerbators showed a 4.4 fold increased risk to be frail compared to subjects without COPD (OR 4.4, 95%CI 1.41-14.04, p<0.011; *Table 2*).

COPD, comorbidities, drug-use and frailty

We performed a stepwise regression analysis with backward elimination including age, sex, smoking status, pack years of cigarette smoking, BMI, COPD, current use of diuretics, beta-blockers, calcium channel blockers, drugs acting on the renin-angiotensin system, drugs for obstructive airway diseases and current and cumulative use of oral corticosteroids, and all lab values and individual comorbidities of *Table 1*. This resulted in a final model which is represented in *Table 3*. Importantly, COPD remained significantly associated with frailty, independent of age, sex, smoking pack years, cholesterol and hematocrit levels, stroke, osteoporosis, current beta-blocker and calcium channel blocker use, and cumulative use of corticosteroids (OR 2.2, 95% CI 1.34-3.54, p=0.002, *Table 3*).

Table 3: Final step of a backward regression model determining the most informative factors for frailty.

	OR	95% CI	P-value
COPD	2.2	1.34-3.54	0.002
Age	1.9	1.14-3.21	0.014
Female sex	1.1	1.11-1.19	<0.001
Cholesterol (mmol/l)	0.7	0.59-0.93	0.011
Hemoglobin (mmol/l)	0.7	0.52-0.96	0.025
Pack years	1.0	1.00-1.02	0.011
Stroke	3.0	1.54-5.94	0.001
Osteoporosis	1.9	1.10-3.22	0.021
Beta-blockers	0.5	0.28-0.84	0.010
Calcium channel blockers	1.9	1.02-3.53	0.044
Cumulative oral corticosteroids	1.0	1.01-1.06	0.008

Final step of a stepwise regression with backward elimination including age, sex, smoking status, pack years of cigarette smoking, body mass index, COPD, current use of diuretics, beta-blockers, calcium channel blockers, drugs acting on the renin-angiotensin system, drugs for obstructive airway diseases and current and cumulative use of oral corticosteroids, hemoglobin (mmol/l), white blood cell count (count/mm^3), cholesterol (mmol/l), glucose (mmol/l), creatinin (µmol/l), coronary disease, heart failure, hypertension, stroke, diabetes, osteoporosis, cancer, anemia and kidney disease.

DISCUSSION

This is the first general population study showing that subjects with COPD have a more than twofold increased risk to develop frailty, independent of age, sex, smoking, systemic corticosteroid use and comorbidities. We demonstrated that the association was stronger in COPD subjects with severe airflow limitation and moreover with frequent exacerbations and more symptoms (GOLD group D). The results further suggest that with increasing COPD severity of airflow limitation more intermediate frail elderly become frail, while the prevalence of subjects without any of the frailty criteria does not change substantially.

Our results demonstrating that COPD is independently associated with frailty are in line with previous suggestions of an association between frailty and respiratory impairment.[410, 411] Vaz Fragoso *et al.* have demonstrated that airflow limitation is associated with frailty, independent of age, gender, smoking history, height, BMI, health status and several chronic conditions.[411] In this cross-sectional and longitudinal cohort study, the researchers stated that the association between airflow limitation and frailty is likely due to COPD although subjects suggestive of a restrictive syndrome were also included. In addition, this study did not take into account corticosteroid use which has been associated with significant morbidity, including osteoporosis, falls, fractures, hyperglycemia, hypertension and muscle wasting, and mortality.[412-414] These side effects of systemic corticosteroids are all clinical characteristics related to frailty. Our study is the first to demonstrate that the use of corticosteroids, frequently prescribed in COPD, does not explain the association of COPD and frailty. Park *et al.* also have argued that frailty is prevalent in people with COPD.[410] In line with our results, those COPD subjects with shortness of breath were more likely to be frail. However, unlike the assessment of physical frailty criteria in our study, the used frailty index was primarily based on survey responses.[410] Furthermore, in this study an odds ratio of COPD and frailty has not been estimated. In our study, not only we have demonstrated a significant association of frailty and COPD, we also have shown that this association was stronger with increasing COPD severity. The percentage of prevalent frailty increased from mild, over moderate to severe COPD. However, the percentage of non-frail subjects did not differ significantly over the different groups. Therefore, it seems that intermediate frailty converts into frailty with increasing COPD severity. Fried *et al.* have found that intermediate frail elderly are at an increased risk of becoming frail.[38] Possibly, COPD places intermediate frail elderly at an even more increased risk of frailty. This could result in an aggravation of intermediate frailty towards frailty with increasing COPD severity. Elderly people without any of the frailty characteristics might be less susceptible to the negative impact of increasing COPD severity, because of sufficient physiologic reserves and thus a preserved resilience.[37]

The regulatory pathway responsible for the association between COPD and frailty has not yet been elucidated, but might involve impaired innate immune defenses.[13] Both frailty and COPD are furthermore associated with systemic inflammation as evidenced by increased levels of inflammatory markers, such as white blood cell (WBC) count, high-sensitivity C-

reactive protein (hsCRP), interleukin 6 (IL6) and tumor necrosis factor-α (TNF-α).[34, 37, 45-51, 415] IL6 in particular has been associated with anemia, decreased lean body mass and sarcopenia and is inversely correlated with circulating hormones like insulin-like growth factor-1 (IGF-I) and dehydroepiandrosterone sulfate (DHEA-S) in frail elderly.[47, 52, 53] Possibly, the subset of elderly subjects with COPD that have a persistent inflammation are particularly prone to develop frailty.[34]

The strengths of this study are the high quality and detail of medical information, the objective measurement of comorbidities and frailty, the general population based setting and the large number of elderly subjects that participated in the Rotterdam Study. Possible limitations include the cross-sectional design implying that we cannot infer causal mechanisms between COPD and frailty. However, the association is biologically plausible and the risk of frailty increased according to the severity of COPD. The identification of frail COPD patients could be of importance to identify those COPD subjects at high risk of mortality. Especially in COPD subjects with mild airflow limitation, preventive measures might be required to prohibit them of becoming frail, a state wherein adverse health events are inevitable.

In conclusion, our results suggest that COPD is associated with frailty independent of age, sex, smoking and comorbidities. Potentially, a more holistic approach is required to improve life of patients with COPD.

ABSTRACT

Background

Gait disturbances are a potential systemic manifestation of chronic obstructive pulmonary disease (COPD), which may lead to disability and falls in patients with COPD. Although walking endurance tests are extensively performed in patients with COPD, studies assessing the gait pattern including gait kinematics, are sparse.

Objectives

To investigate associations of COPD and lung function parameters with various gait domains and to explore a potential link with falling.

Methods

Cross-sectional analysis within the Rotterdam Study, a prospective population-based cohort study in persons aged ≥ 55 years. Spirometry was used to assess lung function and confirm the diagnosis of COPD. Gait was measured using an electronic walkway and summarized into seven gait domains: Rhythm, Phases, Pace, Base of Support, Tandem, Turning, and Variability.

Results

Persons with COPD (n = 196) exhibited worse Rhythm (z-score -0.21, 95%CI: -0.36; -0.06) compared to persons with normal lung function (n = 898) independent of age, sex, height, primary education, smoking and use of analgesics. This effect was most pronounced in COPD persons with dyspnea and severe airflow limitation or frequent exacerbations (GOLD D, -0.83 SD, 95%CI: -1.25; -0.41). Similarly, all lung function measurements except FVC associated with Rhythm. Additionally, FEV_1 associated with Pace, FVC with Pace and Phases, FEV_1/FVC with Phases, and DLCOc with Pace and Turning. Fallers with COPD had significantly worse Rhythm compared to non-fallers with COPD, fallers without COPD, and non-fallers without COPD.

Conclusions

This study demonstrates that people with COPD have worse Rhythm, especially fallers with COPD. Additionally, impaired lung function parameters and frequent exacerbations associated with worse gait. These results suggest that COPD may affect gait quality in addition to walking endurance.

14. Gait patterns in COPD

INTRODUCTION

Worldwide, COPD is a leading cause of morbidity and mortality.[1] COPD is primarily characterized by persistent airflow limitation that is associated with an abnormal inflammatory response of the lungs to noxious particles or gases.[13] However, its systemic effects also have an important impact on symptoms and prognosis.[1] Even in COPD patients with mild disease severity, cardiovascular comorbidities and muscle weakness precede the development of functional limitations.[178] Since COPD also affects function and mobility, persons with COPD have an increased risk of falling.[177, 178, 359, 416-418] Falling is a major health concern, representing one of the main causes of pain, disability and death in the elderly.[417, 419] Even without injuries, a fall can negatively impact the quality of life of an individual through fear of falling, which often inhibits performing activities.[420]

Poor gait is one of the main risk factors of falls and an important indicator of general health.[421-423] Gait is affected by various organ systems, such as the central nervous system, cardiovascular system, and musculoskeletal system, and poor gait is a strong risk factor of death.[424-428] COPD may affect gait through any of these systems by inducing hypoxia or systemic inflammation.

Gait is a complex concept that can be assessed using many parameters, which can be summarized into seven gait domains.[427] As of yet, COPD has mostly been associated with a shorter walking distance. Associations of COPD and lung function parameters with specific gait domains may give an indication of the pathways underlying their associations with gait. In turn, this knowledge may aid in identifying better intervention strategies to prevent future falling in patients with COPD.

The aim of this study was to investigate the associations of COPD and lung function parameters with various gait domains, in a community-dwelling population. Moreover, we evaluated the influence of COPD severity by airflow limitation, symptoms and/or frequent exacerbations. Additionally, we explored a potential link between gait differences in COPD and falling.

METHODS

Study design

The present study is embedded within the Rotterdam Study (RS), a population based cohort study aimed at assessing the occurrence and risk factors of chronic diseases in the elderly.[108] The study was initiated in 1990 (RS-I), when all inhabitants aged ≥55 of the suburb Ommoord, in Rotterdam, were invited to participate. This study was extended in 2000 (RS-II) and another time in 2006 (RS-III), this last time inviting all inhabitants aged ≥45. At baseline and every 3 to 4 years of follow-up participants undergo a home interview and medical

examinations at the research centre, including spirometry. From March 2009 onwards, gait assessment has been implemented in the core protocol.[108] The home interview included standardized questionnaires on smoking including cumulative smoking history and history of falls in the past 12 months. The present study comprises all participants from the first two cohorts (RS-I and RS-II) who completed gait assessment and spirometry successfully until December 2011. The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. A written informed consent was obtained from all participants.

Assessment of lung function parameters and COPD

Spirometry and diffusing capacity were performed using a Master Screen® PFT Pro (CareFusion, San Diego, CA) by trained paramedical personnel according to the ATS/ERS guidelines.[22, 23] The diagnosis of COPD was based on an obstructive spirometry examination according to the modified Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria [proportion of the forced vital capacity exhaled in the first second (FEV1/FVC) < 70%] and classified into mild and moderate/severe airflow limitation by forced expiratory volume in one second (FEV1)% predicted of ≥ 80%, <80% respectively.[24, 232] Furthermore, participants with COPD were also classified according to the updated GOLD group categorization into group A (low risk, less symptoms), B (low risk, more symptoms), C (high risk, less symptoms) and D (high risk, more symptoms).[1] Exacerbations were counted as the total number of moderate and severe exacerbations in the year 2010. Frequent exacerbators were defined as COPD persons having at least two moderate or severe exacerbations.

Assessment of gait

Gait was assessed with a 5.79 m long electronic walkway with 1.27 cm wide pressure sensors (4.88 m active area; GAITRite Platinum; CIR systems Inc., Sparta, New Jersey USA).[427] The walkway checked for activation of the pressure sensors at 120Hz. The standardized gait protocol includes three different walking conditions *(Figure 1)*: normal walk, turn and tandem walk. In normal walk, participants were asked to walk at their usual pace across the walkway, until they were of the walkway. The normal walk was performed four times in both directions (eight recordings). In turn, participants were asked to walk at their usual pace across the walkway, turn halfway and return to their starting position (one recording). In tandem walk, participants were asked to walk tandem (heel-to-toe) over a line visible on the walkway until they were off the walkway (one recording). The first recording of the normal walks was considered a practice walk and not included in the analyses.

All gait parameters from the normal walk were automatically quantified by the walkway software. Parameters were quantified for both steps and strides, e.g. step length is the distance between two opposite feet on the line of progression, while stride length is the distance between two feet of the same side on the line of progression. Variability measures were quantified as standard deviations of the gait parameters among steps or strides.

Turning time was calculated from the walkway generated data as the time between foot off of the first foot and first contact of the last foot (Figure 1). Turning step count was calculated as the automatically quantified number of steps in the turn minus one. Errors in tandem walking were calculated as the distance of sidesteps from the line and the surface of these sidesteps, with aid of the automatically generated surface and distance parameters from the walkway software. Additionally, we scored the number of double steps, i.e. two consecutive steps with the same foot on the line, as errors in tandem walking.

As previously described, principal components analysis was used to summarize 30 gait parameters, including 25 from normal walking, 2 from turn and 3 from tandem walking, into fewer independent gait domains, while capturing the largest amount of variance. Each gait domain had to explain at least as much variance as a single gait parameter. Varimax rotation was used to ensure that the gait domains were mutually independent. We found seven independent gait domain: Rhythm, Variability, Phases, Pace, Tandem, Turning and Base of Support.[427] Among others, Rhythm reflects cadence and stride time; Variability reflects stride length and time variability; Phases reflects double support time and double support as a percentage of the gait cycle; Pace reflects stride length and velocity; Tandem reflects errors in tandem walking; Turning reflects the number of turning steps and turning time; and Base of Support reflects stride width and its variability. Finally, Global Gait was calculated by summing all gait domains, dividing by the number of gait domains, and subsequently calculating a new z score.[425] To facilitate interpretation of our findings, we additionally included the two most commonly assessed gait parameters, velocity and cadence, and the highest constituting gait parameters of the three domains strongest associated with COPD and lung function: single support time for Rhythm, stride length for Pace, and single support percentage for Phases. Single support time reflects the absolute time (in seconds) that a person supports on one leg during one stride, while single support percentage reflects the relative time (in %) that a person supports on one leg during one stride. For interpretation, lower velocity, cadence, stride length and single support percentage is considered worse gait, while lower single support time is considered better.

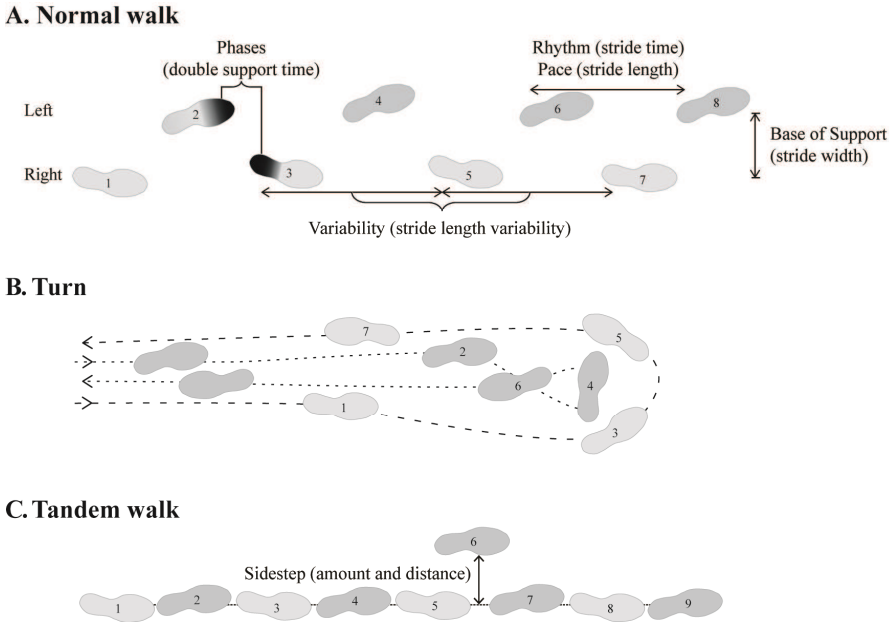


Figure 1: The three walking conditions, including the five gait domains of normal walking. The gait domains of normal walking are visualized using constituting gait parameters, which are mentioned in parenthesis. For turn, only the feet used in the calculation of turning time and turning step count are numbered. Turning time was calculated as the time between foot off of foot 1 until first contact of foot 7. Turning step count was calculated as the number of steps in the turn minus one. In this figure, turning step count would be five.

Statistical analyses

Differences between persons with and without COPD were studied using Mann-Whitney U test for continuous variables and Chi-Square test for categorical variables. Univariate ANCOVAs and linear regression analyses were used to investigate associations of COPD and lung function respectively, with the gait domains. Age, sex, height, weight, primary education, MMSE, pack years and use of analgesics (N02) within 90 days preceding gait assessment were considered as potential confounders and models were adjusted for those showing trends of an association with both COPD and gait ($p<0.10$). Models including tandem walk were additionally adjusted for step count and mean step length. We repeated these analyses to investigate associations of lung function and COPD with velocity, cadence, stride length, single support time and single support percentage. For gait domains that associated with COPD, we further investigated an association with falls by univariate ANCOVAs. Since cognition has been shown to be very strongly associated with gait, we performed a sensitivity analysis repeating all analyses additionally adjusted for MMSE. In this way we could whether any of our associations were the result of residual confounding by cognition. Statistical analyses were performed using SPSS, version 20.0 for Windows (IBM, North Castle, NY).

RESULTS

Between March 2009 and December 2011, 1791 participants were invited for gait assessment. Of these, 199 participants did not perform all walking conditions for the following reasons: 152 for physical inability, 36 for technical reasons, and 11 for refusal. Of remaining 1592 participants, 182 had to be excluded for technical reasons, 26 for completing less than 16 steps in the normal walks, lowering validity of the measurements[429], 7 for not following instructions, and 2 for using walking aids. Of the 1375 participants with complete and valid gait data, 1247 had interpretable spirometry data available. Of these, participants with a restrictive spirometry (n=46) or asthma (n=107) were excluded. Baseline characteristics of the study population (n=1094) are presented in *Table 1.* Among participants, 196 (17.9%) persons had COPD (96 mild COPD and 100 moderate to severe COPD). COPD persons were slightly older, less often female, smoke(d) more frequently and presented with worse lung function parameters.

We found COPD to associate with lower Rhythm (-0.21 SD [95%CI: -0.36; -0.06], *Table 2*) independent of age, sex, height, primary education, pack-years of cigarette smoking and use of analgesics. Regarding the lung function parameters, higher FEV_1 associated significantly with higher Global Gait, Rhythm and Pace; higher FEV_1/FVC with higher Rhythm but lower Phases.(*Table 2*) Additionally, higher FVC associated significantly with higher Global Gait, Phases and Pace; higher diffusing capacity $DL_{CO,c}$ with higher Global Gait, Rhythm and Pace but lower Turning; and higher $DL_{CO,c}$/Va with higher Rhythm.(*Table 2*)

Regarding the influence of disease severity, COPD persons with moderate/severe airflow limitation significantly related to lower Rhythm compared to persons without COPD.(*Table 3*) Both COPD with and without frequent exacerbations associated with lower Rhythm, with the effect size for COPD with frequent exacerbations being four times larger than for COPD without frequent exacerbations (-0.65 SD [95%CI: -1.04; -0.27] compared to -0.16 SD [95%CI: -0.31; 0.00] respectively, *Table 3*).

Figure 2 represents the gradual decrease of the age and sex-adjusted Z-scores of walking Rhythm over the GOLD 2011 categories. GOLD group C and D were significantly related to lower Rhythm and GOLD group D related moreover to lower Global Gait score and Pace compared to persons without COPD.(*Table 3*) In contrast, GOLD group A associated with higher Global Gait and Pace.

234 (21.4%) participants reported that they had fallen in the past 12 months, including 195 (21.7%) subjects without COPD versus 39 (19.9%) subjects with COPD ($p_{difference}$=0.564). *Figure 3* shows Rhythm to be lowest in COPD persons who fell in the previous year. Fallers with COPD had significantly lower Rhythm than fallers without COPD (p=0.004), non-fallers with COPD (p=0.022), and non-fallers without COPD (p<0.001), after adjustment for age and sex. These differences remained significant after additional adjustment for height, primary education, pack-years of cigarette smoking and use of analgesics.

We found a very similar pattern of associations for the original gait parameters as for the gait domains. COPD was associated with a lower cadence and longer single support time. GOLD group A associated with higher velocity, stride length and single support percentage.

GOLD groups C and D associated with lower cadence and longer single support time and group D additionally with lower velocity. With regard to lung functions, we found higher FEV_1 to associate with higher velocity and cadence, shorter single support time and larger stride length; higher FEV_1/FVC to associate with shorter single support time but also shorter single support percentage; higher $DL_{CO,c}$ with higher velocity and cadence, shorter single support time and larger stride length; and higher $DL_{CO,c}$/Va to associate with higher cadence and shorter single support time. When additionally adjusting for MMSE, our associations hardly changed. Only the association of COPD without frequent exacerbations became borderline non-significant (p=0.055).

Table 1: Baseline characteristics of the study population.

Characteristic	No COPD (n = 898)	COPD (n = 196)	p-value[a]
Age [years]	74.2 (5.2)	75.5 (5.5)	0.003
Sex [females]	483 (53.8)	76 (38.8)	<0.001
Height [cm]	167.3 (8.9)	170.5 (8.9)	<0.001
Weight [kg]	76.2 (12.7)	80.8 (18.7)	0.877
Body Mass Index [kg/m²]	27.2 (3.7)	26.6 (3.7)	0.049
Primary education [n]	89 (10.1%)	33 (17.1%)	0.013
MMSE-score [points]	27.9 (1.8)	27.8 (1.9)	0.957
Past smoker [n]	508 (56.6%)	125 (63.8%)	<0.001
Current smoker [n]	67 (7.5%)	35 (17.9%)	<0.001
Pack-years [pack-years]	12.6 (18.3)	25.2 (25.9)	<0.001
Analgesics [n]	37 (4.1%)	14 (7.1%)	0.051
Velocity [cm/s]	118.2 (18.3)	115.1 (19.1)	0.122
FEV1 [%]	110.9 (16.3)	80.8 (18.7)	<0.001
FVC [%]	111.9 (16.7)	99.2 (20.9)	<0.001
FEV_1/FVC [%]	78.6 (4.7)	63.4 (6.0)	<0.001
$DL_{CO,c}$ [%][b]	97.5 (15.3)	89.1 (18.5)	<0.001
$DL_{CO,c}$/Va [%][b]	111.6 (17.2)	105.3 (21.1)	<0.001

Categorical variables are expressed as numbers (percentage). Values of continuous variables are expressed as mean (standard deviation). [a] p-values for differences between participants with COPD and without COPD, adjusted for age and sex (if applicable). [b] Total n = 942.
Abbreviations: COPD=Chronic Obstructive Pulmonary Disease; $DL_{CO,c}$ = diffusing capacity of the lungs measured using carbon monoxide and corrected for the hemoglobin concentration, FEV_1= forced expiratory volume in one second, FEV_1/FVC= proportion of the forced vital capacity exhaled in the first second, MMSE = mini–mental state examination, Va= alveolar volume.

Table 2: Associations between COPD, lung function and the gait domains, adjusted for potential confounders.

	COPD	FEV_1 (/10%)	FEV_1/FVC (/10%)
Global gait	0.02 (-0.14; 0.17)	**0.05 (0.03; 0.08)**	-0.01 (-0.09; 0.07)
Rhythm	**-0.21 (-0.36; -0.06)**	**0.04 (0.01; 0.07)**	**0.11 (0.04; 0.19)**
Variability	0.11 (-0.05; 0.27)	0.00 (-0.03; 0.03)	-0.04 (-0.13; 0.04)
Phases	0.12 (-0.03; 0.28)	0.01 (-0.02; 0.04)	**-0.10 (-0.18; -0.02)**
Pace	-0.05 (-0.19; 0.08)	**0.06 (0.04; 0.09)**	0.02 (-0.04; 0.09)
Tandem[a]	0.00 (-0.16; 0.15)	0.03 (0.00; 0.06)	0.05 (-0.03; 0.13)
Turning	0.03 (-0.13; 0.20)	0.00 (-0.03; 0.03)	-0.01 (-0.10; 0.07)
Base of Support	0.04 (-0.12; 0.20)	-0.01 (-0.04; 0.03)	-0.05 (-0.13; 0.04)
	FVC (/10%)	$DL_{CO,c}$ (/10%)	$DL_{CO,c}$/Va (/10%)
Global gait	**0.08 (0.04; 0.11)**	**0.05 (0.01; 0.08)**	0.02 (-0.02; 0.05)
Rhythm	0.03 (-0.01; 0.06)	**0.06 (0.03; 0.10)**	**0.06 (0.03; 0.10)**
Variability	0.01 (-0.02; 0.05)	0.01 (-0.03; 0.05)	0.02 (-0.02; 0.05)
Phases	**0.04 (0.00; 0.07)**	0.00 (-0.04; 0.04)	-0.03 (-0.07; 0.00)
Pace	**0.08 (0.05; 0.11)**	**0.07 (0.03; 0.10)**	0.03 (0.00; 0.06)
Tandem[a]	0.03 (-0.01; 0.06)	0.00 (-0.04; 0.04)	-0.01 (-0.04; 0.03)
Turning	0.01 (-0.03; 0.05)	**-0.05 (-0.09; -0.01)**	-0.03 (-0.07; 0.00)
Base of Support	0.00 (-0.03; 0.04)	0.02 (-0.02; 0.06)	0.01 (-0.03; 0.05)

Values represent differences in z-scores of gait (95% Confidence Interval). Values in bold survived thresholds of nominal significance ($p < 0.05$). A lower value of gait represents worse gait. All analyses were adjusted for age, sex, height, primary education, pack-years and use of analgesics.

[a] Additionally adjusted for the step count and step size within the tandem walk.

Abbreviations: COPD= Chronic Obstructive Pulmonary Disease; FEV_1= forced expiratory volume in one second; FEV_1/FVC = proportion of the forced vital capacity (FVC) exhaled in the first second; $DL_{CO,c}$ = diffusing capacity of the lungs measured using carbon monoxide and corrected for the hemoglobin concentration, Va= alveolar volume.

Table 3: Stratified analyses of the associations between COPD and the gait domains, adjusted for potential confounders.

	Mild COPD	Moderate/severe COPD	Infrequent exacerbator	Frequent exacerbator
Global gait	0.14 (-0.06; 0.34)	-0.11 (-0.32; 0.09)	0.05 (-0.11; 0.21)	-0.23 (-0.63; 0.17)
Rhythm	-0.16 (-0.35; 0.04)	**-0.27 (-0.46; -0.07)**	**-0.16 (-0.31; 0.00)**	**-0.65 (-1.04; -0.27)**
Variability	0.06 (-0.15; 0.27)	0.17 (-0.04; 0.39)	0.09 (-0.07; 0.26)	0.26 (-0.16; 0.68)
Phases	0.06 (-0.14; 0.27)	0.18 (-0.03; 0.39)	0.12 (-0.04; 0.28)	0.13 (-0.28; 0.54)
Pace	0.06 (-0.11; 0.23)	-0.17 (-0.35; 0.00)	-0.03 (-0.16; 0.11)	-0.30 (-0.65; 0.05)
Tandem[a]	0.16 (-0.04; 0.37)	-0.18 (-0.39; 0.03)	-0.02 (-0.18; 0.15)	0.10 (-0.32; 0.51)
Turning	0.20 (-0.01; 0.42)	-0.14 (-0.36; 0.07)	0.04 (-0.13; 0.21)	-0.04 (-0.47; 0.39)
Base of Support	-0.04 (-0.25; 0.17)	0.12 (-0.09; 0.34)	0.05 (-0.11; 0.22)	-0.07 (-0.49; 0.36)
	COPD A	COPD B	COPD C	COPD D
Global gait	**0.26 (0.05; 0.46)**	-0.19 (-0.42; 0.04)	0.02 (-0.56; 0.61)	**-0.48 (-0.92; -0.05)**
Rhythm	-0.06 (-0.26; 0.13)	-0.21 (-0.43; 0.01)	**-0.73 (-1.29; -0.17)**	**-0.83 (-1.25; -0.41)**
Variability	0.11 (-0.11; 0.32)	0.08 (-0.17; 0.32)	0.32 (-0.30; 0.93)	0.15 (-0.31; 0.61)
Phases	**0.30 (0.09; 0.51)**	-0.10 (-0.33; 0.14)	0.33 (-0.26; 0.93)	-0.08 (-0.53; 0.37)
Pace	0.12 (-0.06; 0.29)	-0.18 (-0.38; 0.02)	-0.24 (-0.75; 0.27)	**-0.40 (-0.78; -0.02)**
Tandem[a]	0.02 (-0.19; 0.24)	-0.07 (-0.31; 0.17)	0.17 (-0.44; 0.78)	0.04 (-0.41; 0.50)
Turning	0.08 (-0.14; 0.30)	-0.01 (-0.26; 0.24)	-0.01 (-0.64; 0.62)	-0.01 (-0.48; 0.47)
Base of Support	0.11 (-0.11; 0.32)	-0.04 (-0.28; 0.20)	0.26 (-0.36; 0.88)	-0.13 (-0.59; 0.34)

Values represent differences in z-scores of gait (95% Confidence Interval). A lower value of gait represents worse gait. Values in bold survived thresholds of nominal significance ($p < 0.05$). All analyses were adjusted for age, sex, height, primary education, pack-years, and use of analgesics. [a] Additionally adjusted for the step count and step size within the tandem walk. COPD was defined as $FEV_1/FVC < 70\%$ and categorized according to the modified Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification of airflow limitation 2007 into mild COPD (GOLD1; $FEV_1 \geq 80\%$pred) or moderate/severe COPD (GOLD2&3; $FEV_1 < 80\%$pred) and according to the updated GOLD group categorization 2013. A (low risk, less symptoms), B (low risk, more symptoms), C (high risk, less symptoms) and D (high risk, more symptoms). Exacerbations were counted as the total number of moderate and severe exacerbations in the year 2010. Frequent exacerbators were defined as COPD persons having at least two moderate or severe exacerbations.
Abbreviation: COPD= Chronic Obstructive Pulmonary Disease

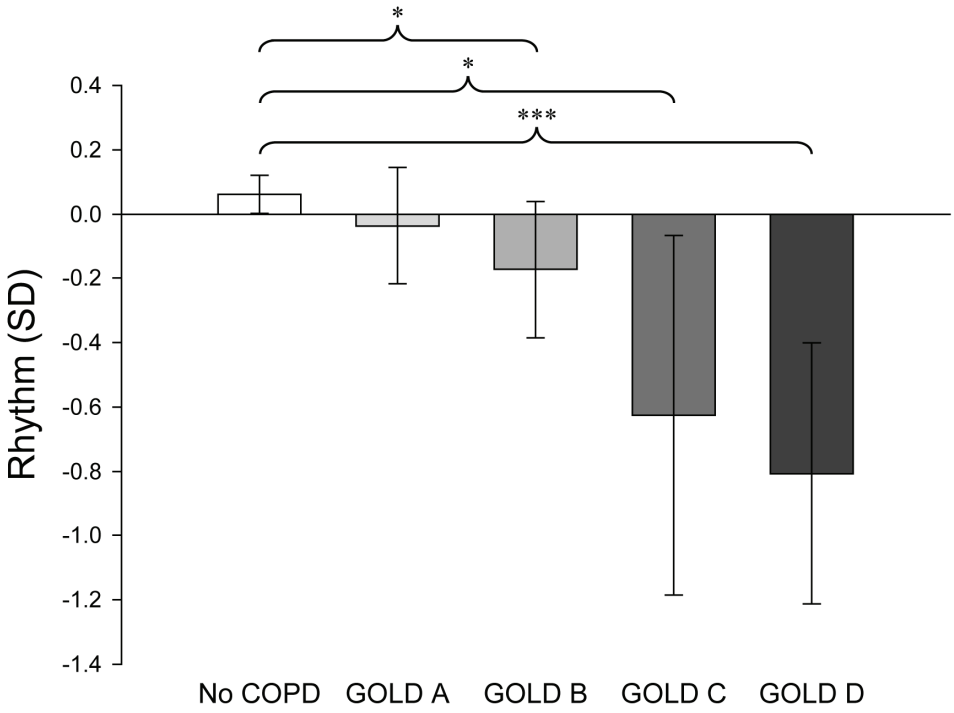


Figure 2: Means of walking Rhythm across the COPD GOLD 2011 categories, adjusted for age and sex. Error bars represent the 95% confidence interval.
* $p < 0.05$; ** $p < 0.005$; ***$p < 0.001$ by ANCOVA

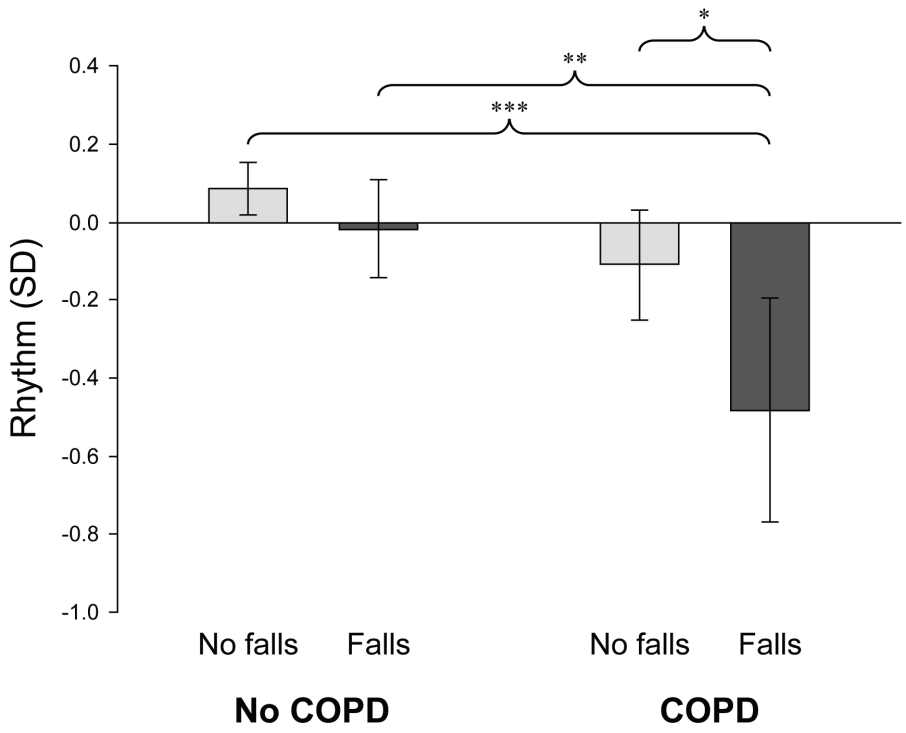


Figure 3: Means of walking Rhythm stratified for COPD and a history of falls, adjusted for age and sex. Error bars represent the 95% confidence interval.
* $p < 0.05$; ** $p < 0.005$; ***$p < 0.001$ by ANCOVA

DISCUSSION

In this large population based cohort study, we demonstrated that COPD and continuous lung function parameters associated with gait. We found COPD to be specifically associated with taking slower steps (lower Rhythm). Moreover, a clinical association between Rhythm and falls according to the COPD status, was observed. Additionally, we found better lung function in all parameters to associate with better gait, especially in Rhythm and Pace.

We are the first study to comprehensively investigate the associations of COPD and lung function parameters with the gait pattern. A lower walking distance during a six-minute walk or shuttle walk test, has been extensively reported in people with COPD; however, literature on gait velocity and other gait deficits is scarce.[418] Our results show that COPD is especially related with taking slower steps (lower Rhythm). This indicates that any slowing in gait velocity is mainly driven by a lower cadence instead of taking smaller steps. This notion was supported by the associations found with the original gait parameters. A possible explanation for this decrease in cadence may be an adaption mechanism to cope with impaired walking endurance due to lack of oxygen supply. Taking slower steps may decrease oxygen need of the leg muscles and thus allow for long distance walking in people with impaired lung function. The lower Rhythm might further result from systemic effects of COPD on the brain, cardiovascular system, muscle strength or bone mineralization.[141, 232, 418, 430-432] Beauchamp *et al.* found that gait deficits in patients with COPD were similar to patients with an elevated fall risk or neuromuscular disease.[359] The increased risk of falling in patients with COPD is thought to be caused by an inability to respond quickly in circumstances of instability.[359] Our finding of an especially low Rhythm in fallers with COPD may reflect this inability, because people who take steps slowly may also be slower at reacting to imbalances. However, further research is needed to determine whether this association of low Rhythm with a history of falling in people with COPD is also reflected in future falling.

The effect of COPD on Rhythm was especially strong in more severe COPD, which is in line with a recent study by Yentes *et al.*[433] Similarly, we found the severest COPD GOLD category to associate with worse Global Gait and Pace. In contrast, GOLD A was related to better Global Gait and Phases. These positive associations may be the result of a healthy selection effect, i.e. GOLD A represents the most healthy people with COPD who may have better gait than the general population.

Our study also demonstrates that continuous lung function parameters associate with gait. Less airflow limitation (i.e. higher FEV_1) was associated with better Global Gait, Rhythm, and Pace (larger steps). Less airflow obstruction (FEV_1/FVC) associated with better Rhythm, but worse Phases (more double support). The associations of higher FVC and lower FEV_1/FVC with better Phases may indicate that Phases is more related to restrictive lung disorders. A preserved diffusing capacity $_{DLCO,c}$ associated with better Global Gait, Rhythm and Pace, but

worse Turning (longer turning time). The latter result suggests that when the lungs transfer less oxygen to the blood and carbon dioxide from the blood, persons have a lower cadence, take smaller steps and more turning steps and time. When dividing the $DL_{CO,c}$ by the alveolar volume, it only remained significantly associated with Rhythm. We found very similar associations of lung function with the original gait parameters, supporting our suggestions.

The main strength of our study is that we for the first time investigated associations of different gait domains, including different walking conditions, with COPD and lung function in a community-dwelling population. Furthermore, we investigated the influence of disease severity – taking into account exacerbations and the updated GOLD categories besides the severity of airflow limitation- on the association of COPD with the gait domains and explored associations of the related gait domain with falling. To cope with confounding, all analyses in our observational study were adjusted for potential confounders that showed a trend of an association with both COPD and gait.[416, 427, 434]

A first potential limitation of this study is that persons with severe COPD and very poor lung function might be less likely to come to the research centre for gait assessment. However, if anything, this selective refusal of participation would have underestimated the strength of association of COPD and worse lung function with gait disturbances. Second, given the cross-sectional analysis and the retrospective interrogation of falls, we cannot infer causality. Consequently, the worse Rhythm in COPD patients may lead to more falls, or, falls in COPD patients may aggravate anxiety and unsecured gait leading to worse Rhythm.[416]

In conclusion, COPD associates with worse gait, especially with taking slower steps, in a community-dwelling population. Additionally, less airflow limitation, more forced vital capacity, and better oxygen diffusion capacity associate with better gait, as especially reflected by quicker and larger steps. Further research should investigate the underlying mechanisms of these associations, to enable development of proper intervention strategies to prevent falling in patients with COPD.

PART V: GENERAL DISCUSSION

COPD is a common and progressive chronic pulmonary inflammatory disease affecting millions of people worldwide. In addition to the degree of airflow limitation, the presence of symptoms, exacerbations and comorbidities has been increasingly recognized to have a large impact on the severity of COPD. Since comorbidities have a profound influence on the morbidity and mortality in patients with COPD, the identification of (early predictors of) comorbidities is crucial in the management of patients with COPD. Therefore, the main objective of this thesis was to identify and explore (novel) co-morbid conditions and systemic effects of COPD within the Rotterdam Study, a large population-based cohort study. In this general discussion, I will first summarize and discuss the most important findings. Second, I will highlight some of the methodological issues regarding our studies. Finally, I will conclude with potential implications of my research and some suggestions for future research.

MAIN FINDINGS

Systemic inflammation in COPD

The knowledge on COPD has evolved from a specific lung disease characterized by poorly reversible airflow limitation towards a multicomponent disease characterized by airflow limitation associated with an abnormal inflammatory response of the lungs, exacerbations and extrapulmonary effects that further impact the disease severity and prognosis.[1] A mosaic of processes contribute to the pathogenesis of COPD including variable gene expression, abnormal immune responses, influence of hormones and damaging effects of environmental factors.[435] During my PhD, I collaborated on several genome wide association studies to further elucidate the genetic susceptibility.[436-438] COPD research has largely focused on the damaging smoking effects as disease initiator. However, little is known on the underlying predisposing conditions explaining why COPD develops in only 20% of smokers and how it develops in never smokers, why the inflammation persists in COPD after smoking cessation and how COPD leads to comorbidities independent of smoking. The role of innate and adaptive immunity in these predisposing conditions is intriguing. Next to local airway inflammation, COPD is also associated with systemic inflammation and both correlate with each other, at least when measured during an exacerbation.[166] Relating to the unanswered questions regarding the predisposing conditions in COPD and the potential role of systemic inflammation herein, the aim in **chapter 5** was to investigate whether increased levels of systemic inflammatory markers (i.e. plasma levels of IL6) were causally related with the risk of developing COPD. Together with previous findings on hsCRP, our results do suggest that systemic inflammation is associated with COPD, but is not causally driving the pathogenesis of COPD.[61]

The systemic inflammation in COPD might be explained by a direct spill-over of pulmonary inflammation or by pathophysiologic changes due to COPD, including processes of increased

oxidative stress, hypoxia and hyperinflation. In addition, physical inactivity, obesity and comorbidities of COPD might contribute to systemic inflammation. Furthermore, the bone marrow as production site of inflammatory cells might be involved in the initiation or aggravation of systemic inflammation too.[266, 439] Regarding the bones, we demonstrated within the Rotterdam Study that low bone mineral density is associated with COPD mortality.[432]

Interestingly, elevated levels of systemic inflammatory markers are associated with an increased risk of future exacerbations and major comorbidities in subjects with COPD.[440, 441] Although the specific causal role of systemic inflammation herein requires further research, elevated levels of systemic inflammatory markers can have a clinically prognostic value. For example in **chapter 6**, we demonstrated that the cumulative survival of COPD patients with higher levels of a systemic inflammatory marker (i.e. serum hsCRP), was worse than for those COPD patients without an elevated hsCRP at baseline. Furthermore, we demonstrated in the same chapter that statin use was associated with a reduced mortality, particularly in COPD subjects having increased systemic inflammation at baseline. In addition to their lipid-lowering effects, statins also possess pleiotropic anti-inflammatory and immunomodulating properties, reducing levels of inflammatory markers such as CRP and IL6.[104, 107] Finally, in **chapter 7**, we observed that the risk of COPD frequent exacerbators on sudden cardiac death was modified by higher levels of systemic inflammation (i.e. serum hsCRP) at baseline. From part II of this thesis, we might thus conclude that increased levels of systemic inflammatory markers are present in a subgroup of subjects with COPD and may have a clinical prognostic value by identifying COPD subjects with an increased mortality risk. Overall, systemic inflammation is likely to be one mechanism underlying the development of cardiovascular comorbidities in COPD and frequent exacerbations might aggravate this inflammatory pathway.

Angiopathy in COPD

Angiopathy can be divided into two types, namely macro- and microangiopathy affecting large and small blood vessels, respectively. Because COPD has been associated with vascular dysfunction, we explored both angiopathies in chapters 9 and 10 respectively.

Regarding macroangiopahty, we investigated the presence of increased carotid intima-media wall thickness (IMT) on ultrasonography as well as the plaque content on Magnetic Resonance Imaging (MRI). Both measurements provide valuable information and may reflect different underlying mechanisms. The carotid IMT is an indicator of subclinical generalized atherosclerosis and a strong predictor of stroke as well of coronary heart disease and left ventricular hypertrophy.[442-445] We demonstrated in **chapter 9** that COPD subjects have a twofold increased risk of carotid artery wall thickening compared to controls with a normal lung function. Moreover, an increased carotid IMT was more prevalent in COPD subjects

with a higher degree of airflow limitation, clinical symptoms of dyspnea or chronic bronchitis.

The composition of the atherosclerotic plaque can be differentiated into vulnerable plaques versus more stable calcified plaques. Especially plaques with a lipid core are along with a thin fibrous cap more prone to rupture.[334] The MRI data from subjects with increased carotid IMT in our study, showed that plaques are more lipid-rich in COPD subjects compared to control subjects, and that lipid core plaques are also more prevalent in COPD subjects suffering from dyspnea. In contrast, effects on lipid core plaques were not as strongly modified by the degree of airflow limitation or chronic bronchitis as they were on an increased carotid IMT. Interestingly, COPD patients with increased systemic inflammation are more characterized by obesity, dyspnea and cardiovascular disease than by the presence of chronic bronchitis or increased airflow limitation.[34] Although numbers were lower in the MRI than ultrasound part of our study, we hypothesize from this observation that the systemic inflammation might play a bigger or more direct role in making plaques vulnerable than in initiating plaques. Mechanisms on how systemic inflammation might lead to vulnerable plaques are in detail discussed in an editorial by Sin and MacNee and presented in Part I, *Figure* 4 of this thesis.[266]

Regarding microangiopathy, we investigated the prevalence and incidence of cerebral microbleeds which are small areas of hypointensity on MRI. Cerebral microbleeds are markers of cerebral small vessel disease and thus indicative of specific underlying microscopic pathological changes in cerebral small vessels.[202] The findings from **chapter 10** indicate that the prevalence of cerebral microbleeds is higher in subjects with COPD compared to subjects with a normal lung function, even after adjustment for potential confounders such as smoking. Furthermore, we investigated the location of these cerebral microbleeds since the location appears to reveal the underlying disease mechanism. In particular, microbleeds in deep or infratentorial locations are suggestive of hypertensive or arteriolosclerotic microangiopathy, whereas those occurring in strictly lobar brain sites are indicative of cerebral amyloid angiopathy.[202, 204] We observed that cerebral microbleeds within subjects with COPD occurred more frequently in deep or infratentorial locations. In contrast, COPD was not significantly associated with microbleeds occurring in strictly lobar brain sites. This preferential location suggests that cerebral microbleeds in patients with COPD occur by arteriolosclerosis on the basis of hypertensive vasculopathy and lipohyalinosis.

It is important here to differentiate arteriolosclerosis used in chapter 10 from atherosclerosis used in chapter 9. The term “arteriolosclerosis” refers to microangiopathy caused by stiffening of small arteries and arterioles. This loss of compliance can be caused by increased protein deposition in the vessel wall, seen in hypertension or diabetes. Arteriolosclerosis is pathophysiologically different from atherosclerosis. Atherosclerosis is a process of fat depositing which over time leads to stiffening of the wall of large/middle arteries. To further

explore causality between COPD and deep or infratentorial microbleeds, we performed longitudinal analyses in participants who had two MRI brain scans and no cerebral microbleed at the time of the first MRI scan. Herein, we confirmed that COPD was significantly associated with an increased risk of developing deep or infratentorial microbleeds in the subsequent three years. Although the association between COPD subjects and deep or infratentorial microbleeds seemed to be more modified by an increased IMT than additionally by the presence of a lipid core, the association did not disappear after adding carotid artery wall thickening to the model. This suggests that although macro- and microangiopathy in COPD may share common risk factors, treating generalized atherosclerosis may not entirely prevent arteriolosclerosis in patients with COPD.

Comorbidities in COPD

Although COPD is a chronic disease of the lungs which eventually can lead to death from respiratory failure, the majority of COPD patients suffers and dies from comorbidities before they reach end stage lung diseas. In **chapter 6**, we observed that most COPD participants died due to cardiovascular causes. The protective effect of statins on the all cause mortality in COPD observed in this chapter, might therefore represent effects on the cardiovascular comorbidities. However, our results also suggest that statin use affected respiratory mortality, potentially by downregulating the expression of CCL2, CXCL8 and the matrix metalloproteinases (MMPs) involved in COPD matrix remodelling, such as MMP2, MMP9 and MMP12.[114-117] In **chapter 7**, we demonstrated that COPD is an independent risk factor for sudden cardiac death in the general population. These observations are in line with the previous observed increased cardiovascular morbidity and mortality risk, and with results from studies in specific patient populations at high risk of sudden cardiac death.[131, 132, 135-138, 154-156]

Since both carotid artery plaques and cerebral microbleeds are associated with cerebrovascular morbidity, we dedicated **chapter 8** to a poorly explored field of research, namely that of interactions between the lung and the brain in the elderly. Although neurological disorders have a profound impact on wellbeing and functional capacity, their assessment in patients with COPD is not common practice. However, several central nervous system disorders have been described in relation to COPD. Since current techniques make it possible to even visualize early markers of central nervous system disorders, future research might bridge the current knowledge gaps regarding the potential underlying mechanisms.

Carotid artery plaques and cerebral microbleeds are associated with ischemic and hemorrhagic stroke respectively.[446] Consequential to our results and mechanistic insights from chapters 9 and 10, we explored an association between COPD and stroke subtypes in **chapter 11**. Herein, we showed that subjects with COPD have an increased risk of stroke during follow-up. We found age and sex independent associations with both ischemic and

hemorrhagic stroke. Smoking had again an important influence and the relationship between COPD and stroke was not independent of the effects of smoking. Interestingly, the association between COPD and incident hemorrhagic stroke was still borderline significant after adjusting for smoking and we found for ischemic stroke larger associations in persons with cortical stroke or strokes due to large artery atherosclerosis. Overall, we conclude from this part that (I) COPD is associated with both subclinical macro- and microvascular disease in the cerebral circulation, and (II) that smoking amplifies risks and is most likely crucial in developing clinical events as stroke.

Cachexia and skeletal muscle weakness are two other important comorbidities in COPD.[265, 447] This has driven us to further explore frailty in patients with COPD. Because of a global interest in healthy aging, the prevention of frailty among elderly gets increasing attention. Since frailty was not yet explored within the Rotterdam Study, we identified physically frail subjects and estimated their prevalence in **chapter 12** of this thesis. The observed frailty prevalence (5.8%) was in line with literature increasing the generalizability of the Fried frailty criteria.[38] Furthermore, frail elderly were more likely to be older and female, to have worse quality of life, more falls and hospitalizations and an increased risk of dying within three years. Frail subjects had also more comorbidities including COPD.

Intriguingly, both frailty and COPD are associated with decline in function across multiple systems that in composite contribute to geriatric syndromes, including osteoporosis, cognitive decline, anemia, immune deficiency, weight loss and muscle dysfunction. In line with studies suggesting an association between frailty and respiratory impairment, we demonstrated that COPD was significantly associated with frailty in **chapter 13**.[410, 411] Since osteoporosis and muscle wasting are also known side effects of systemic corticosteroids and since corticosteroids are frequently prescribed in COPD, we added oral corticosteroid use together with other potential confounders to our models. COPD remained significantly associated with frailty, even after adjusting for age, sex, smoking, dyslipidemia, anemia, stroke, osteoporosis, antihypertensive use, and cumulative use of systemic corticosteroids. These results suggest that COPD is linked with frailty beyond common comorbidities or the use of systemic corticosteroids. Systemic inflammation may underlie this association since both frailty and COPD subjects present with increased levels of inflammatory markers, such as white blood cell count, hsCRP, IL6 and tumor necrosis factor-α.[34, 37, 45-51, 415] In turn, inflammation and oxidative stress have synergistic effects on muscle breakdown.[265] Importantly, the results suggest that with increasing degree of airflow limitation more intermediate frail elderly become frail, while the prevalence of COPD subjects without any of the frailty criteria did not change substantially across the stages. This might imply that prevention of frailty should focus on mild-to-moderate COPD subjects.

In **chapter 14**, we explored whether devastating effects of COPD on the cardiovascular system, central nervous system, and musculoskeletal system observed in previous chapters

might have an impact on the gait pattern. COPD appeared to be specifically associated with taking slower steps (lower Rhythm) and an association between gait Rhythm and falls according to the COPD status was observed. However, the cross-sectional analysis and the retrospective interrogation of falls did not allow us to conclude whether the worse Rhythm in COPD patients leads to more falls, or conversely, whether falls in COPD patients aggravate anxiety and unsecured gait leading to worse walking Rhythm.[416] Additionally, we found better lung function in all parameters to associate with better gait, especially with Rhythm and pace. From this part, we might conclude that interactions between the lung and the brain, cardiovascular, and musculoskeletal system might result in observable deficits in physical functioning.

METHODOLOGICAL CONSIDERATIONS

Study setting and design

All studies described in this thesis were embedded within the Rotterdam Study (RS), a large prospective population-based cohort study aimed at assessing the occurrence of, and risk factors for chronic diseases in the elderly.[20] The Rotterdam Study (RS) has multiple strengths including the large number of participants and continued prospective follow-up, making this study very well suited to investigate most of our COPD research questions. All inhabitants of one district of Rotterdam (Ommoord) were invited to the original cohort in 1990 and the response rate was high (78%).[20, 21] The sole inclusion criteria was the age, i.e. 55 years or older for RS-I and RS-II, 45 years or older for RS-III. Participants were recruited irrespective of their disease status and the majority of elderly chronic diseases were diagnosed during follow-up. Selection bias was thus avoided as much as possible.

Assessment of COPD

Previously, 928 (11.6%) subjects with COPD were identified in RS-I. **Chapter 5** and **6** describe studies based on these COPD patients. During my PhD, I further identified incident COPD cases in RS-I and all prevalent and incident cases within RS-II and RS-III. Until January 1st 2011, 1615 (10.8%) subjects with COPD and without asthma or asthma-COPD Overlap Syndrome (ACOS) were identified within the three cohorts of the RS. **Chapters 7** and **11** include all identified COPD cases. Controls were subjects without COPD or asthma or ACOS during follow-up. The diagnosis of 883 subjects was based on an obstructive lung function measurement at the research centre, 487 (55.2%) participants of them had moderate to severe airflow limitation and 396 (44.8%) participants had mild airflow limitation. The manuscripts investigating the association between COPD and angiopathy, frailty and gait included only subjects who recently performed an interpretable spirometry at the research centre (**chapters 9,10,13,14**). Subjects with an obstructive lung function measurement were cases, subjects with a normal lung function were controls. Subjects with a restrictive lung function measurement, asthma or ACOS were excluded.

The diagnosis of the remaining 447 and 285 subjects was based on a validated diagnosis by the pulmonologist or general practitioner (GP) respectively based on the combination of clinical history, physical examination, and spirometry. We evaluated whether the exclusion of COPD patients diagnosed by GP affected our results substantially in **chapter 6**, but it did not. Pharmacy dispensing data, questionnaires on respiratory symptoms, hospital discharge letters and mortality reports were used next to lung function tests for continued case-finding. During my PhD, I also identified frequent COPD exacerbators by validating moderate exacerbations, i.e. exacerbations of COPD treated with antibiotics and/or systemic corticosteroids, and severe exacerbations, i.e. hospitalizations due to exacerbations of COPD during the complete follow-up.

Since the spirometry at the research centre was only introduced in the study protocol since the fourth cross-sectional round of RSI, we might have missed some subjects with clinically silent but spirometrically identifiable COPD in the beginning, underestimating the incidence of COPD in RS-I in the nineties. Other limitations might be that reversibility tests were not conducted (although we identified and excluded all asthma subjects from our analyses) and that the simple, generally recommended fixed ratio was used to diagnose COPD instead of the lower limit of normal (LLN). Although the fixed ratio might overestimate COPD in healthy elderly, subjects identified with the fixed cut-off but not with LLN do have poorer prognosis.[448, 449]

Assessment of comorbidities

All participants are very meticulous monitored for the onset of diseases and carefully examined at the research centre with non-invasive state of the art techniques.[20] This thesis was accomplished through the fruitful cooperation with doctors and researchers from different disciplines and departments, who are all experts in their field. The specific assessment of the relevant comorbidities are discussed in detail in the respective chapters. The prospective independent data collection has the advantage that our research hypotheses did not impact data collection, preventing information bias. A downside might be that one is restricted to the questionnaires, examinations and collections which were part of the study protocol in the past. However, some tests are too invasive or time-consuming and investigating everything would be impossible for the participants or budget holders, and might limit the researcher's creativity in finding answers.

Bias and confounding

Despite our best efforts to minimize biases through high response rates, independent prospective data collection and adjustment for known confounders, the validity of our studies may still to some extent have been affected by selection bias, information bias and confounding. Details are given in the respective chapters, however, I like to present here a general discussion regarding bias and confounding with respect to my thesis.

First, some selection might have occurred by those participants who declined the invitation to participate to the Rotterdam study for unknown reasons. Since most of COPD onset occurred during follow-up and COPD incidence rates within the Rotterdam Study are in line with general figures, it is unlikely that reasons were related to COPD.

Second, some information might have been missed on our investigated exposures and outcomes. However, information on dispensed prescriptions for medication exposure is gathered prospectively and automatically, and COPD and the described comorbidities were very meticulous assessed. Differential misclassification of the outcome was reduced as the outcomes under analyses were collected independently of the exposure of interest, mostly COPD. However, we have to acknowledge that few deaths occurred under extensive monitoring or were autopsied, that radiation exposure has been limited and contrast material has not been administered, that sometimes participation to tests at the research centre or questionnaires was refused and that all tests are limited by their sensitivity and specificity. Because non-differential misclassification or misclassification by underestimation are generally thought to underestimate true effects, associations with COPD may be rather higher than we have found.

Third, some unknown or unmeasured confounders might have attenuated our associations. Potential confounding is inherent to observational study designs and COPD and the described comorbidities frequently share major risk factors as old age and smoking history. COPD is moreover a very heterogeneous disease associated with accelerated aging and multiple comorbid conditions. Although we carefully evaluated the influence of potential confounders and appropriately adjusted models, residual confounding might have been present.

Causal inference

Some studies in this thesis were limited to a cross-sectional design which makes it difficult to judge the sequence of cause and effect. Therefore, we cannot infer causal mechanisms between COPD and carotid artery plaques, nor between COPD and frailty and gait respectively. There might exist a phenotype more sensitive to smoking, simultaneously developing cardiovascular, cerebral, musculoskeletal and pulmonary abnormalities. Although longitudinal studies are necessary to further explore the temporal sequence of association, we did observe some biological gradient since the associations were stronger along with the COPD severity and we presented several potential biological mechanisms in the concerning chapters.[450] Furthermore, to control for reverse causation and unmeasured confounding in the association between interleukin 6 and COPD, we used the Mendelian randomization method, described in **chapter 5**. Herein, we use genetic variation in the *IL6* gene as an instrument for strengthening causal inference between IL6 and COPD.

POTENTIAL IMPLICATIONS AND FUTURE DIRECTIONS

The general aim was to increase our knowledge on comorbidities and systemic effects in COPD, a common disease in the elderly associated with a high burden on healthcare costs, morbidity and mortality. To achieve healthy aging and to keep costs of our aging population affordable, we have to find ways to reverse or optimally prevent vulnerability in elderly. Our results on COPD, cardio- and cerebrovascular comorbidities, angiopathy and frailty might aid herein by identifying vulnerable elderly. Moreover, the results of this thesis might enhance further research into the underlying mechanisms to find new targets for preventive strategies or more effective management of chronic diseased people. Despite the enormous global impact of COPD, no current drug therapies have shown conclusively to prevent disease progression or reduce mortality. The results from this thesis might help to shift the sole focus on lung function decline towards a more multidisciplinary approach to tackle general decline. Especially in the early stages of the disease where COPD is still a silent killer through its comorbidities.

First, more research is needed into the origin of the systemic inflammation in COPD since it even persists in ex-smokers, and into the differentiation of highly prognostic inflammatory markers and causal makers of the disease. Furthermore, it is of particular interest whether systemic inflammation influences the innate defenses of the lungs against microbial pathogens, or whether it reflects impaired lung defense and consequent immune-inflammatory dysregulation.[451] However, since a recent study showed that elevated levels of systemic inflammatory markers are only present in a subset of COPD patients and sometimes merely intermittent, it remains generally questionable whether the number or severity of acute flares of inflammation is not even more important than the persistence of a low grade inflammation.[34]

Second, more research is needed into the mechanisms underlying the comorbidities in COPD. Regarding the association of COPD with macro- and microangiophaty, it would be of particular interest to further unravel genetic susceptibility or systemic inflammatory markers. Regarding the genetic susceptibility, the receptor for Advanced Glycation End products (RAGE) has been associated with atherosclerosis and arteriolosclerosis in diabetic patients.[452-454] Furthermore, genetic variation in the gene of this receptor has been shown to be associated with pulmonary function.[11] Since both COPD and lipohyalinosis are associated with advanced glycation end products, I like to further explore whether there is a potential genetic susceptibility underlying cerebral microangiopathy in COPD. Regarding the systemic inflammatory markers, this requires longitudinal follow-up and potentially multiple CRP measurements. Since for example in patients with rheumatoid arthritis, the magnitude and chronicity of the inflammatory response over time (and not the cross-sectional CRP measurement at the time of the ultrasound study) was associated with the carotid IMT.[455]

Third, we like to further investigate the vascular status of patients with COPD and explore whether the (systemic) atherosclerosis is associated with cognitive impairment. Since we now have identified frail subjects, we will evaluate prospectively the direction of the association with COPD. In addition, we like to explore whether certain phenotypes of COPD- for example the frequent exacerbator or chronic bronchitis phenotype- are more prone to develop frailty. Longitudinal follow-up is also required in our gait research. Both gait disturbances and COPD have independently been linked to cerebral small-vessel disease, however, little is known about underlying mechanisms.[200, 416, 456] In this thesis, we demonstrated that COPD persons have an increased risk of developing cerebral microbleeds in deep or infratentorial locations.[141] Since the deep and infratentorial regions harbour structures crucial to gait regulation, such as the cerebellum (coordination, adaptation), the basal ganglia (initiation, automatisation), brainstem (integration) and the thalamus, cerebral microbleeds in these regions might contribute to gait disturbances in persons with COPD.[457,458]

To tailor current and future treatment strategies, there is also a need for better characterization of the COPD heterogeneity. An example where we could meet herein might be the overlap with asthma. Since we also identified elderly with asthma or asthma-COPD Overlap Syndrome (ACOS), many opportunities are left to answer research questions about these underinvestigated phenotypes. We further aim to conduct more research into gender differences in patients with COPD. It would be of particular interest to know whether woman are more (or less) susceptible to smoking, acute exacerbations, early onset of the disease or with regard to this thesis, to certain comorbidities.

Lastly, the way from bench to bedside might be shortest for our research regarding statins. Randomized clinical trials (RCTs) are performed to confirm the protective effects of statin use we and others have seen in COPD observational studies.[101, 103, 140, 459] The large 'real-life' effect sizes of statins seem sometimes improbable and warrant further study. The great advantage of RCTs is that the exposure is randomly assigned and the design is very well suited to evaluate efficacy of a treatment. However, the first RCT recently published regarding statins in COPD demonstrated that simvastatin did not affect exacerbation rates or mortality.[459] Besides potential confounding in our study as discussed previously, three other factors might explain the discordant mortality results and generally reflect the major differences between RCTs and observational studies. First, the STATCOPE study results may not hold for COPD patients with mild airflow limitation or cardiovascular comorbidities. Although the majority of COPD patients has comorbidities, many have mild airflow limitation and a substantial amount did not smoke or had less than twenty packyears, they are all consistently excluded from clinical trials. These exclusions impede generalizability of the results and increase off-label use. Second, the protective effect of statins on mortality was especially observed in our study after prolonged treatment, while the mean follow-up of the STATCOPE study was less than two years since the study terminated early for futility on the primary endpoint.[140, 459] Because long-term follow-up is very expensive and hard endpoints

are also harder to measure, many RCTs in COPD focus on surrogate endpoints as lung function and lung function-associated parameters. Third, since we observed in our study that the protective effect of statins on mortality in subjects with COPD was modified by the degree of systemic inflammation at baseline, an enrichment design enrolling COPD patients with systemic inflammation or stratification by measured systemic biomarkers of inflammation would be of interest. Management of the COPD patient would optimally integrate the treatment of comorbidities. However, prevention of the systemic effects would be even better and it is very tempting to find solutions in minimizing the systemic inflammation. In addition to smoking cessation, increasing physical activity and treating impaired immunity might be effective preventive strategies too. Another prevention could consist of screening all COPD patients for the presence of concomitant atherosclerosis and patients with ischemic heart disease for the presence of airflow limitation.[30]

In conclusion, COPD and its potential role in other diseases clearly demonstrates that COPD is a very heterogenous disease deserving a multidisciplinary approach and much more scientific investigation. I hope that the results and suggestions in this thesis give oxygen to novel ideas for research and may be translated one day into making patients with COPD breathe better and live longer.

PART VI: SUMMARY

COPD is a common respiratory disease among elderly which is characterized by a progressive airflow limitation. In addition to the chronic inflammatory response in the lungs, systemic effects and comorbidities are frequently present and determine the prognosis in patients with COPD. A first step towards a better prevention and treatment of comorbidities in COPD is the identification of those COPD subjects in the general population at high risk of co-morbid conditions. The main objective of this thesis was therefore to identify and explore (novel) comorbid conditions and systemic effects of COPD within the Rotterdam Study, a large population-based cohort study.

Part II of this thesis focuses on the systemic inflammation and cardiovascular mortality in subjects with COPD. The systemic inflammation is potentially involved in the underlying mechanisms leading to COPD comorbidities. First, we investigated the role of interleukin 6 (IL6) and high sensitivity C-reactive protein (hsCRP). In **chapter 5**, we demonstrated that increased IL6 plasma levels at baseline were associated with the risk of developing COPD during follow-up. In contrast, our study could not find strong evidence for an association between common variation in the *IL6* gene and the risk of developing COPD. Our findings suggest that IL6, next to hsCRP, is a marker of systemic inflammation in patients with COPD, but is not causally driving the pathogenesis of the pulmonary dysfunction in COPD (maker of disease). However, further research is needed to study their specific role in the development of COPD comorbidities and the subsequent mortality. The results of **chapter 6** already suggest a potential role of systemic inflammation in the all cause mortality of patients with COPD. COPD subjects with an increased level of systemic inflammation at baseline had worse survival. Statins were associated with a reduced mortality, particularly in COPD subjects having increased serum levels of hsCRP at baseline. Although the protective effect of statins in COPD patients could represent solely an indirect effect on the cardiovascular comorbidities associated with COPD, our results also suggest an effect on respiratory mortality by statin use compared to never use. The results further imply that in COPD elderly, CRP levels rather than total cholesterol levels, might guide the clinician in the decision to start statin therapy. In this study, we also observed that most COPD cases died due to cardiovascular causes. Therefore, we focused on the cardiovascular mortality in **chapter 7**. Taking advantage of the enormous person-years of follow-up within the Rotterdam Study, we reported that COPD is an independent risk factor for sudden cardiac death in the general population. The risk especially increased five years after the diagnosis of COPD in frequent exacerbators having increased serum levels of hsCRP at baseline. This study might provide directions for better targeted actions in the general population for the prevention of sudden cardiac death.

Part III is dedicated to angiopathy and cerebrovascular comorbidities in COPD. **Chapter 8** presents a general overview of the current literature regarding the interplay between the lung and the brain in an aging population. Because COPD has been associated with vascular dysfunction, we investigated in the subsequent chapters the effects of COPD on the large and small blood vessels supplying the brain of oxygen and nutrients. In **chapter 9**, we demonstrated that COPD is associated with an increased risk of carotid artery wall thickening on ultrasonography compared to controls with a normal lung function. Importantly, vulnerable lipid core plaques on magnetic resonance imaging (MRI) were more frequent in COPD cases than in control subjects. Since persons with vulnerable carotid artery plaques are at risk for ischemic stroke through disruption of the plaque surface and thromboembolism, these observations offer new insights into the epidemiologic link between COPD and ischemic stroke. In **chapter 10**, we describe that COPD is associated with a higher prevalence of cerebral microbleeds, a marker of cerebral small-vessel disease determined by MRI. These findings tend to be driven by a greater occurrence of microbleeds in deep or infratentorial locations in subjects with COPD. In contrast, COPD was not significantly associated with microbleeds occurring in strictly lobar brain sites. The preferential location gives more insight into the potential underlying mechanism because microbleeds in deep or infratentorial locations are thought to occur by arteriolosclerosis on the basis of hypertensive vasculopathy and lipohyalinosis. Moreover, in this study, we were able to follow-up subjects without a cerebral microbleed at baseline and demonstrated that COPD is an independent risk factor to develop deep or infratentorial cerebral microbleeds during follow-up. After measuring brain angiopathy in chapters 9 and 10, we explored in **chapter 11** whether subjects with COPD have an increased risk of stroke during follow-up. We observed that COPD was associated with both the development of ischemic and hemorrhagic stroke. However, these findings tend to be driven by the shared etiology of smoking.

Part IV elaborates on another common syndrome in the elderly, frailty. In **chapter 12**, we estimated that the prevalence of physically frail elderly in the Rotterdam Study is 5.8%. Furthermore, we found that frail elderly were more likely to be older and female, to have an impaired quality of life and to have fallen or to have been hospitalized. Moreover, frail elderly had a significantly increased risk of dying within three years compared to the non-frail elderly, even when adjusted for age, sex and comorbidities. **Chapter 13** elaborates on the interrelationship between COPD and frailty. In this study, we evaluated the added value of the identification of the frail COPD phenotype. We were particularly interested in whether clinicians should mainly treat the individual comorbidities in patients with COPD or that a more holistic approach towards the most vulnerable COPD patients is necessary. In **chapter 14**, we further investigated the disability in subjects with COPD by exploring the impact of COPD on their gait pattern. In this study, we demonstrated that COPD was specifically associated with taking slower steps (lower Rhythm). We also observed that the Rhythm was

worst in COPD persons with dyspnea, severe airflow limitation and frequent exacerbations (GOLD category D) and moreover, in COPD fallers.

Part V finally, gives a general interpretation of the most important results and places the findings into perspective. In addition, I highlighted some of the methodological issues regarding our studies and discussed some implications and suggestions for the future.

SAMENVATTING

COPD is een veel voorkomende respiratoire ziekte bij ouderen die wordt gekenmerkt door een progressieve luchtwegobstructie. Naast de chronische ontstekingsreactie in de longen, zijn systemische effecten en comorbiditeiten vaak aanwezig en bepalen ze in belangrijke mate mee de prognose van patiënten met COPD. Een eerste stap naar een betere preventie en behandeling van comorbiditeiten bij COPD is de identificatie ervan in de algemene bevolking. Het belangrijkste doel van dit proefschrift was dan ook (nieuwe) comorbiditeiten en systemische effecten van COPD te onderzoeken in de Rotterdam Study, een grote cohort studie bij de algemene bevolking.

Deel II van dit proefschrift richtte zich op de systemische inflammatie en cardiovasculaire mortaliteit bij patiënten met COPD. De systemische ontsteking is namelijk mogelijks betrokken in de onderliggende mechanismen die leiden tot comorbiditeiten bij COPD. Vooreerst hebben we de rol van interleukine-6 (IL6) en hoog sensitief C-reactief proteïne (hsCRP) onderzocht. In **hoofdstuk 5** hebben we aangetoond dat verhoogde IL6 plasma spiegels bij aanvang van de studie geassocieerd waren met het risico op het ontwikkelen van COPD tijdens de follow-up. Daarentegen kon onze studie geen sterk bewijs aanleveren dat veel voorkomende variatie in het IL6-gen geassocieerd zou zijn met het risico op COPD. Onze bevindingen suggereren dus eerder dat IL6, naast hsCRP, een merker is van systemische inflammatie bij COPD-patiënten, maar niet causaal betrokken is bij de pulmonale pathogenese van COPD (maker van de ziekte). Er is echter verder onderzoek nodig om de specifieke rol van deze twee markers in de ontwikkeling van COPD comorbiditeiten en de daaropvolgende mortaliteit te bestuderen. De resultaten van **hoofdstuk 6** suggereren alvast een mogelijke rol van systemische ontsteking in de mortaliteit bij patiënten met COPD. COPD-patiënten met een verhoogd niveau van systemische ontsteking bij aanvang van de studie, hadden namelijk een slechtere overleving. Statines waren bovendien geassocieerd met een verminderde mortaliteit, vooral bij COPD patiënten met verhoogde serumspiegels van hsCRP bij aanvang van de studie. Hoewel het beschermend effect van statines bij COPD patiënten mogelijks volledig te wijten is aan een indirect effect op de cardiovasculaire comorbiditeiten, suggereren onze resultaten ook een effect op de respiratoire mortaliteit. Mogelijks is de aanwezigheid van systeeminflammatie dus een betere indicatie dan een verhoogd cholesterol om statines op te starten bij ouderen met COPD. In deze studie konden we ook vaststellen dat de meeste personen met COPD overlijden ten gevolge van cardiovasculaire oorzaken. Daarom hebben we ons gericht op de cardiovasculaire mortaliteit in **hoofdstuk 7**. Gebruikmakend van het enorme aantal persoonsjaren aan follow-up binnen de Rotterdam Study, hebben we een verband aangetoond tussen COPD en plotse hartdood in de algemene bevolking. De relatie was vooral sterker vijf jaar na de COPD diagnose en in COPD personen die frequent exacerbaties ondergingen én bij aanvang van de studie verhoogde serumspiegels van hsCRP hadden. Dit onderzoek zou mede een betere preventie van plotse hartdood kunnen bewerkstelligen.

Deel III is volledig gewijd aan angiopathie en cerebrovasculaire comorbiditeiten bij COPD. **Hoofdstuk 8** geeft hierbij een algemeen overzicht van de huidige literatuur omtrent de interacties tussen de longen en de hersenen bij ouderen. Omdat de literatuur suggereert dat COPD onder andere geassocieerd is met subklinische hersenschade, onderzochten we in de daaropvolgende hoofdstukken de effecten van COPD op de grote en kleine bloedvaten die de hersenen van zuurstof en voedingsstoffen voorzien. In **hoofdstuk 9** hebben we aangetoond dat COPD geassocieerd is met een verhoogd risico op halsslagader wandverdikking op echografie in vergelijking met personen met een normale longfunctie. Belangrijk hierbij is dat kwetsbare plaques met een lipide kern op magnetische resonantie beeldvorming (MRI) vaker voorkwamen bij personen met COPD dan bij controle personen. Gezien kwetsbare plaques ter hoogte van de halsslagader een risico vormen op ischemische beroerte door het ontstaan van trombo-embolie aan de plaque-oppervlakte, bieden deze observaties nieuwe inzichten in de relatie tussen COPD en ischemische beroerte. In **hoofdstuk 10**, beschrijven we dat COPD geassocieerd is met een hogere prevalentie van cerebrale microbloedingen, een MRI-merker voor cerebrale kleine vaatziekte. Deze bevindingen blijken vooral gedreven door een grotere aanwezigheid van microbloedingen in diep of infratentoriële hersengebieden in personen met COPD. Daarentegen was COPD niet significant geassocieerd met microbloedingen die zich strikt lobair in de hersenen bevinden. De preferentiële locatie geeft meer inzicht in de mogelijks onderliggende mechanismen omdat microbloedingen in diep of infratentoriële locaties zouden ontstaan door arteriolosclerose op basis van hypertensieve vasculopathie en lipohyalinosis. Bovendien konden we in deze studie nagaan of deelnemers zonder microbloedingen bij aanvang van de studie, microbloedingen ontwikkelden tijdens follow-up. Hierbij hebben we aangetoond dat COPD wel degelijk een onafhankelijke risicofactor blijkt te zijn om diep of infratentoriële cerebrale microbloedingen te ontwikkelen.
Na het meten van de bloedvataantasting in hoofdstukken 9 en 10, onderzochten we in hoofdstuk 11 of deelnemers met COPD een verhoogd risico hadden op een beroerte tijdens follow-up. We vonden dat COPD geassocieerd is met zowel de ontwikkeling van ischemische als hemorragische beroerte. Echter, deze bevindingen waren in belangrijke mate gedreven door de gemeenschappelijke etiologie van roken.

Deel IV gaat dieper in op een ander veel voorkomend syndroom bij ouderen, namelijk frailty. In **hoofdstuk 12**, schatten we dat de prevalentie van fysiek fraile ouderen in een Nederlandse populatie 5,8% is. Verder vonden we dat frailty onder andere gekenmerkt wordt door een hogere leeftijd, vrouwelijk geslacht, een verminderde kwaliteit van leven en een verhoogde aanwezigheid van vallen en hospitalisaties. Fraile ouderen hadden bovendien een significant verhoogd risico op overlijden binnen de drie jaar in vergelijking met niet-fraile ouderen, zelfs na correctie voor leeftijd, geslacht en comorbiditeiten. **Hoofdstuk 13** gaat dieper in op de onderlinge relatie tussen COPD en frailty. In deze studie identificeerden we fraile personen binnen COPD. Hierbij vroegen we ons af of sommige kwetsbare personen met COPD eerder baat zouden hebben bij een holistische benadering of dat hun

kwetsbaarheid vooral gedreven lijkt te zijn door de individuele comorbiditeiten. In **hoofdstuk 14** hebben we verder de invaliditeit van personen met COPD onderzocht door de effecten van COPD op hun looppatroon te onderzoeken. In deze studie hebben we specifiek aangetoond dat personen met COPD langzamer stappen nemen dan personen zonder COPD. Verder konden we ook opmerken dat deze gang voornamelijk was aangetast in COPD personen met kortademigheid, met een ernstige beperking van de luchtstroom, met frequente exacerbaties en bovendien, met vallen.

Deel V tenslotte, geeft een algemene interpretatie van de belangrijkste resultaten en plaatst de bevindingen in een breder perspectief. Daarnaast wou ik hierin een aantal methodologische kwesties met betrekking tot onze studies aanstippen en een aantal implicaties en suggesties voor de toekomst bespreken.

PART VII: DANKWOORD

Dit proefschrift is een joint PhD, niet alleen internationaal, maar ook interdisciplinair werden de krachten gebundeld. Heel wat personen verdienen dan ook een bijzonder woord van dank gezien dit doctoraat zonder hen niet mogelijk was geweest.

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En dan is er gelukkig nog een kleine filou die op tijd en stond laat weten dat het leven niet draait om een PhD maar om hemzelf, onze grootste schat Leander! Niets werkt meer relativerend dan jouw lieve lach. Afsluiten doe ik met de persoon waarvoor woorden te kort schieten. Kenny, gedurende deze PhD periode zijn we getrouwd, hebben we een (t)huis gebouwd, zijn we papa en mama geworden, jij dokter en ik hopelijk doctor. We hebben het graag druk maar boeiend geloof ik. De wereld is allezinds mooier met jou aan mijn zijde…

LIST OF PUBLICATIONS

1. Smolonska J, Koppelman GH, Wijmenga C, Vonk JM, Zanen P, Bruinenberg M, Curjuric I, Imboden M, Thun GA, Franke L, Probst-Hensch NM, Nürnberg P, Riemersma RA, van Schayck CP, Loth DW, Brusselle GG, Stricker BH, Hofman A, Uitterlinden AG, **Lahousse L**, London SJ, Loehr LR, Manichaikul A, Barr RG, Donohue KM, Rich SS, Pare P, Bossé Y, Hao K, van den Berge M, Groen HJ, Lammers JW, Mali W, Boezen HM, Postma DS. **Common genes underlying asthma and COPD? Genome-wide analysis on the Dutch hypothesis.** *Eur Respir J. 2014 Jul 3.*

2. Tang W, Kowgier M, Loth DW, Soler Artigas M, Joubert BR, Hodge E, Gharib SA, Smith AV, Ruczinski I, Gudnason V, Mathias RA, Harris TB, Hansel NN, Launer LJ, Barnes KC, Hansen JG, Albrecht E, Aldrich MC, Allerhand M, Barr RG, Brusselle GG, Couper DJ, Curjuric I, Davies G, Deary IJ, Dupuis J, Fall T, Foy M, Franceschini N, Gao W, Gläser S, Gu X, Hancock DB, Heinrich J, Hofman A, Imboden M, Ingelsson E, James A, Karrasch S, Koch B, Kritchevsky SB, Kumar A, **Lahousse L**, Li G, Lind L, Lindgren C, Liu Y, Lohman K, Lumley T, McArdle WL, Meibohm B, Morris AP, Morrison AC, Musk B, North KE, Palmer LJ, Probst-Hensch NM, Psaty BM, Rivadeneira F, Rotter JI, Schulz H, Smith LJ, Sood A, Starr JM, Strachan DP, Teumer A, Uitterlinden AG, Völzke H, Voorman A, Wain LV, Wells MT, Wilk JB, Williams OD, Heckbert SR, Stricker BH, London SJ, Fornage M, Tobin MD, O Connor GT, Hall IP, Cassano PA. **Large-Scale Genome-Wide Association Studies and Meta-Analyses of Longitudinal Change in Adult Lung Function.** *PLoS One. 2014 Jul 1;9(7)*

3. **Lahousse L***, Maes B*, Ziere G, Loth DW, Verlinden VJA, Zillikens MC, Uitterlinden AG, Rivadeneira F, Tiemeier H, Franco OH, Ikram MA, Hofman A, Brusselle GG, Stricker BH. **Adverse outcomes of frailty in the elderly: the Rotterdam Study.** *European Journal of Epidemiology. 2014 Jun;29(6):419-27.*

4. Loth DW, Artigas MS, Gharib SA, Wain LV, Franceschini N, Koch B, Pottinger TD, Smith AV, Duan Q, Oldmeadow C, Lee MK, Strachan DP, James AL, Huffman JE, Vitart V, Ramasamy A, Wareham NJ, Kaprio J, Wang XQ, Trochet H, Kähönen M, Flexeder C, Albrecht E, Lopez LM, de Jong K, Thyagarajan B, Alves AC, Enroth S, Omenaas E, Joshi PK, Fall T, Viñuela A, Launer LJ, Loehr LR, Fornage M, Li G, Wilk JB, Tang W, Manichaikul A, **Lahousse L**, Harris TB, North KE, Rudnicka AR, Hui J, Gu X, Lumley T, Wright AF, Hastie ND, Campbell S, Kumar R, Pin I, Scott RA, Pietiläinen KH, Surakka I, Liu Y, Holliday EG, Schulz H, Heinrich J, Davies G, Vonk JM, Wojczynski M, Pouta A, Johansson A, Wild SH, Ingelsson E, Rivadeneira F, Völzke H, Hysi PG, Eiriksdottir G, Morrison AC, Rotter JI, Gao W, Postma DS, White WB, Rich SS, Hofman A, Aspelund T, Couper D, Smith LJ, Psaty BM, Lohman K, Burchard EG, Uitterlinden AG, Garcia M, Joubert BR, McArdle WL, Musk AB, Hansel N, Heckbert SR, Zgaga L, van Meurs JB, Navarro P, Rudan I, Oh YM, Redline S, Jarvis DL, Zhao JH, Rantanen T, O'Connor GT, Ripatti S, Scott RJ, Karrasch S, Grallert H, Gaddis NC, Starr JM, Wijmenga C, Minster

RL, Lederer DJ, Pekkanen J, Gyllensten U, Campbell H, Morris AP, Gläser S, Hammond CJ, Burkart KM, Beilby J, Kritchevsky SB, Gudnason V, Hancock DB, Williams OD, Polasek O, Zemunik T, Kolcic I, Petrini MF, Wjst M, Kim WJ, Porteous DJ, Scotland G, Smith BH, Viljanen A, Heliövaara M, Attia JR, Sayers I, Hampel R, Gieger C, Deary IJ, Boezen HM, Newman A, Jarvelin MR, Wilson JF, Lind L, Stricker BH, Teumer A, Spector TD, Melén E, Peters MJ, Lange LA, Barr RG, Bracke KR, Verhamme FM, Sung J, Hiemstra PS, Cassano PA, Sood A, Hayward C, Dupuis J, Hall IP, Brusselle GG, Tobin MD, London SJ. **Genome-wide association analysis identifies six new loci associated with forced vital capacity.** *Nat Genet. 2014 Jun 15.*

5. Dijkstra AE, Smolonska J, van den Berge M, Wijmenga C, Zanen P, Luinge MA, Platteel M, Lammers JW, Dahlback M, Tosh K, Hiemstra PS, Sterk PJ, Spira A, Vestbo J, Nordestgaard BG, Benn M, Nielsen SF, Dahl M, Verschuren WM, Picavet HS, Smit HA, Owsijewitsch M, Kauczor HU, de Koning HJ, Nizankowska-Mogilnicka E, Mejza F, Nastalek P, van Diemen CC, Cho MH, Silverman EK, Crapo JD, Beaty TH, Lomas DA, Bakke P, Gulsvik A, Bossé Y, Obeidat MA, Loth DW, **Lahousse L**, Rivadeneira F, Uitterlinden AG, Hofman A, Stricker BH, Brusselle GG, van Duijn CM, Brouwer U, Koppelman GH, Vonk JM, Nawijn MC, Groen HJ, Timens W, Boezen HM, Postma DS; LifeLines Cohort study. **Susceptibility to chronic mucus hypersecretion, a genome wide association study.** *PLoS One. 2014 Apr 8;9(4).*

6. Campos-Obando N, Castano-Betancourt MC, Oei L, Franco OH, Stricker BH, Brusselle GG, **Lahousse L**, Hofman A, Tiemeier H, Rivadeneira F, Uitterlinden AG, Zillikens MC. **Bone mineral density and chronic lung disease mortality: the rotterdam study.** *J Clin Endocrinol Metab. 2014 May;99(5):1834-42.*

7. **Lahousse L**, Loth DW, Vernooij MW, Darweesh SKL, Akoudad S, Joos GF, Hofman A, Stricker BH, Ikram MA, Brusselle GG. **Chronic Obstructive Pulmonary Disease and cerebral microbleeds: the Rotterdam Study.** *Am J Respir Crit Care Med. 2013;188(7):783-8*

8. Loth DW, Brusselle GG, **Lahousse L**, Hofman A, Leufkens HG, Stricker BH. **Beta-blockers and pulmonary function in the general population: the Rotterdam Study.** *Br J Clin Pharmacol. 2013 Jun 17.*

9. Loth DW, Ittermann T, **Lahousse L**, Hofman A, Leufkens HG, Brusselle GG, Stricker BH. **Normal spirometry values in healthy elderly: the Rotterdam Study.** *Eur J Epidemiol. 2013 Apr;28(4):329-34.*

10. **Lahousse L**, van den Bouwhuijsen Q, Loth DW, Joos GF, Hofman A, Witteman JC, van der Lugt A, Brusselle GG, Stricker BH. **Chronic Obstructive Pulmonary Disease and Lipid Core Carotid Artery Plaques in the Elderly: the Rotterdam Study.** *Am J Respir Crit Care Med. 2013 Jan 1;187(1):58-64*

11. **Lahousse L**, Loth DW, Joos GF, Hofman A, Leufkens HG, Brusselle GG, Stricker BH. **Statins, systemic inflammation and risk of death in COPD: the Rotterdam Study.** *Pulm Pharmacol Ther. 2013 Apr;26(2):212-7*

12. Wilk JB, Shrine NR, Loehr LR, Zhao JH, Manichaikul A, Lopez LM, Smith AV, Heckbert SR, Smolonska J, Tang W, Loth DW, Curjuric I, Hui J, Cho MH, Latourelle JC, Henry AP, Aldrich M, Bakke P, Beaty TH, Bentley AR, Borecki IB, Brusselle GG, Burkart KM, Chen TH, Couper D, Crapo JD, Davies G, Dupuis J, Franceschini N, Gulsvik A, Hancock DB, Harris TB, Hofman A, Imboden M, James AL, Khaw KT, **Lahousse L**, Launer LJ, Litonjua A, Liu Y, Lohman KK, Lomas DA, Lumley T, Marciante KD, McArdle WL, Meibohm B, Morrison AC, Musk AW, Myers RH, North KE, Postma DS, Psaty BM, Rich SS, Rivadeneira F, Rochat T, Rotter JI, Artigas MS, Starr JM, Uitterlinden AG, Wareham NJ, Wijmenga C, Zanen P, Province MA, Silverman EK, Deary IJ, Palmer LJ, Cassano PA, Gudnason V, Barr RG, Loos RJ, Strachan DP, London SJ, Boezen HM, Probst-Hensch N, Gharib SA, Hall IP, O'Connor GT, Tobin MD, Stricker BH. **Genome-wide association studies identify CHRNA5/3 and HTR4 in the development of airflow obstruction.** *Am J Respir Crit Care Med. 2012 Oct 1;186(7):622-32.*

13. van Durme YMTA*, **Lahousse L***, Verhamme KMC, Stolk L, Eijgelsheim M, Loth DW, Uitterlinden AG, Breteler MMB, Joos GF, Hofman A, Stricker BH, Brusselle GG. **Mendelian Randomization Study of Interleukin 6 in Chronic Obstructive Pulmonary Disease*. Respiration.*** *2011;82(6):530-8.*

** Both authors contributed equally*

PHD PORTFOLIO

1 Information

- Name: Lies Lahousse
- Faculty: Medicine and health sciences
- Erasmus MC: Department of Epidemiology
- Doctoral School: Life Sciences and Medicine
- Research School: Netherlands Institute for Health Sciences (NIHES)
- PhD period: September 2010 - November 2014
- Promoteres: Prof. Dr. Guy Brusselle
  Prof. Dr. Bruno Stricker

2 PhD training

- **2011-2013:** Master of Science in Health Sciences, Erasmus University Rotterdam
  Specialization Clinical Epidemiology - Organized by NIHES
- **2010-2014:** UGent Doctoral Training Programme - *Organized by UGent*
- **Specialist courses:**
  - Principles of Research in Medicine and Epidemiology (August 15-19, 2011) *Organized by NIHES, accepted by UGent*
  - Methods of Clinical Research (August 22-26, 2011) *Organized by NIHES, accepted by UGent*
  - Pharmaco-epidemiology (August 29 – September 2, 2011) *Organized by NIHES, accepted by UGent*
  - Registration of Medicines (October-December 2013)- *Organized by UGent*
- **Research and validation:**
  - Endnote (November 18, 2010) - *Organized by UGent/UZGent*
  - Advanced SPSS (January, 2011) - *Organized by UGent/UZGent*
  - Study Design (September, 2011) - *Organized by NIHES, accepted by UGent*
  - Good Clinical Practice (October 17, 2012) - *Organized by UGent/UZGent*
- **Career management:**
  - Welcome Day. Everything you need to know about PhD studies in the Doctoral School of Life Sciences and Medicine (November 22, 2010) - *Organized by UGent*
  - Knowledge for Growth (May 5th, 2011) - *Organized by FlandersBio-UGent*
  - PhD-day (Juyl 4, 2014), including workshops on "Hora est and PhD training" and "How to print your thesis" – *Organized by ErasmusMC*
  - From PhD to Job Market: From PhD to Life (September 20, 2013) - *Organized by UGent*
  - PhD-day (Juyl 3, 2014), including workshops on "Popularizing your research" and "Defend your thesis" – *Organized by ErasmusMC*
- **Leadership and efficiency:**
  - Leadership Foundation Course (November 12,20&27, 2012) - *Organized by UGent*

3 Presentations

- Interleukin 6 plasma levels, common variation in the IL6 gene and the risk of chronic obstructive pulmonary disease: a prospective population-based cohort study.
  - *Poster presentation at the 2011 ATS International Conference in Denver, Colorado*

- *Oral presentation at the 2011 5th IUAP Meeting Ghent, October 28th 2011*
- *Poster presentation at the 2012 Science Day Ghent, March 14th 2012*

- Statins, systemic inflammation and the risk of death in patients with COPD: the Rotterdam Study
  - *Oral presentation at the 2012 BVP Meeting Brussel, April 25th 2012*
  - *Poster discussion at the 2012 ATS International Conference in San Francisco*
- Frailty affects quality of spirometry: the Rotterdam Study
  - *Poster presentation at the 2013 ATS International Conference in Philadelphia*
- COPD exacerbations and carotid artery plaques: the Rotterdam Study
  - *Oral presentation at the 2013 BVP Meeting Brussel, March 27th 2013*
  - *Poster discussion at the 2013 ERS International Conference in Barcelona*
- COPD and Sudden Cardiac Death: the Rotterdam Study
  - *Oral presentation at the 2014 BVP Meeting Brussel, June 11th 2014*
- Cause-specific mortality in mild COPD: the Rotterdam Study
  - *Poster presentation at the 2014 ERS International Conference in Munich*

4 International conferences

2014 European Respiratory Society International Conference in Munich
2013 European Respiratory Society International Conference in Barcelona
2013 CHARGE International meeting in Rotterdam
2013 American Thoracic Society International Conference in Philadelphia
2012 European Respiratory Society International Conference in Amsterdam
2012 American Thoracic Society International Conference in San Francisco
2011 American Thoracic Society International Conference in Denver

5 Supervising Master students

2013 Bastiaan Maes, "Frailty and COPD: the Rotterdam Study"
2012 Kenny Vlaemynck, "COPD and the risk of Open-Angle Glaucoma: the Rotterdam Study"
2011 Tom Holvoet, "Genetische determinanten en klinische risico's van Staphylococcus Aureus neusdragerschap"

6 Awards & Fellowships

International Trainee Travel Award 2011 from the National Emphysema Foundation honouring Claude Lenfant, ATS International Conference, Denver, 2011
European Respiratory Society Short-term research fellowship, 2011
Belgian Society for Pneumology (BVP) Short-term Research fellowship, 2012
Travel Award 2012 to the ATS International Conference in San Francisco, 2012
Belgian Society for Pneumology (BVP) Short-term Research fellowship, 2013
ERS Young Scientist Sponsorship, ERS International Conference, Barcelona, 2013

7 Other

- Reviewing articles
- Numerous intern presentations at the Department of Respiratory Medicine, Ghent University Hospital and at the Department of Epidemiology, Erasmus MC, Rotterdam

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11. Hancock DB, Eijgelsheim M, Wilk JB, et al. Meta-analyses of genome-wide association studies identify multiple loci associated with pulmonary function. *Nature Genetics*. 2009;42(1):45-52.
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