Chronic Obstructive Pulmonary Disease and cerebral microbleeds: The Rotterdam Study

Lies Lahousse [a,b], Meike W.Vernooij [b,c], Sirwan K.L.Darweesh [b], Saloua Akoudad [b-d], Daan W. Loth [b,e], Guy F. Joos [a], Albert Hofman [b,f], Bruno H. Stricker [b,e-g], M. Arfan Ikram [b-d], Guy G. Brusselle [a,b]

a. Department of Respiratory Medicine, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium.
b. Department of Epidemiology, Erasmus Medical Center, PO Box 2040, 3000 CA Rotterdam, the Netherlands.
c. Department of Radiology, Erasmus Medical Center, PO Box 2040, 3000 CA Rotterdam, the Netherlands.
d. Department of Neurology, Erasmus Medical Center, PO Box 2040, 3000 CA Rotterdam, the Netherlands.
e. Inspectorate of Healthcare, The Hague, the Netherlands.
f. Members of the Netherlands Consortium on Healthy Aging (NCHA), Leiden, the Netherlands.
g. Department of Medical Informatics, Erasmus Medical Center, PO Box 2040, 3000 CA Rotterdam, the Netherlands

Corresponding author: Bruno H. Stricker; Department of Epidemiology; Erasmus Medical Center; PO Box 2040; 3000 CA Rotterdam, the Netherlands; Phone: +31 (0)10 70 44294; E-mail: b.stricker@erasmusmc.nl

Authors' contributions: *Study Design:* A.H., M.W.V., M.A.I., B.H.S. ; *Data collection:* L.L., M.W.V., S.A., D.W.L ; *Data-analysis and writing:* L.L., S.K.L.D; *Data-interpretation:* L.L., S.K.L.D, D.W.L., B.H.S., M.W.V., M.A.I., G.G.B.; *Critical Review:* S.A., D.W.L., G.F.J., A.H., B.H.S., M.W.V., M.A.I., G.G.B.

Funding: This study was supported by the Fund for Scientific Research Flanders (FWO) project 3G019309, the Netherlands Organization for Scientific Research (NWO) grants 904-61-093 and 918-46-615. Lies Lahousse is the recipient of a Belgian Thoracic Society Fellowship. Meike Vernooij is supported by an Erasmus MC Fellowship grant. Sirwan Darweesh is supported by a grant from the Royal Netherlands Academy of Arts and Sciences (KNAW). The funding source had no involvement in the collection, analysis, writing, interpretation, nor in the decision to submit the paper for publication.

Running title: COPD and cerebral microbleeds

Subject Code List: 9.4 COPD: Comorbidities

Total word count manuscript body: 2363

At a Glance Commentary:

What is the current scientific knowledge on this subject? Two cross-sectional studies suggest that COPD is related with white matter lesions through cerebral small vessel disease.(1, 2) However, the association between COPD and cerebral microbleeds as marker of cerebral small vessel disease, and the cerebral microbleed location indicative for the underlying disease mechanism, needs to be elucidated.

What does this study add to the field? Our study shows for the first time, in a large, population-based cohort study, that COPD increases the risk of developing cerebral microbleeds in deep or infratentorial locations, cross-sectionally and moreover, longitudinally in subjects without microbleed at baseline.

This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org

ABSTRACT

Rationale: 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).

Main 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.

Word count abstract: 246 - Key words: Cerebral Small Vessel Disease; Airflow Obstruction, Chronic; Magnetic Resonance Imaging; strictly lobar cerebral microbleed; deep or infratentorial cerebral microbleed

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.(3) 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.(4) 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.(5-8) 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.(5, 9) These microbleeds, which consist of hemosiderin deposits in macrophages, can be visualized on Magnetic Resonance Imaging (MRI) as small areas of hypointensity.(10) Microbleeds rarely disappear, making them suitable markers for cumulative cerebrovascular damage.(11) 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.(9, 12)

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.(13) The study started in 1990 and all participants are invited every 3 to 4 years to the research centre 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.(14) 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).(11, 14) 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 Centre, 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 and in more detail in the Online Data Supplement.(15) 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.(16, 17) 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 and in more detail in the Online Data Supplement, all participants of the Rotterdam Scan Study underwent a multisequence MRI protocol on a 1.5-T scanner and cerebral 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.(9, 14). 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).(9, 18, 19) 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).(11)

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%. More detail on the covariables is provided in the Online Data Supplement. 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 centre 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 (20) into 84 (50.9%) mild, 73 (44.2%) moderate and 8 (4.8%) severe COPD; and according to the updated GOLD group categorization (4) 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. 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)* 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). *Figure E1* 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)).

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 E2)* 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 E1* 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 E2)*

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 packyears, 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.

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.(9) 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.(2, 21)

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.(9) 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.(15) 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.(15, 22, 23) 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.(24) 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.(25, 26) The high percentage of subjects in group A and the very small number in group C are in line with other general population studies.(25-27)

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.(17) 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.(21) 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.(3) 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.(2, 15, 28, 29) 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.(30) 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.(21) 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.(21)

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.(9)

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 DLCO, it is not the gold standard to measure emphysema.(31)

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.

Acknowledgments

The authors thank the study participants, the staff from the Rotterdam Study and the participating general practitioners.

REFERENCES

1. van Dijk EJ, Vermeer SE, de Groot JC, van de Minkelis J, Prins ND, Oudkerk M, Hofman A, Koudstaal PJ, Breteler MM. Arterial oxygen saturation, copd, and cerebral small vessel disease. *J Neurol Neurosurg Psychiatry* 2004;75:733-736.
2. Dodd JW, Chung AW, van den Broek MD, Barrick TR, Charlton RA, Jones PW. Brain structure and function in chronic obstructive pulmonary disease: A multimodal cranial magnetic resonance imaging study. *Am J Respir Crit Care Med* 2012;186:240-245.
3. Brusselle GG, Joos GF, Bracke KR. New insights into the immunology of chronic obstructive pulmonary disease. *Lancet* 2011;378:1015-1026.
4. Vestbo J, Hurd SS, Agusti AG, Jones PW, Vogelmeier C, Anzueto A, Barnes PJ, Fabbri LM, Martinez FJ, Nishimura M, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: Gold executive summary. *Am J Respir Crit Care Med* 2013;187:347-365.
5. Pantoni L. Cerebral small vessel disease: From pathogenesis and clinical characteristics to therapeutic challenges. *Lancet Neurol* 2010;9:689-701.
6. Dodd JW, Getov SV, Jones PW. Cognitive function in copd. *Eur Respir J* 2010;35:913-922.
7. Beauchamp MK, Sibley KM, Lakhani B, Romano J, Mathur S, Goldstein RS, Brooks D. Impairments in systems underlying control of balance in copd. *Chest* 2012;141:1496-1503.
8. Eisner MD, Blanc PD, Yelin EH, Sidney S, Katz PP, Ackerson L, Lathon P, Tolstykh I, Omachi T, Byl N, et al. Copd as a systemic disease: Impact on physical functional limitations. *Am J Med* 2008;121:789-796.
9. Greenberg SM, Vernooij MW, Cordonnier C, Viswanathan A, Al-Shahi Salman R, Warach S, Launer LJ, Van Buchem MA, Breteler MM. Cerebral microbleeds: A guide to detection and interpretation. *Lancet Neurol* 2009;8:165-174.
10. Fazekas F, Kleinert R, Roob G, Kleinert G, Kapeller P, Schmidt R, Hartung HP. Histopathologic analysis of foci of signal loss on gradient-echo t2*-weighted mr images in patients with spontaneous intracerebral hemorrhage: Evidence of microangiopathy-related microbleeds. *AJNR Am J Neuroradiol* 1999;20:637-642.
11. Poels MM, Ikram MA, van der Lugt A, Hofman A, Krestin GP, Breteler MM, Vernooij MW. Incidence of cerebral microbleeds in the general population: The rotterdam scan study. *Stroke* 2011;42:656-661.
12. Vernooij MW, van der Lugt A, Ikram MA, Wielopolski PA, Niessen WJ, Hofman A, Krestin GP, Breteler MM. Prevalence and risk factors of cerebral microbleeds: The rotterdam scan study. *Neurology* 2008;70:1208-1214.
13. Hofman A, van Duijn CM, Franco OH, Ikram MA, Janssen HL, Klaver CC, Kuipers EJ, Nijsten TE, Stricker BH, Tiemeier H, et al. The rotterdam study: 2012 objectives and design update. *Eur J Epidemiol* 2011;26:657-686.
14. Ikram MA, van der Lugt A, Niessen WJ, Krestin GP, Koudstaal PJ, Hofman A, Breteler MM, Vernooij MW. The rotterdam scan study: Design and update up to 2012. *Eur J Epidemiol* 2011;26:811-824.
15. 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;187:58-64.
16. Celli BR, MacNee W, Agusti A, Anzueto A, Berg B, Buist AS, Calverley PMA, Chavannes N, Dillard T, Fahy B, et al. Standards for the diagnosis and treatment of patients with copd: A summary of the ats/ers position paper. *Eur Respir J* 2004;23:932-946.
17. Macintyre N, Crapo RO, Viegi G, Johnson DC, van der Grinten CP, Brusasco V, Burgos F, Casaburi R, Coates A, Enright P, et al. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J* 2005;26:720-735.
18. Jeerakathil T, Wolf PA, Beiser A, Hald JK, Au R, Kase CS, Massaro JM, DeCarli C. Cerebral microbleeds: Prevalence and associations with cardiovascular risk factors in the framingham study. *Stroke* 2004;35:1831-1835.
19. Roob G, Schmidt R, Kapeller P, Lechner A, Hartung HP, Fazekas F. Mri evidence of past cerebral microbleeds in a healthy elderly population. *Neurology* 1999;52:991-994.
20. Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, Fukuchi Y, Jenkins C, Rodriguez-Roisin R, van Weel C, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: Gold executive summary. *Am J Respir Crit Care Med* 2007;176:532-555.

21. Poels MM, Vernooij MW, Ikram MA, Hofman A, Krestin GP, van der Lugt A, Breteler MM. Prevalence and risk factors of cerebral microbleeds: An update of the rotterdam scan study. *Stroke* 2010;41:S103-106.
22. Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med* 1999;340:115-126.
23. Poels MM, Zaccai K, Verwoert GC, Vernooij MW, Hofman A, van der Lugt A, Witteman JC, Breteler MM, Mattace-Raso FU, Ikram MA. Arterial stiffness and cerebral small vessel disease: The rotterdam scan study. *Stroke* 2012;43:2637-2642.
24. Agusti A, Vestbo J. Current controversies and future perspectives in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2011;184:507-513.
25. Lange P, Marott JL, Vestbo J, Olsen KR, Ingebrigtsen TS, Dahl M, Nordestgaard BG. Prediction of the clinical course of chronic obstructive pulmonary disease, using the new gold classification: A study of the general population. *Am J Respir Crit Care Med* 2012;186:975-981.
26. Johannessen A, Nilsen RM, Storebo M, Gulsvik A, Eagan T, Bakke P. Comparison of 2011 and 2007 gold guidelines for predicting mortality and hospitalization. *Am J Respir Crit Care Med* 2013.
27. Agusti A, Hurd S, Jones P, Fabbri L, Martinez F, Vogelmeier C, Vestbo J, Rodriguez-Roisin R. Frequently asked questions (faqs) about the gold 2011 assessment proposal of copd. *Eur Respir J* 2013.
28. Liao D, Higgins M, Bryan NR, Eigenbrodt ML, Chambless LE, Lamar V, Burke GL, Heiss G. Lower pulmonary function and cerebral subclinical abnormalities detected by mri: The atherosclerosis risk in communities study. *Chest* 1999;116:150-156.
29. Maclay JD, McAllister DA, Mills NL, Paterson FP, Ludlam CA, Drost EM, Newby DE, Macnee W. Vascular dysfunction in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2009;180:513-520.
30. Garcia-Aymerich J, Gomez FP, Benet M, Farrero E, Basagana X, Gayete A, Pare C, Freixa X, Ferrer J, Ferrer A, et al. Identification and prospective validation of clinically relevant chronic obstructive pulmonary disease (copd) subtypes. *Thorax* 2011;66:430-437.
31. Gould GA, Redpath AT, Ryan M, Warren PM, Best JJ, Flenley DC, MacNee W. Lung ct density correlates with measurements of airflow limitation and the diffusing capacity. *Eur Respir J* 1991;4:141-146.

Figure legends

Figure 1: Study profile of cross-sectional analysis.

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.

Figure E1: 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.

Figure E2: Study profile of longitudinal analysis.

Tables

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]			
Never smoker	31 (18.8)	242 (37.5)	<0.001
Former smoker	107 (64.8)	368 (57.1)	
Current smoker	27 (16.4)	35 (5.4)	
Pack-years 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,	152.0 (23.0)	153.0 (28.0)	0.472
Mean diastolic blood pressure,	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 ε3ε3 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

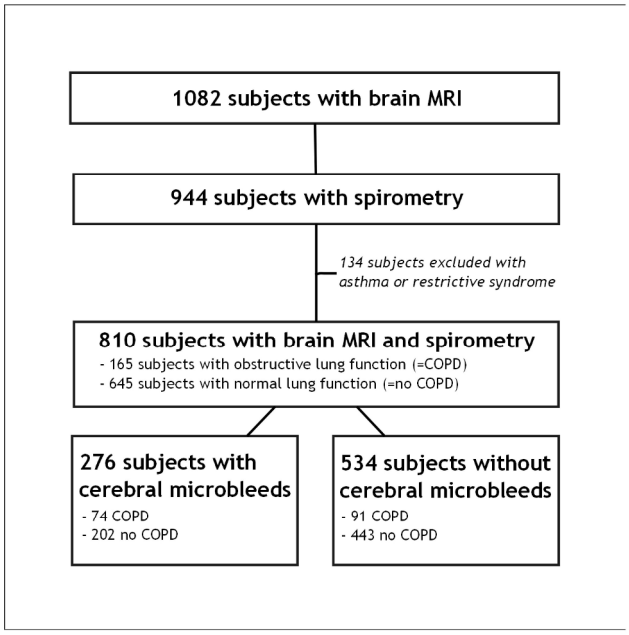
Table 2: COPD and the risk on cerebral microbleeds in deep or infratentorial locations (n=633)

	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 parameters	**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

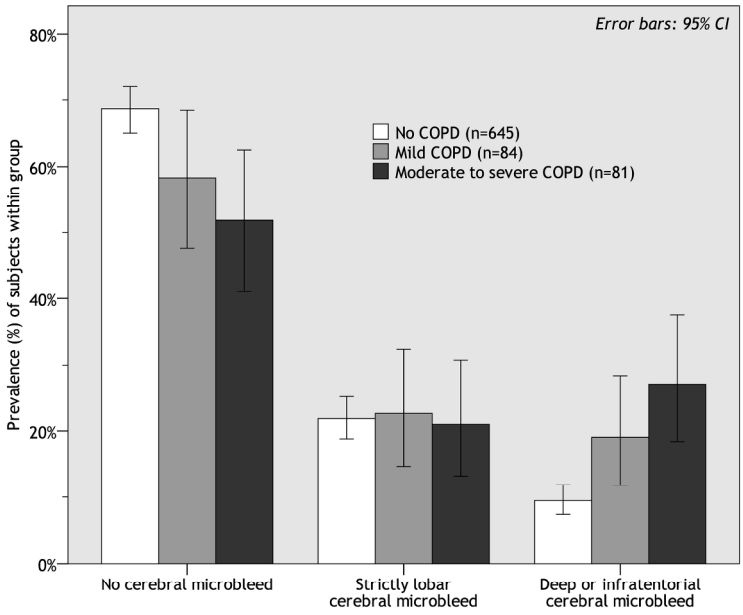
Model 1: age and sex adjusted.
Model 2: adjusted for age, sex, smoking status, and pack-years.

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(20) 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(4) 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



221x426mm (300 x 300 DPI)



228x154mm (300 x 300 DPI)

Online Data Supplement

Chronic Obstructive Pulmonary Disease and cerebral microbleeds: The Rotterdam Study

Lies Lahousse [a,b], Meike W.Vernooij [b,c], Sirwan K.L.Darweesh [b], Saloua Akoudad [b-d], Daan W. Loth [b,e], Guy F. Joos [a], Albert Hofman [b,f], Bruno H. Stricker [b,e-g], M. Arfan Ikram [b-d], Guy G. Brusselle [a,b]

Additional information on the methods

Assessment and staging of 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.(1, 2) The diffusing capacity of the lungs measured using carbon monoxide was corrected for the haemoglobin concentration ($DL_{CO,c}$).(2) 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.(3) In addition, according to the GOLD 2011 update, the influence of respiratory symptoms was evaluated.(4) 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).(5) 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 [$FEV_1/FVC \geq 70\%$ 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 centre within the study period, however, diagnosis was made by a pulmonologist.

Assessment of cerebral microbleeds on MRI

As previously described, all participants of the Rotterdam Scan Study underwent a multisequence MRI protocol on a 1.5-T scanner (GE Healthcare).(6) 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.(7) 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.(6, 8) Intraobserver and interobserver reliabilities for microbleed rating were very good ($\kappa = 0.85–0.87$) and review of the initial ratings by an experienced neuroradiologist yielded a very high accordance.(9)

Covariables

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.(5, 10) 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.(11) With regard to cerebrovascular disease status, a known history of stroke was assessed on entry of study participants into the Rotterdam Study.(12) 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.(13)

Table E1: Baseline characteristics of the longitudinal study population (n=553)

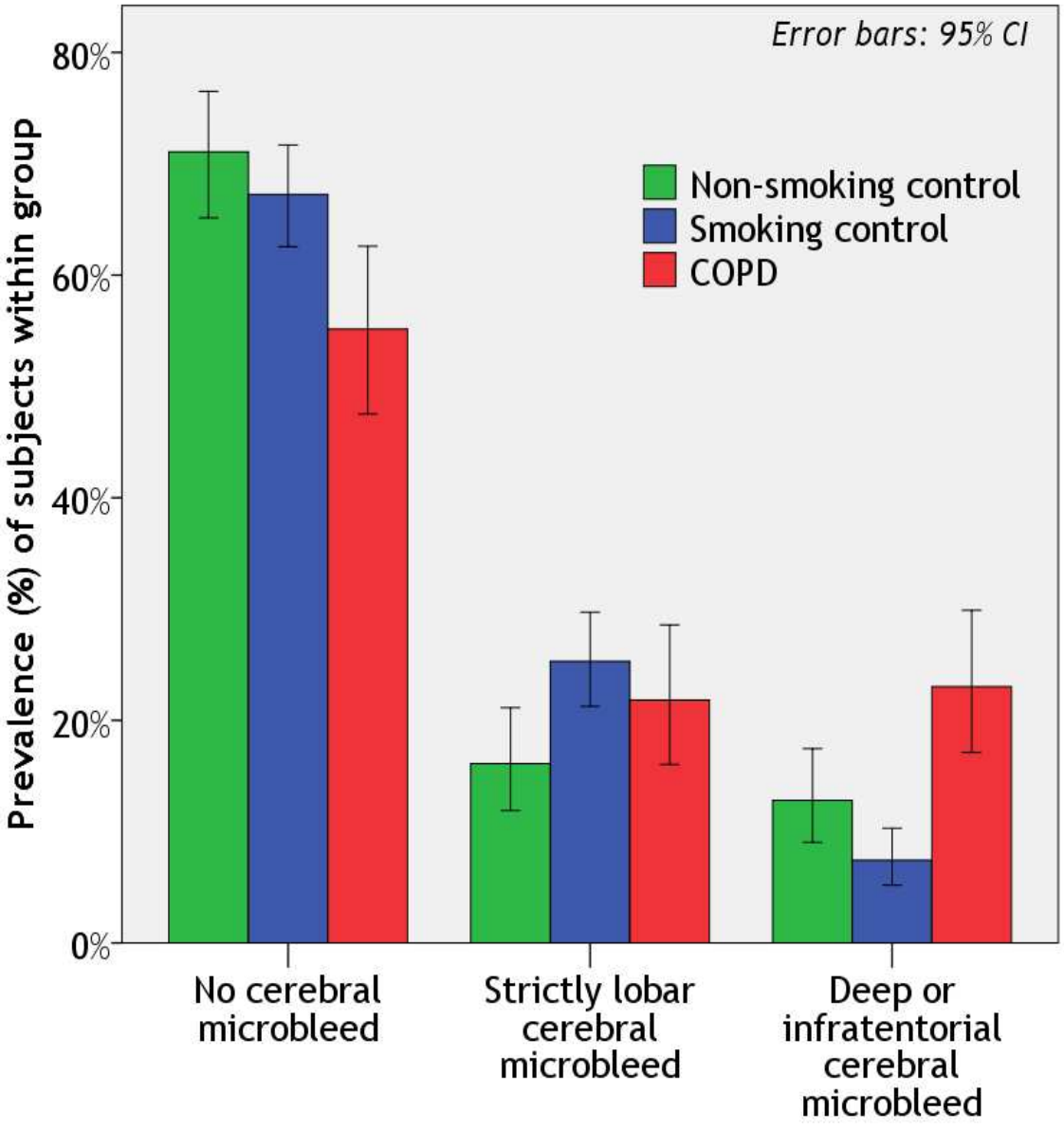
Covariable measured at baseline (=first MRI)	COPD (n=46)	No COPD (n= 507)	p-value
Age, *years*	66 (9)	66 (6)	0.122
Males	30 (65.2)	248 (48.9)	0.034
Smoking status[1]			<0.001
Never smoker	5 (11.1)	181 (36.1)	
Former smoker	23 (51.1)	276 (55.1)	
Current smoker	17 (37.8)	44 (8.8)	
Pack-years cigarette 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

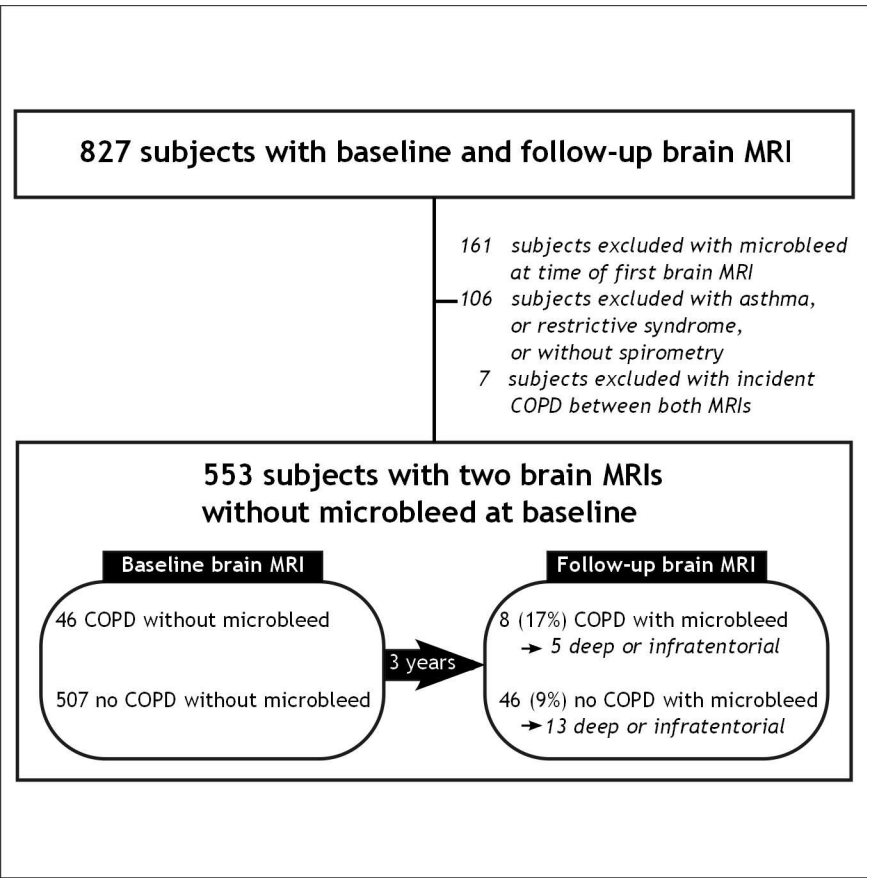
Categorical variables are expressed as numbers (percentage). Values of continuous variables are expressed as median (IQR). [1]Smoking status and pack-years were self-reported. [2]Diabetes mellitus was defined as blood glucose-lowering medication use and/or a non-fasting serum glucose level of ≥11.1 mmol/L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

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

REFERENCES

1. Celli BR, MacNee W, Agusti A, Anzueto A, Berg B, Buist AS, Calverley PMA, Chavannes N, Dillard T, Fahy B, et al. Standards for the diagnosis and treatment of patients with copd: A summary of the ats/ers position paper. *Eur Respir J* 2004;23:932-946.
2. Macintyre N, Crapo RO, Viegi G, Johnson DC, van der Grinten CP, Brusasco V, Burgos F, Casaburi R, Coates A, Enright P, et al. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J* 2005;26:720-735.
3. Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, Fukuchi Y, Jenkins C, Rodriguez-Roisin R, van Weel C, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: Gold executive summary. *Am J Respir Crit Care Med* 2007;176:532-555.
4. Vestbo J, Hurd SS, Agusti AG, Jones PW, Vogelmeier C, Anzueto A, Barnes PJ, Fabbri LM, Martinez FJ, Nishimura M, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: Gold executive summary. *Am J Respir Crit Care Med* 2013;187:347-365.
5. 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;187:58-64.
6. Ikram MA, van der Lugt A, Niessen WJ, Krestin GP, Koudstaal PJ, Hofman A, Breteler MM, Vernooij MW. The rotterdam scan study: Design and update up to 2012. *Eur J Epidemiol* 2011;26:811-824.
7. Vernooij MW, Ikram MA, Wielopolski PA, Krestin GP, Breteler MM, van der Lugt A. Cerebral microbleeds: Accelerated 3d t2*-weighted gre mr imaging versus conventional 2d t2*-weighted gre mr imaging for detection. *Radiology* 2008;248:272-277.
8. Greenberg SM, Vernooij MW, Cordonnier C, Viswanathan A, Al-Shahi Salman R, Warach S, Launer LJ, Van Buchem MA, Breteler MM. Cerebral microbleeds: A guide to detection and interpretation. *Lancet Neurol* 2009;8:165-174.
9. Vernooij MW, van der Lugt A, Ikram MA, Wielopolski PA, Niessen WJ, Hofman A, Krestin GP, Breteler MM. Prevalence and risk factors of cerebral microbleeds: The rotterdam scan study. *Neurology* 2008;70:1208-1214.
10. Vernooij MW, Haag MD, van der Lugt A, Hofman A, Krestin GP, Stricker BH, Breteler MM. Use of antithrombotic drugs and the presence of cerebral microbleeds: The rotterdam scan study. *Arch Neurol* 2009;66:714-720.
11. Vernooij MW, van der Lugt A, Ikram MA, Wielopolski PA, Vrooman HA, Hofman A, Krestin GP, Breteler MM. Total cerebral blood flow and total brain perfusion in the general population: The rotterdam scan study. *J Cereb Blood Flow Metab* 2008;28:412-419.
12. Wieberdink RG, Ikram MA, Hofman A, Koudstaal PJ, Breteler MM. Trends in stroke incidence rates and stroke risk factors in rotterdam, the netherlands from 1990 to 2008. *Eur J Epidemiol*;27:287-295.
13. Bots ML, van der Wilk EC, Koudstaal PJ, Hofman A, Grobbee DE. Transient neurological attacks in the general population. Prevalence, risk factors, and clinical relevance. *Stroke* 1997;28:768-773.


Error bars: 95% CI
Prevalence (%) of subjects within group
80%
60%
40%
20%
0%
Non-smoking control
Smoking control
COPD
No cerebral microbleed
Strictly lobar cerebral microbleed
Deep or infratentorial cerebral microbleed


827 subjects with baseline and follow-up brain MRI
161 subjects excluded with microbleed at time of first brain MRI
106 subjects excluded with asthma, or restrictive syndrome, or without spirometry
7 subjects excluded with incident COPD between both MRIs
553 subjects with two brain MRIs without microbleed at baseline
Baseline brain MRI
46 COPD without microbleed
507 no COPD without microbleed
3 years
Follow-up brain MRI
8 (17%) COPD with microbleed
→ 5 deep or infratentorial
46 (9%) no COPD with microbleed
→ 13 deep or infratentorial

Chronic Obstructive Pulmonary Disease and cerebral microbleeds: The Rotterdam Study

Lies Lahousse [a,b], Meike W.Vernooij [b,c], Sirwan K.L.Darweesh [b], Saloua Akoudad [b-d], Daan W. Loth [b,e], Guy F. Joos [a], Albert Hofman [b,f], Bruno H. Stricker [b,e-g], M. Arfan Ikram [b-d], Guy G. Brusselle [a,b]

a. Department of Respiratory Medicine, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium.
b. Department of Epidemiology, Erasmus Medical Center, PO Box 2040, 3000 CA Rotterdam, the Netherlands.
c. Department of Radiology, Erasmus Medical Center, PO Box 2040, 3000 CA Rotterdam, the Netherlands.
d. Department of Neurology, Erasmus Medical Center, PO Box 2040, 3000 CA Rotterdam, the Netherlands.
e. Inspectorate of Healthcare, The Hague, the Netherlands.
f. Members of the Netherlands Consortium on Healthy Aging (NCHA), Leiden, the Netherlands.
g. Department of Medical Informatics, Erasmus Medical Center, PO Box 2040, 3000 CA Rotterdam, the Netherlands

Corresponding author: Bruno H. Stricker; Department of Epidemiology; Erasmus Medical Center; PO Box 2040; 3000 CA Rotterdam, the Netherlands; Phone: +31 (0)10 70 44294; E-mail: b.stricker@erasmusmc.nl

Authors' contributions: *Study Design:* A.H., M.W.V., M.A.I., B.H.S. ; *Data collection:* L.L., M.W.V., S.A., D.W.L ; *Data-analysis and writing:* L.L., S.K.L.D; *Data-interpretation:* L.L., S.K.L.D, D.W.L., B.H.S., M.W.V., M.A.I., G.G.B.; *Critical Review:* S.A., D.W.L., G.F.J., A.H., B.H.S., M.W.V., M.A.I., G.G.B.

Funding: This study was supported by the Fund for Scientific Research Flanders (FWO) project 3G019309, the Netherlands Organization for Scientific Research (NWO) grants 904-61-093 and 918-46-615. Lies Lahousse is the recipient of a Belgian Thoracic Society Fellowship. Meike Vernooij is supported by an Erasmus MC Fellowship grant. Sirwan Darweesh is supported by a grant from the Royal Netherlands Academy of Arts and Sciences (KNAW). The funding source had no involvement in the collection, analysis, writing, interpretation, nor in the decision to submit the paper for publication.

Running title: COPD and cerebral microbleeds

Subject Code List: 9.4 COPD: Comorbidities

Total word count manuscript body: 2363

At a Glance Commentary:

What is the current scientific knowledge on this subject? Two cross-sectional studies suggest that COPD is related with white matter lesions through cerebral small vessel disease.(1, 2) However, the association between COPD and cerebral microbleeds as marker of cerebral small vessel disease, and the cerebral microbleed location indicative for the underlying disease mechanism, needs to be elucidated.

What does this study add to the field? Our study shows for the first time, in a large, population-based cohort study, that COPD increases the risk of developing cerebral microbleeds in deep or infratentorial locations, cross-sectionally and moreover, longitudinally in subjects without microbleed at baseline.

This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org

ABSTRACT

Rationale: 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).

Main 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.

Word count abstract: 246 - Key words: Cerebral Small Vessel Disease; Airflow Obstruction, Chronic; Magnetic Resonance Imaging; strictly lobar cerebral microbleed; deep or infratentorial cerebral microbleed

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.(3) 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.(4) 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.(5-8) 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.(5, 9) These microbleeds, which consist of hemosiderin deposits in macrophages, can be visualized on Magnetic Resonance Imaging (MRI) as small areas of hypointensity.(10) Microbleeds rarely disappear, making them suitable markers for cumulative cerebrovascular damage.(11) 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.(9, 12)

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.(13) The study started in 1990 and all participants are invited every 3 to 4 years to the research centre 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.(14) 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).(11, 14) 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 Centre, 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 and in more detail in the Online Data Supplement.(15) 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.(16, 17) 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 and in more detail in the Online Data Supplement, all participants of the Rotterdam Scan Study underwent a multisequence MRI protocol on a 1.5-T scanner and cerebral 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.(9, 14). 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).(9, 18, 19) 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).(11)

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%. More detail on the covariables is provided in the Online Data Supplement. 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 centre 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 (20) into 84 (50.9%) mild, 73 (44.2%) moderate and 8 (4.8%) severe COPD; and according to the updated GOLD group categorization (4) 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. 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)* 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). *Figure E1* 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)).

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 E2)* 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 E1* 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 E2)*

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 packyears, 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.

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.(9) 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.(2, 21)

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.(9) 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.(15) 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.(15, 22, 23) 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.(24) 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.(25, 26) The high percentage of subjects in group A and the very small number in group C are in line with other general population studies.(25-27)

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.(17) 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.(21) 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.(3) 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.(2, 15, 28, 29) 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.(30) 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.(21) 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.(21)

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.(9)

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 DLCO, it is not the gold standard to measure emphysema.(31)

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.

Acknowledgments

The authors thank the study participants, the staff from the Rotterdam Study and the participating general practitioners.

REFERENCES

1. van Dijk EJ, Vermeer SE, de Groot JC, van de Minkelis J, Prins ND, Oudkerk M, Hofman A, Koudstaal PJ, Breteler MM. Arterial oxygen saturation, copd, and cerebral small vessel disease. *J Neurol Neurosurg Psychiatry* 2004;75:733-736.
2. Dodd JW, Chung AW, van den Broek MD, Barrick TR, Charlton RA, Jones PW. Brain structure and function in chronic obstructive pulmonary disease: A multimodal cranial magnetic resonance imaging study. *Am J Respir Crit Care Med* 2012;186:240-245.
3. Brusselle GG, Joos GF, Bracke KR. New insights into the immunology of chronic obstructive pulmonary disease. *Lancet* 2011;378:1015-1026.
4. Vestbo J, Hurd SS, Agusti AG, Jones PW, Vogelmeier C, Anzueto A, Barnes PJ, Fabbri LM, Martinez FJ, Nishimura M, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: Gold executive summary. *Am J Respir Crit Care Med* 2013;187:347-365.
5. Pantoni L. Cerebral small vessel disease: From pathogenesis and clinical characteristics to therapeutic challenges. *Lancet Neurol* 2010;9:689-701.
6. Dodd JW, Getov SV, Jones PW. Cognitive function in copd. *Eur Respir J* 2010;35:913-922.
7. Beauchamp MK, Sibley KM, Lakhani B, Romano J, Mathur S, Goldstein RS, Brooks D. Impairments in systems underlying control of balance in copd. *Chest* 2012;141:1496-1503.
8. Eisner MD, Blanc PD, Yelin EH, Sidney S, Katz PP, Ackerson L, Lathon P, Tolstykh I, Omachi T, Byl N, et al. Copd as a systemic disease: Impact on physical functional limitations. *Am J Med* 2008;121:789-796.
9. Greenberg SM, Vernooij MW, Cordonnier C, Viswanathan A, Al-Shahi Salman R, Warach S, Launer LJ, Van Buchem MA, Breteler MM. Cerebral microbleeds: A guide to detection and interpretation. *Lancet Neurol* 2009;8:165-174.
10. Fazekas F, Kleinert R, Roob G, Kleinert G, Kapeller P, Schmidt R, Hartung HP. Histopathologic analysis of foci of signal loss on gradient-echo t2*-weighted mr images in patients with spontaneous intracerebral hemorrhage: Evidence of microangiopathy-related microbleeds. *AJNR Am J Neuroradiol* 1999;20:637-642.
11. Poels MM, Ikram MA, van der Lugt A, Hofman A, Krestin GP, Breteler MM, Vernooij MW. Incidence of cerebral microbleeds in the general population: The rotterdam scan study. *Stroke* 2011;42:656-661.
12. Vernooij MW, van der Lugt A, Ikram MA, Wielopolski PA, Niessen WJ, Hofman A, Krestin GP, Breteler MM. Prevalence and risk factors of cerebral microbleeds: The rotterdam scan study. *Neurology* 2008;70:1208-1214.
13. Hofman A, van Duijn CM, Franco OH, Ikram MA, Janssen HL, Klaver CC, Kuipers EJ, Nijsten TE, Stricker BH, Tiemeier H, et al. The rotterdam study: 2012 objectives and design update. *Eur J Epidemiol* 2011;26:657-686.
14. Ikram MA, van der Lugt A, Niessen WJ, Krestin GP, Koudstaal PJ, Hofman A, Breteler MM, Vernooij MW. The rotterdam scan study: Design and update up to 2012. *Eur J Epidemiol* 2011;26:811-824.
15. 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;187:58-64.
16. Celli BR, MacNee W, Agusti A, Anzueto A, Berg B, Buist AS, Calverley PMA, Chavannes N, Dillard T, Fahy B, et al. Standards for the diagnosis and treatment of patients with copd: A summary of the ats/ers position paper. *Eur Respir J* 2004;23:932-946.
17. Macintyre N, Crapo RO, Viegi G, Johnson DC, van der Grinten CP, Brusasco V, Burgos F, Casaburi R, Coates A, Enright P, et al. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J* 2005;26:720-735.
18. Jeerakathil T, Wolf PA, Beiser A, Hald JK, Au R, Kase CS, Massaro JM, DeCarli C. Cerebral microbleeds: Prevalence and associations with cardiovascular risk factors in the framingham study. *Stroke* 2004;35:1831-1835.
19. Roob G, Schmidt R, Kapeller P, Lechner A, Hartung HP, Fazekas F. Mri evidence of past cerebral microbleeds in a healthy elderly population. *Neurology* 1999;52:991-994.
20. Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, Fukuchi Y, Jenkins C, Rodriguez-Roisin R, van Weel C, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: Gold executive summary. *Am J Respir Crit Care Med* 2007;176:532-555.

21. Poels MM, Vernooij MW, Ikram MA, Hofman A, Krestin GP, van der Lugt A, Breteler MM. Prevalence and risk factors of cerebral microbleeds: An update of the rotterdam scan study. *Stroke* 2010;41:S103-106.
22. Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med* 1999;340:115-126.
23. Poels MM, Zaccai K, Verwoert GC, Vernooij MW, Hofman A, van der Lugt A, Witteman JC, Breteler MM, Mattace-Raso FU, Ikram MA. Arterial stiffness and cerebral small vessel disease: The rotterdam scan study. *Stroke* 2012;43:2637-2642.
24. Agusti A, Vestbo J. Current controversies and future perspectives in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2011;184:507-513.
25. Lange P, Marott JL, Vestbo J, Olsen KR, Ingebrigtsen TS, Dahl M, Nordestgaard BG. Prediction of the clinical course of chronic obstructive pulmonary disease, using the new gold classification: A study of the general population. *Am J Respir Crit Care Med* 2012;186:975-981.
26. Johannessen A, Nilsen RM, Storebo M, Gulsvik A, Eagan T, Bakke P. Comparison of 2011 and 2007 gold guidelines for predicting mortality and hospitalization. *Am J Respir Crit Care Med* 2013.
27. Agusti A, Hurd S, Jones P, Fabbri L, Martinez F, Vogelmeier C, Vestbo J, Rodriguez-Roisin R. Frequently asked questions (faqs) about the gold 2011 assessment proposal of copd. *Eur Respir J* 2013.
28. Liao D, Higgins M, Bryan NR, Eigenbrodt ML, Chambless LE, Lamar V, Burke GL, Heiss G. Lower pulmonary function and cerebral subclinical abnormalities detected by mri: The atherosclerosis risk in communities study. *Chest* 1999;116:150-156.
29. Maclay JD, McAllister DA, Mills NL, Paterson FP, Ludlam CA, Drost EM, Newby DE, Macnee W. Vascular dysfunction in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2009;180:513-520.
30. Garcia-Aymerich J, Gomez FP, Benet M, Farrero E, Basagana X, Gayete A, Pare C, Freixa X, Ferrer J, Ferrer A, et al. Identification and prospective validation of clinically relevant chronic obstructive pulmonary disease (copd) subtypes. *Thorax* 2011;66:430-437.
31. Gould GA, Redpath AT, Ryan M, Warren PM, Best JJ, Flenley DC, MacNee W. Lung ct density correlates with measurements of airflow limitation and the diffusing capacity. *Eur Respir J* 1991;4:141-146.

Figure legends

Figure 1: Study profile of cross-sectional analysis.

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.

Figure E1: 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.

Figure E2: Study profile of longitudinal analysis.

Tables

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]			
Never smoker	31 (18.8)	242 (37.5)	<0.001
Former smoker	107 (64.8)	368 (57.1)	
Current smoker	27 (16.4)	35 (5.4)	
Pack-years 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,	152.0 (23.0)	153.0 (28.0)	0.472
Mean diastolic blood pressure,	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 ε3ε3 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

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 parameters	**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(20) 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(4) 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

Online Data Supplement

Chronic Obstructive Pulmonary Disease and cerebral microbleeds: The Rotterdam Study

Lies Lahousse [a,b], Meike W.Vernooij [b,c], Sirwan K.L.Darweesh [b], Saloua Akoudad [b-d], Daan W. Loth [b,e], Guy F. Joos [a], Albert Hofman [b,f], Bruno H. Stricker [b,e-g], M. Arfan Ikram [b-d], Guy G. Brusselle [a,b]

Additional information on the methods

Assessment and staging of 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.(1, 2) The diffusing capacity of the lungs measured using carbon monoxide was corrected for the haemoglobin concentration ($DL_{CO,c}$).(2) 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.(3) In addition, according to the GOLD 2011 update, the influence of respiratory symptoms was evaluated.(4) 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).(5) 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 [$FEV_1/FVC \geq 70\%$ 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 centre within the study period, however, diagnosis was made by a pulmonologist.

Assessment of cerebral microbleeds on MRI

As previously described, all participants of the Rotterdam Scan Study underwent a multisequence MRI protocol on a 1.5-T scanner (GE Healthcare).(6) 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.(7) 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.(6, 8) Intraobserver and interobserver reliabilities for microbleed rating were very good ($\kappa = 0.85–0.87$) and review of the initial ratings by an experienced neuroradiologist yielded a very high accordance.(9)

Covariables

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.(5, 10) 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.(11) With regard to cerebrovascular disease status, a known history of stroke was assessed on entry of study participants into the Rotterdam Study.(12) 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.(13)

Table E1: 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

REFERENCES

1. Celli BR, MacNee W, Agusti A, Anzueto A, Berg B, Buist AS, Calverley PMA, Chavannes N, Dillard T, Fahy B, et al. Standards for the diagnosis and treatment of patients with copd: A summary of the ats/ers position paper. *Eur Respir J* 2004;23:932-946.
2. Macintyre N, Crapo RO, Viegi G, Johnson DC, van der Grinten CP, Brusasco V, Burgos F, Casaburi R, Coates A, Enright P, et al. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J* 2005;26:720-735.
3. Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, Fukuchi Y, Jenkins C, Rodriguez-Roisin R, van Weel C, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: Gold executive summary. *Am J Respir Crit Care Med* 2007;176:532-555.
4. Vestbo J, Hurd SS, Agusti AG, Jones PW, Vogelmeier C, Anzueto A, Barnes PJ, Fabbri LM, Martinez FJ, Nishimura M, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: Gold executive summary. *Am J Respir Crit Care Med* 2013;187:347-365.
5. 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;187:58-64.
6. Ikram MA, van der Lugt A, Niessen WJ, Krestin GP, Koudstaal PJ, Hofman A, Breteler MM, Vernooij MW. The rotterdam scan study: Design and update up to 2012. *Eur J Epidemiol* 2011;26:811-824.
7. Vernooij MW, Ikram MA, Wielopolski PA, Krestin GP, Breteler MM, van der Lugt A. Cerebral microbleeds: Accelerated 3d t2*-weighted gre mr imaging versus conventional 2d t2*-weighted gre mr imaging for detection. *Radiology* 2008;248:272-277.
8. Greenberg SM, Vernooij MW, Cordonnier C, Viswanathan A, Al-Shahi Salman R, Warach S, Launer LJ, Van Buchem MA, Breteler MM. Cerebral microbleeds: A guide to detection and interpretation. *Lancet Neurol* 2009;8:165-174.
9. Vernooij MW, van der Lugt A, Ikram MA, Wielopolski PA, Niessen WJ, Hofman A, Krestin GP, Breteler MM. Prevalence and risk factors of cerebral microbleeds: The rotterdam scan study. *Neurology* 2008;70:1208-1214.
10. Vernooij MW, Haag MD, van der Lugt A, Hofman A, Krestin GP, Stricker BH, Breteler MM. Use of antithrombotic drugs and the presence of cerebral microbleeds: The rotterdam scan study. *Arch Neurol* 2009;66:714-720.
11. Vernooij MW, van der Lugt A, Ikram MA, Wielopolski PA, Vrooman HA, Hofman A, Krestin GP, Breteler MM. Total cerebral blood flow and total brain perfusion in the general population: The rotterdam scan study. *J Cereb Blood Flow Metab* 2008;28:412-419.
12. Wieberdink RG, Ikram MA, Hofman A, Koudstaal PJ, Breteler MM. Trends in stroke incidence rates and stroke risk factors in rotterdam, the netherlands from 1990 to 2008. *Eur J Epidemiol*;27:287-295.
13. Bots ML, van der Wilk EC, Koudstaal PJ, Hofman A, Grobbee DE. Transient neurological attacks in the general population. Prevalence, risk factors, and clinical relevance. *Stroke* 1997;28:768-773.