ORIGINAL ARTICLE

Eosinophil Subtypes in Adults with Asthma and Adults with Chronic Obstructive Pulmonary Disease

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Abstract

Rationale: There is a differential response to eosinophilic modulation between patients with asthma and those with chronic obstructive pulmonary disease (COPD). There is also evidence of different subtypes of eosinophils in murine models. However, no study has compared eosinophil subtypes in individuals with COPD and in those with asthma.

Objectives: Study the differences in eosinophils subtypes based in the surface protein expression in COPD patients and asthmatic patients.

Methods: We studied 10 stable subjects in each of four groups: subjects with COPD, subjects with asthma, smokers without COPD, and healthy volunteers. Subjects with COPD and those with asthma were matched by age, sex, and FEV_1% predicted. The following variables were determined: anthropometrics, smoking, exacerbation history, medication use, lung function, and comorbidities. Using flow cytometry and confocal microscopy from blood samples, we determined differences in eosinophil surface proteins and classified them as *1*) resident eosinophils (Siglec-8^+CD62L$^+$IL-3R^{lo}) or *2*) inflammatory eosinophils (iEos; Siglec-8^+CD62L^{lo}IL-3R^{hi}). IL-5 receptor was also determined. Findings were validated in 59 patients with COPD and in 17 patients with asthma.

Measurements and Main Results: Patients with asthma had a higher proportion of iEos (25 ± 15%) compared with those with COPD (0.5 ± 1%), smokers without COPD (0.14 ± 0.24%), and healthy volunteers (0.67 ± 1.72%). In patients with asthma, the proportion of iEos was independent of total eosinophil number. iEos had more IL-5 receptors than resident eosinophils (777.02 ± 124.55 vs. 598.35 ± 318.69; $P < 0.01$). In patients with COPD, there was no relation between iEos number and inhaled corticosteroid use, disease severity, or exacerbations rate. The findings in patients with COPD and those with asthma were confirmed in validation cohorts.

Conclusions: There are differences in the subtypes of circulating eosinophils between patients with asthma and those with COPD. This could have clinical implications in the interpretation of eosinophil significance and the approach to therapy in these patients.

Keywords: eosinophils; subtypes; asthma; COPD

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Author Contributions: C.C.L. was involved in the idea and conception of the study and in the recruitment of patients and the procedures performed and also wrote and submitted the manuscript. A.S.S. is the main molecular biologist who carried out the majority of the flow cytometry and is one of the authors who wrote the methodological information in the appendix. A.L.C. was involved in flow cytometry and in selecting the gating strategy for the differentiation of the eosinophils (Hospital Universitario de Gran Canaria Dr. Negrín) and is one of the authors who wrote the methodological information in the appendix. S.C.R. is a molecular biologist who helped with flow cytometry and is one of the authors who wrote the methodological information in the appendix. J.B.A. was involved in flow cytometry and in selecting the gating strategy for the differentiation of the eosinophils (Hospital Nuestra Señora de la Candelaria). E.G.D. conducted all statistical analyses. B.C. was involved in the idea and conception of the study and also provided very valuable insight in writing and correcting the manuscript before submission. C.C.M. was involved in the idea and conception of the study and in the recruitment of patients and the procedures done and also participated in writing the manuscript.

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At a Glance Commentary

Scientific Knowledge on the Subject: Eosinophils are key cells in both chronic obstructive pulmonary disease and asthma. The numbers of these cells in blood are important to direct biological and nonbiological treatment of patients with these conditions. Two subtypes of eosinophils have been described in the murine model and in patients with asthma.

What This Study Adds to the Field: We show how the eosinophil surface protein pattern differs between patients with chronic obstructive pulmonary disease and those with asthma. This could have implications for treatment and might suggest different behavior of eosinophils depending on the pathobiology of the disease.

Eosinophils are pleiotropic leukocytes derived from progenitor cells in the bone marrow that initiate and propagate inflammatory responses and modulate adaptive immunity by activating T cells (1). The concept of eosinophils as pure inflammatory cells has been called into question in recent years (2), as they seem to play several physiological roles in healthy humans; in the intestine, they are involved in the IgA response and in the production of mucus, while in adipose tissue they mediate sensitivity to insulin or conversion to brown fat (3–5). Given this homeostatic role, the eosinophil is believed to also participate in metabolic regulation and organ function in normal subjects (6).

Eosinophils reside in large numbers in the gastrointestinal tract, whereas the lung is not their natural environment. Therefore, the presence of eosinophils in the lung usually indicates a local abnormal inflammatory response. Indeed, eosinophils are known to play an important role in several lung diseases, including eosinophilic pneumonias and granulomatosis, chronic rhinosinusitis with nasal polyps, and asthma. In contrast, there is controversy about the pathobiological role of eosinophils in chronic obstructive pulmonary disease (COPD) (7).

In asthma, eosinophils seem to play a key role. Indeed, elevated blood eosinophil count is associated with poor outcomes (8), and eosinophil count helps differentiate asthma endotypes (9). Moreover, several monoclonal antibodies to different cytokines, specifically IL-5 and its receptor, IL-4, and IL-13, have been beneficial in the management of these patients, alleviating symptoms, decreasing exacerbation rates, and in some cases improving lung function (10–12).

In COPD, the findings are more controversial. On one hand, blood eosinophil count is the first biomarker that has been shown to relate directly to the beneficial effect of inhaled corticosteroids (ICSs) in reducing exacerbations (13, 14). Having more than 300 eosinophils/μl reliably predicts ICS effect (15), and this threshold is currently recommended in different guidelines and documents as valuable for implementing this therapy (16, 17). On the other hand, blood eosinophil counts lower than 100 cells/μl suggest a relatively poor response to ICSs, thereby helping clinicians make informed therapeutic decisions (18). In contrast, two large 1-year trials of monoclonal antibodies (mepolizumab and benralizumab) that significantly decreased blood eosinophil counts in patients with COPD with histories of exacerbation had minimal effects on exacerbation rates, health status, and lung function, suggesting that eosinophils *per se* may not represent a pivotal target for disease modifying therapy in COPD (19, 20).

To explain the differential response to the same biological agents between patients with asthma and those with COPD, we hypothesized that there could be different subtypes of eosinophils in patients with those diseases. This hypothesis is also supported by the presence of two different types of eosinophils in solid tissue in the murine model, showing that eosinophils differed in their location, nuclear morphology, and protein surface expression (21). These differences have given rise to the theory of resident eosinophils (rEos) and inflammatory eosinophils (iEos). However, this has not been properly studied in COPD and compared with these eosinophil subtypes in asthma. Differentiating subtypes of eosinophils may be important, as this could explain different responses to agents targeting eosinophils as a single group. Furthermore, their identification could help select candidates for more precise treatment (biological or nonbiological) in both asthma and COPD.

To test this hypothesis, we explored the differences in protein surface expression of iEos and rEos, as well as markers of specific cell receptors in this cell line (CD125) in patients with asthma, patients with COPD, as well as smokers without COPD and healthy subjects. Some of the results of these studies have been previously reported in the form of an abstract (22).

Methods

Study Design

Core pilot study. Four groups of 10 members each of stable subjects were studied between September 2021 and December 2022. The four groups consisted of *1*) patients with asthma, 2) patients with COPD (matched by age, sex, and FEV_1% predicted with the asthma group), *3*) smokers without COPD, and *4*) healthy subjects. Patients with COPD and smokers without COPD were all part of the CHAIN (COPD History Assessment in Spain [CHAIN cohort]) study (a multicenter, observational, multidimensional, prospective study) and were enrolled at two tertiary hospitals (Hospital Universitario de Gran Canaria Dr. Negrín and Hospital Universitario Nuestra Señora de la Candelaria) (23). Patients with asthma were recruited consecutively as outpatients, with no eosinophilic threshold, and they had to be diagnosed for at least 3 years according to the Global Initiative for Asthma diagnostic criteria (24). Patients with COPD had smoking histories of at least 10 pack-years and had been diagnosed according to the European Respiratory Society/American Thoracic Society criteria (25). Smokers without COPD had smoking histories of more than 10 pack-years, FEV_1/FVC ratios >0.7, and no respiratory symptoms. Healthy subjects were asymptomatic with no comorbidities, had normal findings on spirometry, and were never-smokers. All

This article has a related editorial.

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

subjects and patients were enrolled in the same setting at the same time. Patients with COPD and those with asthma had no exacerbations in the previous 2 months and were treated according to current guidelines. Exacerbations in the previous year were classified according to the therapy received. Systemic steroids were not allowed in the 6 months preceding the blood sample collection. Subjects were excluded if they had any cancer in the past 5 years, if they had eosinophilic diseases different from asthma (eosinophilic granulomatosis with polyangiitis, eosinophilic esophagitis, eosinophilic cystitis, or hypereosinophilic syndrome), or if they were unable to perform spirometry. This study was approved by the ethics committees of Hospital Universitario de Gran Canaria Dr. Negrín and Hospital Universitario Nuestra Señora de la Candelaria.

Blood sampling and eosinophil identification. Freshly unfractionated blood (100 μl) was obtained in the early morning, with the fasting subject in the sitting position. The blood was immediately incubated for 20 minutes at 4 °C with fluorescence-labeled antibodies (*see* Table E1 in the online supplement) to define cell subpopulations, and using flow cytometry, the eosinophils were labeled according to the Mesnil criteria as rEos (Siglec-8^+CD62L$^+$IL-3R^{lo}) or iEos (Siglec-8^+CD62L^{lo}IL-3R^{hi}) (21). We also measured other proteins not included in the Mesnil pattern as CD125 and CD11b, using a FACSCanto (BD Biosciences). In addition, eosinophils from peripheral blood were sorted from the granulocyte suspension using the FACSMelody Cell Sorter (BD Biosciences). Confocal images from eosinophils were obtained using a Zeiss LSM800 with Airyscan microscopy. Eosinophils were sorted using the FACSMelody Cell Sorter on the basis of forward-scatter and side scatter characteristics and CD11b$^+$ CD16$^-$ and Siglec-8^{hi} expression. Methodological details regarding sample processing used for flow cytometry and the gating strategy are described in the online supplement.

COPD and asthma validation cohort. The same measurements and analysis were repeated in 59 patients with COPD and in 17 patients with asthma to confirm the findings observed in the initial COPD and asthma cohorts. In COPD, we explored associations between the proportions of eosinophils subtypes and three outcomes: severity of airflow limitation using the Global Initiative for Chronic Obstructive Lung Disease (GOLD) spirometric criteria, use of ICSs, and history of exacerbations in the year before enrollment.

Statistical Analysis

Numerical variables are expressed as mean ± SD or as median (interquartile range) depending on their Gaussian distribution, and qualitative variables are expressed as frequencies and percentages. The chi-square test or ANOVA and the Kruskal-Wallis test were used for comparisons of qualitative or quantitative variables, respectively. A generalized linear model was used for the comparison, controlling for sex, age, and FEV_1% predicted. Association was assessed using Pearson or Spearman correlation coefficients. *P* values less than 0.05 were considered to indicate statistical significance.

The software used for the analysis was R version 3.6.2 or greater (R Foundation for Statistical Computing) (26).

Results

As planned, in the initial study there were no meaningful differences in age, FEV_1% predicted, or sex between the COPD and asthma groups (Table 1). There were differences in age, number of active smokers, pack-years, and FEV_1% predicted between these patients and the two control groups (smokers without COPD and healthy subjects). Importantly, the number of eosinophils was higher in patients with asthma (Figure 1), as was the measured concentration of IgE, but these were similar among the other three groups. Most patients with asthma were receiving ICSs, but none were receiving any biological treatment.

Table 2 shows that the percentage of iEos was significantly higher in patients with asthma (25.3 ± 15%) compared with the other three groups (0.7 ± 1.1% in the COPD group, 0.1 ± 0.2% in smokers without COPD, and 0.67 ± 1.7% in healthy subjects; $P < 0.001$). No differences in iEos or rEos were found among the healthy subjects, smokers without COPD, and patients with COPD. The maximum observed proportion of iEos in any patient was 5.6% for the whole COPD group, 0.6% among smokers without COPD, 5% among the healthy subjects, and 43.1% among the patients with asthma. The proportion of iEos remained higher in patients with asthma compared with those with COPD matched by eosinophil count.

Differences in the studied biomarkers can be seen in the online supplement (*see* Table E2). We expanded the proteins studied by Mesnil and colleagues (21) and found differences in CD125 (an IL-5 receptor) and CD11b (but this difference was not observed in the larger validation cohorts for COPD and asthma, just in the matched cohorts). There also were statistical differences in CD125 (measured as median fluorescence intensity) in the iEos of the asthmatic population versus rEos from healthy subjects, smokers without COPD, and the whole COPD group. CD125 and CD62L were also confirmed by confocal microscopy (Figures 2 and E4).

In the asthma group, the number of iEos was independent of IgE concentration (*see* Figure E1), the number of exacerbations in the previous year, and the use of rescue medication (all but one patient were on ICSs plus long-acting β_2-agonists). The total number of iEos was higher as the blood count of eosinophils increased, whereas the proportion of iEos remained constant independent of the total number of eosinophils (Figure 3).

COPD and Asthma Validation Cohorts

The characteristics of the validation COPD and asthma cohorts are shown in Table 1. The validation asthma cohort had the same eosinophil blood count and the same proportion of iEos and rEos as the original asthma cohort. The same was observed for concentrations of CD125. Similarly, in the 59 patients with COPD with a wide range of severity of airflow obstruction, the proportion of iEos was similar to that observed in the original patients with COPD (Table 2). In addition, with this larger number of patients, we were able to establish that the number of rEos or iEos was similar across GOLD spirometric grades, and we found no relationship with the number of exacerbations in the previous year, IgE concentration, or the use of ICS (*see* Tables E3–E5). Importantly, the differences persisted between the validation patients with asthma and the extended COPD group (Tables 1 and 2) as observed in the original comparison between matched groups.

Discussion

To our knowledge, this is the first study differentiating subtypes of circulating eosinophils between patients with COPD

Table 1. Main Characteristics of the Different Groups

	Group						
	COPD Matched Group ($n = 10$)	Smokers without COPD ($n = 10$)	Patients with Asthma ($n = 10$)	Healthy Subjects ($n = 10$)	COPD Validation Cohort ($n = 59$)	Asthma Validation Cohort ($n = 17$)	*P* Value (Controlling for Sex, Age, and FEV_1% predicted)
Sex (male)	5 (50)	3 (30)	6 (60)	2 (20)	43 (73)	10 (59)	0.142
Age, yr	62.6 ± 3.0	60 ± 8	53.5 ± 13.0	44.2 ± 12.6	69 ± 9	58.1 ± 11.9	0.001*
Age							
⩽45 yr	—	—	2 (20)	6 (60)	—	2 (12)	<0.001
45–65 yr	7 (70)	9 (90)	6 (60)	4 (40)	20 (34)	9 (53)	
⩾65 yr	3 (30)	1 (10)	2 (20)	—	39 (66)	6 (35)	
BMI, kg/m^2	26.6 ± 4.7	27.5 ± 4.1	24.8 ± 4.9	24.1 ± 4.4	27.3 ± 5.1	29.1 ± 5.4	0.866
Active smokers	8 (80)	9 (90)	1 (10)	—	26 (44)	1 (6)	0.03
Pack-years	42.3 ± 20.9	43 ± 18	7.4 ± 10.0	—	60 ± 27	8.7 ± 17.9	<0.001
FEV_1, L	2.02 ± 0.32	2.69 ± 0.88	2.37 ± 0.87	3.03 ± 0.63	1.22 ± 0.47	2.12 ± 0.76	0.373
FEV_1, %	72.3 ± 8.7	97 ± 12	80.1 ± 20.6	101.0 ± 13.6	47 ± 16	75.7 ± 22.2	0.001*
FVC, L	3.47 ± 0.65	3.48 ± 1.12	3.33 ± 1.25	3.68 ± 1.00	2.64 ± 0.87	3.23 ± 0.90	0.009
FVC, %	96.0 ± 9.9	98 ± 11	92.4 ± 23.6	103.0 ± 16.0	79 ± 18	92.4 ± 18.0	0.005
FEV_1/FVC, %	60.0 ± 6.8	77 ± 5	71.0 ± 6.1	83.2 ± 5.5	46 ± 9	64.9 ± 13.2	0.008
Eosinophils	200 ± 98	192 ± 135	607 ± 429	203 ± 92	239 ± 181	609 ± 826	0.001
Exacerbations	0 (0–0.5)	0 (0–0)	0 (0–2.7)	0 (0–0)	0 (0–1)	—	0.031
0	8 (80)	10 (100)	6 (60)	10 (100)	44 (75)	12 (71)	—
1	—	—	—	—	8 (13)	1 (6)	—
⩾2	2 (20)	—	4 (40)	—	7 (12)	4 (23)	—
Hospitalizations	0 (0–0)	0 (0–0)	0 (0–2.2)	0 (0–0)	0 (0–0)	—	0.015
0	10 (100)	10 (100)	7 (70)	10 (100)	55 (93)	10 (59)	—
1	—	—	—	—	3 (5)	3 (18)	—
⩾2	—	—	3 (30)	—	1 (2)	4 (23)	—
IgE, IU/ml	29 (16–120)	—	135 (48–234)	22 (16–62)	38 (16–127)	101 (25–171)	0.052
ICS, *n* (%)	3 (30)	—	9 (90)	—	27 (46)	13 (77)	<0.001

Definition of abbreviations: BMI = body mass index; COPD = chronic obstructive pulmonary disease; ICS = inhaled corticosteroid.
Data are expressed as *n* (%), mean ± SD, or median (interquartile range).
*No significant difference between the asthma group and the matched COPD group.

and those with asthma. The results show that the proportion of circulating iEos is significantly higher in patients with asthma compared with patients with COPD, smokers without COPD, and healthy control subjects. There were no differences in the proportions of eosinophil subtypes among the latter three groups. Furthermore, the low proportion of iEos observed in the COPD group and the high proportion documented in the patients with asthma in the original cohorts were validated in larger groups of patients with COPD and those with asthma with a wide range of disease severity.

The concept of iEos and (rEos was proposed in recent years (21, 27). These experimental and human studies indicate that there are rEos that have a homeostatic functional role, which is primarily that of dampening responses to T-helper cell type 2–driven stimuli, and a second subtype of iEos, which are recruited by IL-5 and appear to be responsible for the abnormal inflammatory response characteristic of T-helper cell type 2–driven diseases. Januskevicius and colleagues challenged subjects with asthma and observed an increased response in the proportion of iEos that was significantly higher than that observed in healthy control subjects (27). In a different study in patients with eosinophilic esophagitis, Straumann and colleagues showed that administration of mepolizumab (an anti-IL-5 monoclonal antibody) caused eosinophil depletion in the esophagus and blood but not in the duodenum (28). Both studies suggest different sensitivities to IL-5 in different eosinophils, with a population that is independent (or has a lower affinity) of the inflammation driven by IL-5, the rEos, and another subtype with higher sensitivity to this cytokine, the iEos. This concept is further supported by our study, in which we observed more IL-5 receptors in iEos than in rEos from patients with asthma (*see* Table E2).

In patients with asthma, the larger number of IL-5 receptors found in iEos may predispose them to reach full activation at a similar stimulus degree as rEos, which have a smaller number of IL-5 receptors. These findings are apparently contradictory to those of a study that showed a lower count of IL-5 receptors in eosinophils in BAL fluid from patients with asthma (29). However, we can speculate that these differences in the number of IL-5 receptors between the blood and the lung probably arise because once the inflammatory site has been reached, the

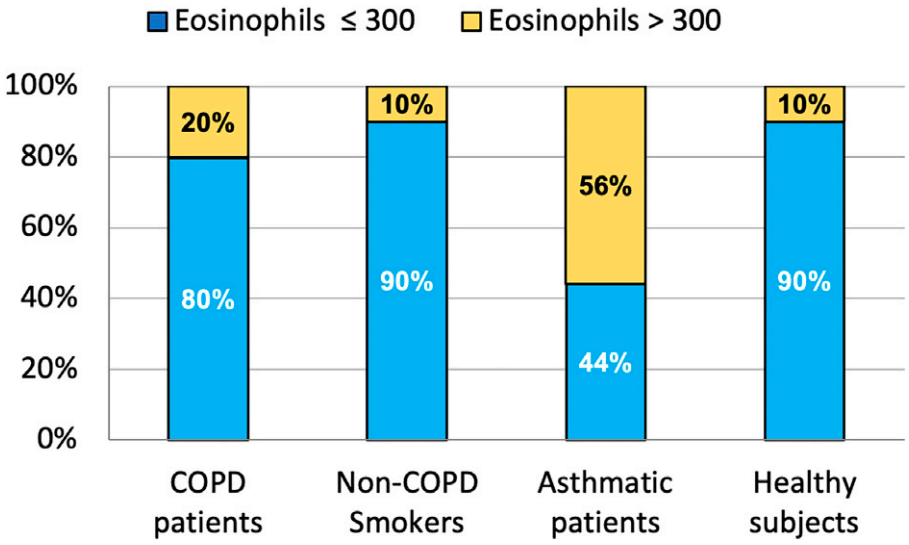

Figure 1. Proportion of individuals per group with <300 eosinophils (the COPD and asthma groups include all patients studied, including both validation cohorts). COPD = chronic obstructive pulmonary disease.

IL-5 binds to the receptors, making them not measurable by flow cytometry and consequently decreasing the CD125 value and lowering their expression. Our findings of high values of CD125 receptors in blood iEos could be useful to expand the characteristics used to define this eosinophil subtype proposed by Mesnil and colleagues (21).

In contrast to the findings observed in patients with asthma, the patients with COPD in our study had a very low number and proportion of iEos in their blood. This may help explain a variety of findings reported in recent studies: *1*) the very limited effect of the monoclonal antibodies against IL-5 and IL-5 receptors mepolizumab and benralizumab in reducing COPD exacerbations in patients with histories of exacerbations and elevated numbers of eosinophils, who were selected as potential candidates to benefit from these therapies (19, 20); and *2*) the observation that well-conducted, careful examinations of airways and lung tissue samples have shown scarce numbers of eosinophils in patients with COPD (30, 31). Coupled with our findings, these observations suggest that the proportion of iEos is low in most patients with COPD. There is only a small proportion of patients with COPD in whom iEos are present and who may represent a targetable treatment group. In our study, even in patients with COPD with high eosinophil blood counts, the presence of iEos did not exceed 5.6%, and the proportions of iEos in both patients with asthma and those with COPD were independent of the number of eosinophils in peripheral blood.

Importantly, blood eosinophils may not relate well to tissue eosinophilia, where local factors can play a role in the differential expression of cell populations. As an example, in a study by Martinez and colleagues in 139 patients from the SPIROMICS (SubPopulations and InteRmediate Outcome Measures In COPD Study) cohort, a larger number of eosinophils was found in BAL fluid from current smokers with COPD compared with never-smokers, current smokers, and former smokers with and without COPD (32). They also found differences in blood compared with sputum counts of eosinophils, but the sputum was collected between 2 and 4 weeks before the blood. Interestingly, the BAL eosinophils showed a low expression of IL-5 receptor, interpreted by the authors as a sign of activation. The results of this study suggest that smoking could alter steady-state localization of eosinophils in the distal lung in patients with COPD relative to smokers (current or former) without COPD. It is important to highlight that Martinez and colleagues did not fully differentiate between iEos and rEos, and assuming that BAL eosinophils are iEos using just one marker could lead to misinterpretation, because eosinophils cannot be accurately differentiated by a single receptor. In our study, we did not perform BAL, and we

Table 2. Differences in Eosinophil Subtypes in the Four Groups of Subjects

	Group				COPD Validation Cohort (*n* = 59)	Asthma Validation Cohort (*n* = 17)	*P* Value (Controlling for Sex, Age and FEV_1% predicted)
	COPD Matched Group (*n* = 10)	Smokers without COPD (*n* = 10)	Patients with Asthma (*n* = 10)	Healthy Subjects (*n* = 10)			
Eosinophils, cells/μl	200 ± 98	192 ± 135	607 ± 429	203 ± 92	239 ± 181	609 ± 826	<0.001
⩽150	3 (30)	4 (40)	—	3 (30)	17 (29)	1 (6)	—
150–299	6 (60)	5 (50)	5 (50)	6 (60)	29 (49)	6 (35)	—
300–499	1 (10)	—	—	1 (10)	8 (14)	6 (35)	—
⩾500	—	1 (10)	5 (50)	—	5 (8)	4 (24)	—
Eosinophils, %	2.7 ± 1.3	2.6 ± 1.8	8.4 ± 7.5	3.0 ± 1.8	2.9 ± 1.9	7.6 ± 8.5	<0.001
rEos, %*	99.3 ± 1.1	99.9 ± 0.2	74.4 ± 15.4	99.3 ± 1.7	99.5 ± 1.1	77.1 ± 13.3	<0.001
iEos, %*	0.70 ± 1.10	0.14 ± 0.24	25.59 ± 15.38	0.67 ± 1.72	0.48 ± 1.1	23.17 ± 13.30	<0.001

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; iEos = inflammatory eosinophils; rEos = resident eosinophils.
Data are expressed as mean ± SD or *n* (%).
*Difference between asthma and any other group.

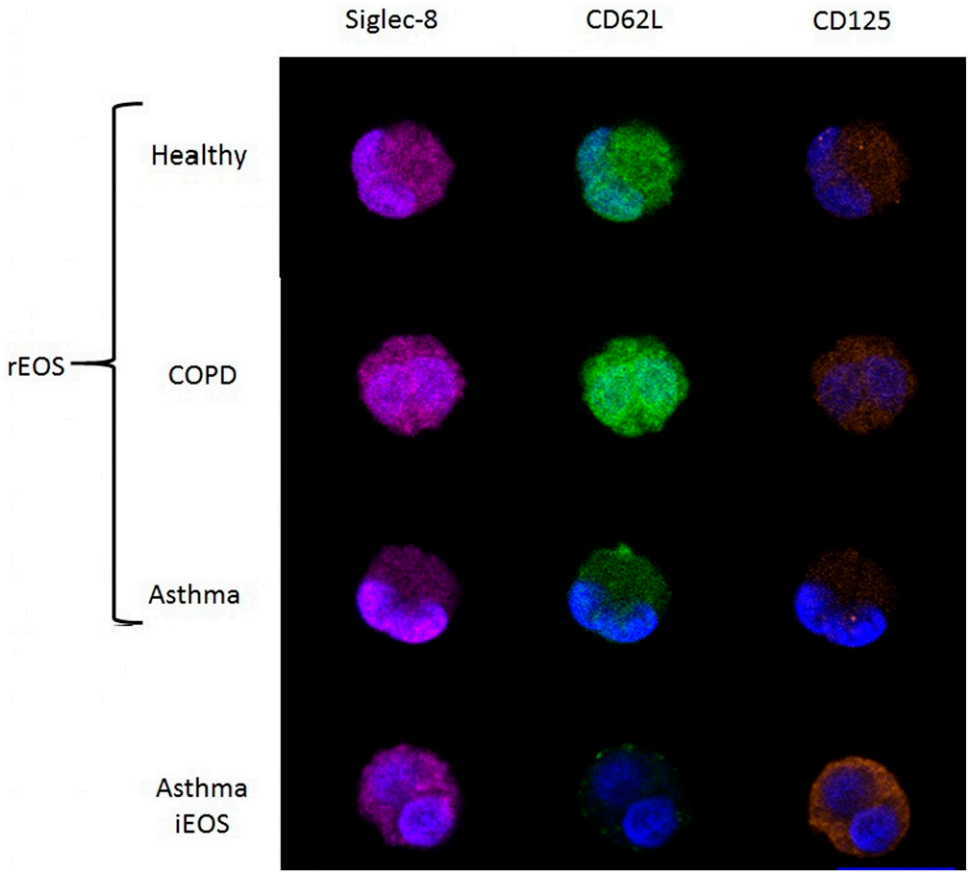

Figure 2. Confocal microscopy of different eosinophil subtypes in the four different groups of subjects. Column 1 (purple) represents Siglec-8, a specific marker for eosinophils, which is similar in the four groups. The second column (green) shows CD62L, an integrin that regulates migration from the vessel to the tissue. IEos in the patients with asthma have significantly less fluorescence than in the rest of the groups. This is because the receptor is already occupied, and it is therefore not possible to detect it on confocal microscopy. The third column (orange) shows CD125 (an IL-5 receptor), which is strikingly different between the patients with asthma and all other groups. COPD = chronic obstructive pulmonary disease; iEos = inflammatory eosinophils; rEos = resident eosinophils.

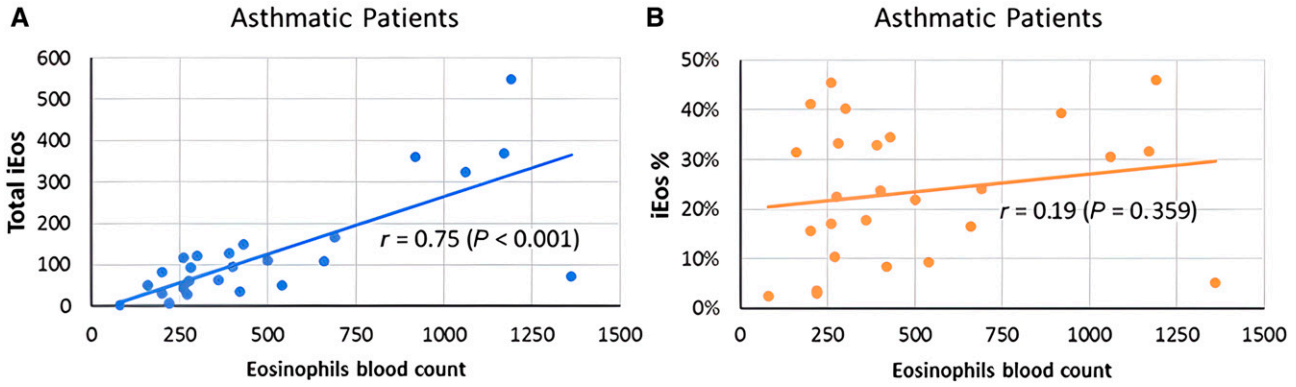

Figure 3. Relation between the proportion and the total number of inflammatory eosinophils (iEos) over the total eosinophil count in all patients with asthma (including the validation cohort). (*A*) Relationship between the total eosinophil count (cells/ml) and the total number of iEos. (*B*) Relationship between the total eosinophil count (cells/ml) and the proportion of iEos. The orange line shows that the percentage of iEos over the total eosinophil count is independent of the total number of eosinophils. Even at low eosinophil count, iEos could account for 40% of the eosinophils.

cannot correlate the differences in blood compared with the lung. We found no difference in iEos or rEos in peripheral blood in current smokers versus former smokers with COPD and current or former smokers without COPD. Importantly, blood eosinophil count is the most widely used biomarker to select patients likely to respond to biological therapies targeting eosinophils. We believe that the presence of iEos versus rEos in peripheral blood complements previous findings and could facilitate precision management of patients likely to respond to those agents, although this needs to be studied.

In the asthma group, the proportion of iEos was independent of the total number of eosinophils. We speculate that the iEos represent eosinophils that could migrate to the site of inflammation mediated by IL-5 and eotaxin, whereas the rEos might distribute to normal eosinophilic destinations (gut, thymus, uterus), with only a small proportion migrating to the lung parenchyma itself. Supporting this concept is the low expression of CD62L, a selectin that facilitates eosinophil transfer from blood to tissue, which we observed in the eosinophils we studied. Our findings could imply that, at the same number of blood eosinophils, the migration of these cells to the inflammation site could be numerically smaller in patients with COPD compared with those with asthma. In the latter group, even at a low eosinophil count, 40% of eosinophils could migrate to the lung, whereas none, or very few, would migrate to the lung in patients with COPD. This effect would be more prominent at a high blood eosinophil count and could explain the effect of monoclonal antibodies such as mepolizumab or benralizumab in patients with asthma with blood eosinophil counts higher than 150 cells/ml. However, this is a hypothesis that must be proved in future research.

It is also interesting that the proportion of iEos in the COPD group was similar across different GOLD spirometric grades, suggesting that eosinophil biology is independent of the severity of disease. The role of eosinophils in COPD exacerbations remains controversial. Some studies have shown that eosinophils are increased in the airways of patients with COPD exacerbations (33, 34), and a large population study reported an association between high blood eosinophil count and the risk of exacerbation (35). However, other studies completed in large observational cohorts of patients with COPD support a minimal, if any, association between blood eosinophil count and risk of COPD exacerbations (36–39). In our study, patients with exacerbations did not have higher iEos counts than those without exacerbations. However, the patients were studied in a stable state, so no correlation could be made with eosinophils during episodes of acute exacerbation. Some authors have postulated that the positive association between elevated circulating eosinophil counts and exacerbations in clinical trials, compared with the lack of such association in observational studies, may be due to the large proportion of patients in observational cohorts who could be receiving ICSs (35). This was not the case in our study, as only one of the patients with COPD in the original cohort and a handful in the validating cohort were receiving ICSs.

Our findings could have several clinical implications. First, the presence of iEos could lead to a better understanding of respiratory eosinophilic diseases. Second, they could be useful in the prescription of biological therapies in asthma, as their number could predict the response to treatment. Third, studies could be designed to precisely target iEos type, while allowing rEos to maintain their homeostatic function. Fourth, it could help to offer the biological therapy currently available for asthma to patients with COPD with the appropriate endotype (those with high iEos counts).

This study has several limitations as well. First, the differences found in this study could be attributed not to the existence of various eosinophil subtypes but rather to different degrees of cell activation, and the cross-sectional design of our study could not establish the longitudinal evolution of cell lines over time. Second, we studied only 10 subjects in each group. However, the differences are very clear and consistent among the groups. Also, to minimize this limitation, we recruited more patients with COPD and with asthma, thus validating the original results for both diseases. Third, because of the technique used to differentiate the eosinophil populations, some of them could have been stimulated and turned into iEos (assuming that the activated cell hypothesis is correct). Nevertheless, this effect would be marginal and would have affected all groups similarly.

Conclusions

This study shows that the proportion of circulating iEos is significantly higher in patients with asthma compared with those with COPD. The amount in these patients with COPD is similar to that in smokers without COPD and healthy control subjects. Differentiating subtypes of eosinophils could help direct the target for biological therapies for both COPD and asthma. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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ORIGINAL ARTICLE

The importance of symptoms in the longitudinal variability of clusters in COPD patients: A validation study

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ABSTRACT

Background and objective: **Cluster analysis has been utilized to explore phenotypic heterogeneity in chronic obstructive pulmonary disease (COPD). To date, little is known about the longitudinal variability of clusters in COPD patients. We aimed to evaluate the 2-year cluster variability in stable COPD patients.**

Methods: **We evaluated the following variables in COPD patients at baseline and 2 years later: age, gender, pack-year history, body mass index (BMI), modified Medical Research Council (MMRC) scale, 6-min walking distance (6MWD), spirometry and COPD Assessment Test (CAT). Patient classification was performed using cluster analysis at baseline and 2 years later. Each patient's cluster variability after 2 years and its parameters associated with cluster change were explored.**

Results: **A total of 521 smokers with COPD were evaluated at baseline and 2 years later. Three different clusters were consistently identified at both evaluation times: cluster A (of younger age, mild airway limitation, few symptoms), cluster B (intermediate) and cluster C (of older age, severe airway limitation and highly symptomatic). Two years later, 70% of patients were unchanged, whereas 30% changed from one cluster to another: 20% from A to B; 15% from B to A; 15% from B to C; 42% from C to B and 8% from C to A. 6MWD, forced expiratory volume in 1 s (FEV_1) % and CAT were the principal parameters responsible for this change.**

Conclusion: **After 2 years of follow-up, most of the COPD patients maintained their cluster assignment. Exercise tolerance, lung function and quality of life were the main driving parameters in those who change their cluster assignment.**

Clinical trial registration: NCT01122758 at ClinicalTrials.gov

SUMMARY AT A GLANCE

This longitudinal analysis of a large chronic obstructive pulmonary disease (COPD) study explored the identification of clusters and their behaviour over 2 years. We validated previous data on the topic but in a multicentre study and with 2 years of follow-up (previously only 1 year). We also identified factors associated with cluster changes.

Key words: chronic obstructive pulmonary disease, clinical factor clusters, longitudinal changes.

Abbreviations: ACOS, asthma–COPD overlap syndrome; ATS, American Thoracic Society; CAT, COPD Assessment Test; CHAIN, COPD History Assessment In SpaiN; GOLD, Global Initiative for Chronic Obstructive Lung Disease; FEV_1, forced expiratory volume in 1 s; HTA, hypertension; MMRC, modified Medical Research Council; OSA, obstructive sleep apnoea.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is an important health problem and is ranked as the third disease burden worldwide since 2010.[1] COPD is characterized by a persistent airflow limitation that is usually progressive and is associated with an enhanced chronic inflammatory response in the airways and lungs to noxious particles or gases, primarily cigarette smoke.[2] COPD is a complex disease with a heterogeneous clinical presentation.[3] Several efforts have been conducted to classify COPD patients into subgroups or clinical phenotypes to allow their easy identification regarding prognostic and therapeutic purposes.[4–9]

Cluster analysis, whose aim is to organize information so that heterogeneous groups of variables can be classified into relatively homogeneous groups,[10] has been proposed for the examination of clinical phenotypic heterogeneity in COPD patients. Several studies have investigated different databases, identifying

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different clusters, but to date, most of them were in cross-sectional studies.[4–9]

Recent data suggest that the most important clinical characteristics of COPD patients could change longitudinally[11] and probably each patient cluster assignment could also change over time. A recent study from Spain reported data on cluster variability in stable COPD patients,[12] suggesting that COPD clusters remain stable over 1 year. To validate these findings, we postulated that clusters of COPD patients are not only stable longitudinally for 1 year but also for 2 years. We therefore explored the identification of clusters at baseline and 2 years after, cluster changes during this time and the parameters associated with these changes.

METHODS

Participants

COPD History Assessment In SpaiN (CHAIN) is a Spanish multicentre study carried out at respiratory medicine clinics. Both active and former smokers with COPD were included. The methodological aspects of the study have been previously described.[13] The definition of the disease was established by a history of smoking of at least 10 pack-years and post-bronchodilator forced expiratory volume in 1 s/forced vital capacity (FEV_1/FVC) ratio <0.70 after the inhalation of 400 μg of albuterol. The main goal of this prospective observational study was to perform a multidimensional evaluation of the evolution of COPD patients to better define the natural history and phenotypes of the disease (ClinicalTrials.gov Identifier: NCT01122758).

The recruitment period was from 15 January 2010 to 31 March 2012. The participated patients are currently being followed up, but the available data at the time of analysis (February 2016) for the present study were collected from the baseline and the 2-year appointments. We evaluated anthropometric data, that is, age, gender, height, weight and body mass index (BMI), pack-year history, respiratory symptoms (modified Medical Research Council (MMRC) dyspnoea scale), health-related quality of life using the COPD Assessment Test (CAT), respiratory function (post-bronchodilation FEV_1 and FVC % of predicted) and exercise tolerance by the 6-min walking distance (6MWD) test. A detailed questionnaire about the presence of co-morbidities was also administered to all patients. The presence of the following co-morbidities was registered: obstructive sleep apnoea (OSA), bronchiectasis, diabetes mellitus, hypertension (HTA), dyslipidaemia, metabolic syndrome, ischaemic cardiomyopathy, heart failure, atrial fibrillation, renal failure, peptic ulcer disease, anxiety, depression, cancer and osteoporosis. Patients with past history of asthma and asthma–COPD overlap syndrome (ACOS) were included in the analysis.

Patient data were anonymized in a database with hierarchical access control in order to guarantee secure access to the information. To be included in the study, the participants provided informed consent as it was approved by each of the ethics committees of the participating centres (Comité de Etica de la Investigación, Hospital Universitario la Candelaria, Tenerife, Institutional Review Board No.: 258/2009).

Clinical and physiological measurements

During a personal interview, trained personnel obtained the following information at the time of recruitment and at subsequent follow-up appointments yearly: age, gender and BMI. Smoking status (current or former) and smoking history (age at initiation and discontinuation, as well as intensity) were registered. From this information, we calculated the total smoking exposure and expressed it as pack-years. Pulmonary function tests were performed following the American Thoracic Society (ATS) guidelines and using the ATS reference values.[14] The 6MWD test was measured after selecting the best of two walks separated by at least 30 min.[15] Dyspnoea was evaluated by the MMRC scale (from 0 to 4).[16] To evaluate health-related quality of life, we used the CAT, a validated eight-item questionnaire, designed to assess and quantify the impact of COPD symptoms on patient health status.[17] We used the Spanish validated version of CAT, and it was self-administered by each patient.

The ACOS was defined using some of the usual features stated by the Global Initiative for Asthma/Global Initiative for Chronic Obstructive Lung Disease (GINA/GOLD) joint project stratified by major and minor criteria to increase the sensitivity and specificity to detect the overlap between COPD and asthma in this cohort as previously described.[18]

Statistical analysis

Quantitative data with a normal distribution were described using mean and SD. Quantitative data with non-normal distribution were described by median and interquartile range (IQR). Categorical data were described using relative frequencies. Paired sample *t*-test was used to compare normally distributed data and Wilcoxon signed-ranks test for non-normally distributed data.

K-means cluster method was used to classify patients into one of the three clinical phenotypic categories. The number of variables to include in the analysis was based on clinical criteria and availability of the data at baseline and 2 years. To represent the most important domains of the disease age, nutritional status, symptoms, exercise capacity and quality of life were included. The variables included were: age, pack-year history, BMI, FEV_1%, FVC%, 6MWD, MMRC and CAT. The number of the categories was selected a priori, using results of the previous studies as guidance,[4–9] which indicated that patients might fall into one of the three different clinical phenotypes: A, B and C. The stability of the clustering solutions was checked by repeating the procedure several times using different starting points, and checking the distance of cases from their cluster, to verify that there were no outliers. K-means cluster is an efficient procedure that attempts to identify relatively homogeneous groups of cases based on the selected characteristics. The algorithm requires the number of clusters in advance, so we selected three based on prior knowledge on the number of different

Table 1 Characteristics of all participants at baseline and 2 years later

Parameter	Baseline $n = 521$ Mean ± SD	2 years later $n = 521$ Mean ± SD	*P*-value
Gender (male %)	83	83	Same population
Age (years)	64.7 ± 9.6	66.7 ± 9.6	2 years later
Pack-year history	54.3 ± 27.9	54.2 ± 28.1	0.54
Active smoking	28%	28%	0.52
BMI (kg/m^2)	28.0 ± 5.0	27.8 ± 4.9	0.05
FEV_1%	63.9 ± 22.4	64.8 ± 23.5	0.07
FVC%	87.7 ± 22.2	88.7 ± 23.1	0.07
FEV_1/FVC	56.3 ± 14	55 ± 14	<0.001
6MWD (m)	453.3 ± 106.6	454.3 ± 119.4	0.49
MMRC	1.6 ± 0.8	1.6 ± 0.8	0.96
CAT points	12.0 ± 7.2	10.5 ± 7.3	<0.001
ACOS (%)	15	15	0.95

6MWD, 6-min walking distance; ACOS, asthma–COPD overlap syndrome; CAT, COPD Assessment Test; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; MMRC, modified Medical Research Council.

phenotypes to be found.[4–9] Ordering of the initial cluster centres may affect the solution if there are tied distances from cases to cluster centres. To assess the stability of a given solution, we have compared results from analyses with different permutations of the initial order of the cases. We used statistical package SPSS version 20.0 Inc. (Chicago, IL, USA). A *P*-value <0.05 was considered statistically significant.

RESULTS

A total of 521 smokers with COPD were evaluated at recruitment and at 2 years follow-up. Figure 1 shows the CONSORT diagram of the participants. Their clinical and physiological characteristics are provided in Table 1. This large sample of mainly males, mostly former smokers with moderate degree of airway limitation patients, had a good exercise capacity, mild degree of dyspnoea and quality of life impairment. Their clinical characteristics remained mainly unchanged after 2 years of follow-up except for the CAT. Their treatment remained unchanged during the follow-up time.

Table S1 and Figure S1 (Supplementary Information) show the baseline characteristics of patients who died or were lost during the follow-up time compared with those included in the final analysis. Statistically significant differences were only found in those lost in the follow-up in age, 6MWD, MMRC, prevalence of HTA, metabolic syndrome and atrial fibrillation.

Table 2A shows the baseline characteristics of the three 'clusters' identified by the analysis and Table 2B shows the characteristics 2 years later. Cluster A is represented by younger COPD patients with better lung function, exercise tolerance and mild symptoms. Cluster C includes older patients with worse lung function, exercise tolerance and severe symptoms. Cluster B has COPD patients with clinical characteristics between the two previously described clinical clusters. Interestingly, patients assigned to this cluster have a higher prevalence of ACOS at baseline but the prevalence is the same in the three clusters after 2 years.

Figure 2 shows the co-morbidities associated with the entire population and with each cluster. Patients included in cluster C have a higher prevalence of OSA, diabetes mellitus, HTA, dyslipidaemia, metabolic syndrome, atrial fibrillation, congestive heart failure and renal failure. Patients in cluster A have a lower percentage of co-morbidities.

Figure 3 shows the percentage of patients who changed their cluster assignment during the 2 years follow-up time. An important percentage of patients changed their cluster assignment longitudinally: 20% of the patients at recruitment in A, 30% in B and 50% in C. Table S2 (Supplementary Information) shows in its different panels, the comparison between the parameters at recruitment and 2 years later of those patients

Table 2 Clinical characteristics of the clusters identified at baseline and 2 years later

	Baseline clusters						Clusters 2 years later					
	A $n = 188$ (36%)		B $n = 235$ (45%)		C $n = 98$ (19%)		A $n = 208$ (40%)		B $n = 234$ (45%)		C $n = 79$ (15%)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	61.6	8.1	67.8	7.7	69.5	9.3	64.1	8.5	68.3	8.3	71.1	8.9
Pack-years	50.9	24.1	55.8	29.2	60.1	30.4	50.5	24.6	55.7	28.0	63.8	35.7
BMI (kg/m^2)	27.2	4.2	28.5	4.9	29.2	6.2	27.0	4.2	28.2	4.9	28.7	5.8
FEV_1%	66.7	21.3	56.5	18.3	51.7	18.9	69.6	21.0	57.0	19.5	45.6	17.6
FVC%	95.1	23.2	82.8	22.0	78.0	19.9	97.6	22.5	83.2	21.6	76.9	23.5
6MWD (m)	549.8	45.5	428.2	38.3	285.1	62.8	554.4	44.9	426.6	38.4	264.9	71.6
MMRC	1.3	0.5	1.8	0.7	2.2	0.9	1.3	0.5	1.7	0.7	2.4	0.9
CAT (points)	11.0	6.9	13.3	6.8	13.9	8.1	9.6	6.8	11.8	7.6	13.9	7.4
ACOS (%)	14		13		20		13		14		14	

6MWD, 6-min walking distance; ACOS, asthma–COPD overlap syndrome; CAT, COPD Assessment Test; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; MMRC, modified Medical Research Council.

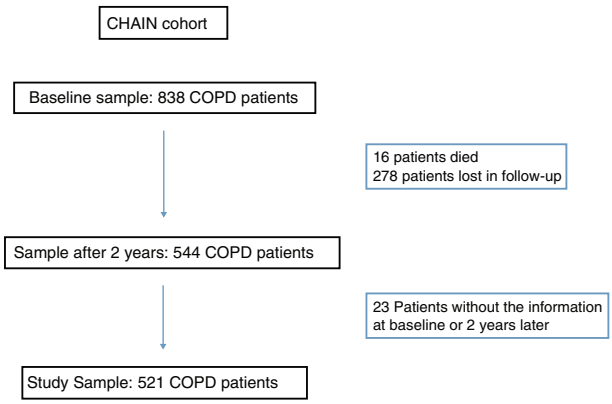

Figure 1 CONSORT diagram of the participants. Patients with the following information at baseline and after 2 years were included: age, gender, height, weight, BMI, pack-year history, respiratory symptoms (MMRC), health-related quality of life using CAT, respiratory function (post-bronchodilation FEV_1 and FVC% predicted) and 6MWD exercise tolerance. 6MWD, 6-min walking distance; CAT, COPD Assessment Test; CHAIN, COPD History Assessment In SpaiN; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; MMRC, modified Medical Research Council.

who changed their cluster assignment. Regarding the patients in cluster A or B, exercise tolerance measured by the 6MWD was the main parameter driving these changes. Patients assigned to cluster C could only improve to B or A clusters and lung function, exercise capacity and quality of life were the main parameters responsible for their changes.

DISCUSSION

The main findings of this longitudinal analysis of clusters in stable COPD patients are: (i) patients were grouped into similar clusters at recruitment and 2 years later; (ii) an important percentage of patients (70% of them) remained in the same cluster at recruitment and 2 years later; and (iii) exercise tolerance, lung function and quality of life modifications were responsible for the changes in cluster assignment regarding the patients who changed after 2 years.

The technique of 'cluster analysis' has been recently applied to COPD patients in an effort to organize heterogeneous variables into relatively homogenous groups.[4–9] This could allow clinicians to identify different groups of patients with similar characteristics mainly for prognostic and therapeutic purposes.

Several studies have been published describing different COPD clusters, with variations in the type of clusters identified according to the population study and the variables included in the analysis: clinical,[4,5] radiological,[8,9] biochemical[7,9] or genetic.[8] Two main clusters have been consistently found in most studies. One that included older patients with high BMI, highly symptomatic and with concomitant cardiovascular co-morbidities as described by Burgel *et al.*,[4,5] Garcia Aymerich *et al.*,[6] Vanfleteren *et al.*[7] and Rennard *et al.*[9] A second one that included patients with severe airflow limitation, disease exacerbations, nutritional depletion, muscle weakness and emphysema as described by Burgel *et al.*,[4,5] Vanfletteren *et al.*[7] and Rennard *et al.*[9]

In the present study, patients included in cluster C could be represented by the first of the above-mentioned cluster: older patients with lower FEV_1%, high BMI, highly symptomatic and with concomitant co-morbidities such as diabetes mellitus, high blood pressure, metabolic syndrome, OSA and cardiovascular problems (atrial fibrillation, ischaemic cardiomyopathy and heart failure). Those in cluster A could be represented by the second cluster: younger patients with significant impairment of their lung function for their age, lower BMI and lower incidence of cardiovascular co-morbidities. Interestingly Pinto *et al.*[19] performed a systematic review of previously published studies on cluster analysis in COPD patients, including eight studies in patients with similar characteristics (male from tertiary university hospitals, in this case with severe disease), reporting that the number of identified clusters goes from 2 to 5, although two were consistently identified similar to clusters C and A from our study.

Cluster B, the most prevalent, is an intermediate one that includes patients with clinical and physiological

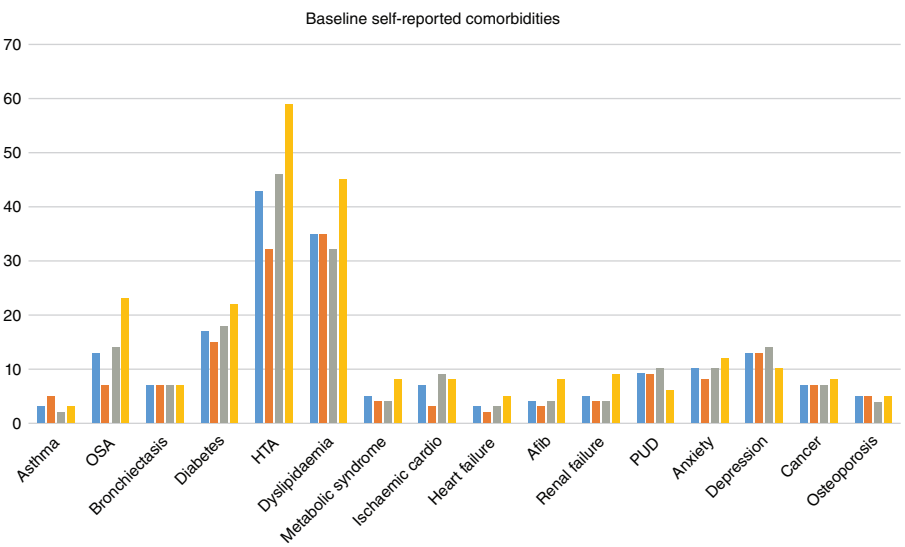

Figure 2 Co-morbidities associated to the entire population (■) and to each clinical cluster (■, A; ■, B; ■, C). Afib, atrial fibrillation; Cardio, cardiomyopathy; HTA, hypertension; OSA, obstructive sleep apnoea; PUD, peptic ulcer disease.

characteristics between the other two. This cluster could be similar to phenotypes 3 and 4 as described by Burgel *et al.* in their landmark studies of cluster analysis in COPD patients.[4,5] The present finding is important, because it reproduces and most importantly validates previously described clusters, but in this occasion is a result of a multicentre, tertiary care level study with a large cohort of COPD patients.

The most relevant information provided by the present study is the longitudinal change pattern of clusters in a population of stable COPD patients. First, we proved that cluster analysis like the one performed in this study identified the same clusters at recruitment and 2 years later. Their characteristics did not change, which was an expected finding as it involves the same population. The selection of number of clusters was done a priori based on clinical judgement and previous reports,[4–9] representing the different domains of the disease: age, symptoms, exercise capacity, quality of life and airway obstruction. The present study findings confirmed those from Esteban *et al.*[12] giving further longitudinal reproducibility to a technique that is becoming more popular for the identification of common clinical characteristics in COPD patients.[20]

Second and most importantly, we also investigated the longitudinal variability of these clusters. We found that some patients assigned to one cluster at recruitment changed to a different cluster 2 years later (A: 20%, B: 30% and C: 50%). Although this information is not entirely novel, it is clinically relevant since it could imply that if a patient is identified as part of one cluster at a specific time, 2 years later he or she could belong to a different one even if the patient is under standard recommended therapy as it was the case with the patients included in this study.

Interestingly, the present study findings are similar to the ones from Esteban *et al.*[12] who also found that approximately 28% of their large population of stable COPD patients migrated to another cluster the year after, although there are differences in the study population and in the time of follow-up between the two studies. The present study population included patients from several Spanish centres, who were younger, had less degree of airway limitation and most importantly were followed up for a longer period of time.

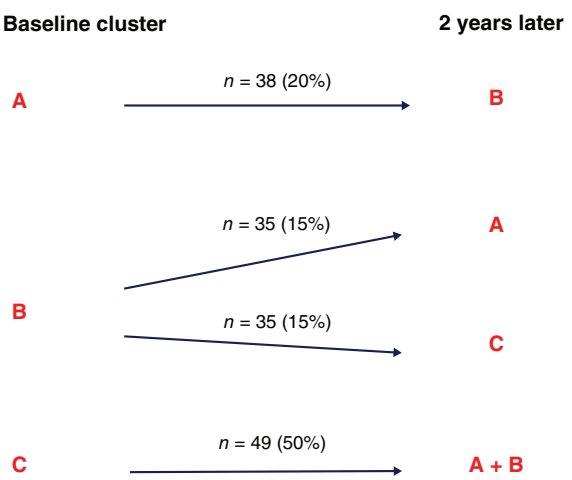

Figure 3 Percentage of patients who changed their cluster assignment during the 2 years of follow-up.

The present study also confirms previous findings from Agusti *et al.*,[21] who indicated that patients' classification (using the new GOLD classification) could change longitudinally in certain types of COPD patients (in their study those in groups B and C), although they did not use cluster analysis. Exploring this finding from another perspective, we could also say that those clusters with a more 'benign' clinical profile like clusters A and B are mainly stable after 2 years. However, half of the patients in cluster C, the ones with 'worse' clinical characteristics, progressed to a better clinical profile cluster after 2 years. This confirms previous findings from another COPD cohort,[22] indicating that in the longitudinal evolution of the disease, not all patients progress unfavourably, suggesting a more favourable prognosis and a better response to standard therapy. This could imply that regular standard therapy as the one applied to the patients included in the present work is effective in moving longitudinally patients into a more favourable cluster with likely a better prognosis, although that was not explored in the present work.

Third, we also explored the parameters associated with changes in cluster assignment and found that 6MWD, FEV_1 and CAT were the ones responsible for these changes and the magnitude of the change is well beyond the clinical significance of each parameter: 25 m for 6MWD, 4 points for CAT and 100 mL for FEV_1. The fact that the clinical characteristics of COPD patients change longitudinally is not novel. Previous studies already reported that important parameters of the disease change longitudinally: 6MWD, dyspnoea MMRC and FEV_1.[11] Esteban *et al.*[12] also reported that the parameters that changed after 1-year follow-up in their patients were exercise tolerance, certain lung function measurements and physical activity. This highlights the importance of longitudinal evaluation of clinical parameters in COPD patients, which mirrors better the natural history of the disease and the patients 'real life'. It also reinforces the importance of the longitudinal evaluation of exercise tolerance with the 6MWD, which provides relevant information about the longitudinal behaviour of the disease.[11] Again, these findings are relevant because they could have implications in the clinical management of these patients, as different studies have demonstrated that these parameters could be modified by therapeutic interventions such as bronchodilation[23] or pulmonary rehabilitation.[24]

Interestingly and as described in previous works, patients in cluster C (older patients with lower FEV_1%, high BMI and highly symptomatic) have a higher prevalence of co-morbidities such as diabetes mellitus, high blood pressure, metabolic syndrome, OSA, cardiovascular problems and interestingly a higher prevalence of ACOS. Clusters A and B have a similar prevalence of most of the described co-morbidities. Unfortunately, the small number of deaths (n = 16), does not allow to perform a solid statistical analysis to explore the potential role of the co-morbidities in each cluster mortality. Longer follow-up studies could answer this important question.

There are several limitations in this study. First, we did not validate these clinical clusters against any important outcomes of the disease such as exacerbations or death. The number of exacerbations and deaths were small not allowing for a solid statistical analysis. The main purpose of the present study was to explore the longitudinal behaviour of clinical clusters in stable COPD patients. Second, the type of cluster analysis used (K means) has its own limitations: the number of clusters (K) is determined before the analysis and the algorithms locate the cluster centre and assign the subjects to the nearest cluster centre. This could limit the number of clusters and may accidentally exclude small but otherwise important group of subjects.[25] Also, K-means analysis does not allow the inclusion of categorical variables limiting the evaluation of potentially important ones such as the presence of co-morbidities that were not considered in this analysis. Third, the present multicentre study included only a small percentage of female patients, thus limiting the applicability of the findings mainly to male COPD patients. Larger samples of female COPD patients should be investigated to determine whether the longitudinal behaviour of clusters is the same between female and male COPD patients. Fourth, the present study only include changes that occurred during a 2-year period of follow-up that seems to be a short time frame for a disease with a long evolution time.

Lastly, the selection of patients only involves those who were alive and have complete data at baseline and 2 years, excluding from the analysis those patients who dropped out or died that tend to be more impaired, which could affect the construction of the baseline cluster locations. As shown in Table S1 and Figure S1 (Supplementary Information), the baseline characteristics, cluster assignment and co-morbidities of those patients that were lost in the follow-up differed from those included in the final analysis. This could have affected the final results of our analysis and therefore the main conclusion of the present work, although this is an event inherent to any longitudinal study.

In conclusion, the present longitudinal, multicentre study of stable COPD patients confirmed and validated previous findings that the same clusters could be identified at recruitment and 2 years later. It also confirmed that an important percentage of patients remained in the same cluster longitudinally and in those who changed are driven by important clinical modifiable parameters such as exercise tolerance, lung function and symptoms. Further longitudinal studies, especially with the inclusion of female patients, should be carried out to confirm these findings.

Acknowledgements

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Disclosure statement

Details are provided in Appendix S2 (Supplementary Information).

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Supplementary Information

Additional supplementary information can be accessed via the *html* version of this article at the publisher's website.

Appendix S1. Collaborators.
Appendix S2. Disclosure statement.
Figure S1. Baseline self-reported co-morbidities.
Table S1. Baseline characteristics of patients who died or were lost during the follow-up time compared with those included in the final analysis.
Table S2. (A) Evaluated parameters in those patients in cluster A at baseline who changed to cluster B 2 years later. (B) Evaluated parameters in those patients in cluster B at baseline who changed to cluster A 2 years later. (C) Evaluated parameters in those patients in cluster B at baseline who changed to cluster C 2 years later. (D) Evaluated parameters in those patients in cluster C at baseline who changed to cluster A + B 2 years later.

CHEST

Natural Course of the Diffusing Capacity of the Lungs for Carbon Monoxide in COPD

Importance of Sex

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BACKGROUND: The value of the single-breath diffusing capacity of the lungs for carbon monoxide (D_{LCO}) relates to outcomes for patients with COPD. However, little is known about the natural course of D_{LCO} over time, intersubject variability, and factors that may influence D_{LCO} progression.

RESEARCH QUESTION: What is the natural course of D_{LCO} in patients with COPD over time, and which other factors, including sex differences, could influence this progression?

STUDY DESIGN AND METHODS: We phenotyped 602 smokers (women, 33%), of whom 506 (84%) had COPD and 96 (16%) had no airflow limitation. Lung function, including D_{LCO}, was monitored annually over 5 years. A random coefficients model was used to evaluate D_{LCO} changes over time.

RESULTS: The mean (± SE) yearly decline in D_{LCO} % in patients with COPD was 1.34% ± 0.015%/y. This was steeper compared with non-COPD control subjects (0.04% ± 0.032%/y; $P = .004$). Sixteen percent of the patients with COPD, vs 4.3% of the control subjects, had a statistically significant D_{LCO} % slope annual decline (4.14%/y). At baseline, women with COPD had lower D_{LCO} values (11.37% ± 2.27%; $P < .001$) in spite of a higher FEV_1 % than men. Compared with men, women with COPD had a steeper D_{LCO} annual decline of 0.89% ± 0.42%/y ($P = .039$).

INTERPRETATION: Patients with COPD have an accelerated decline in D_{LCO} compared with smokers without the disease. However, the decline is slow, and a testing interval of 3 to 4 years may be clinically informative. The lower and more rapid decline in D_{LCO} values in women, compared with men, suggests a differential impact of sex in gas exchange function.

TRIAL REGISTRY: ClinicalTrials.gov; No.: NCT01122758; URL: www.clinicaltrials.gov

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KEY WORDS: COPD; diffusing capacity of the lungs for carbon monoxide; lung function decline; sex

FOR EDITORIAL COMMENT, SEE PAGE 389

ABBREVIATIONS: ATS = American Thoracic Society; BODE = BMI, airflow obstruction, dyspnea, and exercise capacity; D_{LCO} = diffusing capacity of the lungs for carbon monoxide; ERS = European Respiratory Society

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Take-home Points

Study Question: Is a low value of diffusing capacity of the lungs for carbon monoxide (DLCO) associated with poor outcomes in patients with COPD? What is the natural course of DLCO in these patients over time, and which other factors, including sex differences could influence this progression?

Results: Patients with COPD have an accelerated decline in DLCO compared with smokers without the disease. Sixteen percent of the patients with COPD, vs 4.3% of the control subjects, had a statistically significant DLCO % slope annual decline (4.14%/y). Women with COPD have a lower DLCO than men even though they have less airflow limitation. Women also appear to have a greater DLCO decline over time compared with men.

Interpretation: These results provide information about the testing frequency (3-4 years) needed to use of DLCO as a marker of COPD progression in clinical practice, as well as in trials of therapies aimed at improving emphysema. Women seem to have a different susceptibility to cigarette smoke in the alveolar or pulmonary vascular domains.

COPD is now the third leading cause of death worldwide and a major public health problem.[1] COPD is a complex and heterogeneous disease, and although there have been advances in the knowledge of its natural history, they have focused mostly on changes in FEV_1 over time.[2-5] Information about the natural course of other important phenotypic domains continues to be significantly limited because of the lack of prospective longitudinal studies.[2,6,7] One such important domain is that of the gas transfer properties of the lungs.

It was more than 100 years ago that Marie Krogh first studied the use of carbon monoxide (CO) to measure the diffusing capacity of gases in the lungs of humans.[8] However, its introduction into clinical practice became possible only after a single breath-holding technique (DLCO) was standardized 50 years later.[9] Since then, this variable, which at first was of interest only to physiologists, has been shown to provide important practical clinical information and has been identified as a surrogate marker of outcomes in diverse lung diseases.[10] In patients with COPD, cross-sectionally obtained low values of DLCO are associated with decreased exercise capacity[11,12] and worse health status.[13] In addition, low DLCO values help preclude surgical lung resection in patients with cancer[14] and relates to mortality independent of other clinical variables.[15] Also, a low DLCO value, as a marker of emphysema in smokers without airflow limitation, signals an increased risk for developing COPD over time.[16] Recently, the first longitudinal study completed in a small cohort (n = 155) of patients from Korea[17] provided information about the slow time course of DLCO progression; however, it did not use a control group of smokers without COPD and included only nine women. Importantly, it reported the change only as the annual median decline for the group and not as individual decline, providing no information about individual variability.

We hypothesized that, just as it has been shown for FEV_1, the gas transfer domain, as measured by the DLCO, indicates a heterogeneous progression of COPD in individuals with the disease. We also hypothesized that other factors, including sex differences, could influence this progression. To test this hypothesis, we analyzed the long-term evolution of patients with COPD and smoker control subjects, in a well-characterized cohort using DLCO measurements prospectively obtained. This information should help define the implementation and frequency of this pulmonary test in the longitudinal assessment of patients with COPD, a practice gap that remains unfilled.

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Methods

Subject Study Cohort

The COPD History Assessment in Spain (CHAIN) is an ongoing observational study of patients with COPD that began enrollment in January 2010 at 24 university hospitals in Spain.[18] COPD was defined by a smoking history of ≥ 10 pack-years and a postbronchodilator $FEV_1/FVC < 0.7$ after administration of 400 μg of albuterol. Patients were stable for at least 6 weeks and received guideline-directed optimal medical therapy.[1] Exclusion criteria included alpha-1 antitrypsin deficiency or uncontrolled comorbidities such as malignancy or other confounding diseases that could interfere with the study. Data analyzed in the present study were taken at baseline recruitment and then annually over 5 years; the last visit for patients occurred on May 31, 2020. Patient data were anonymized with hierarchical access control to guarantee that information was secured. All participants signed the informed consent form approved by the ethics committee (Comité de Etica de Investigación, Hospital Universitario Nuestra Señora la Candelaria, Tenerife, IRB No. 258/2009).

Clinical and Physiologic Measurements

The methodologic aspects of the CHAIN study have been published previously.[18] In summary, trained staff recorded information on age, sex, and BMI at baseline and at subsequent yearly visits. Smoking status was determined by history and confirmed by CO-oximetry (piCO Smokerlyzer; Bedfont Scientific) during each visit, performed at the same time as the lung function tests. All tests were performed in the early morning. A questionnaire helped determine current or former smoker status and pack-years. Pulmonary function tests were performed in accordance with the American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines.[19] Diffusing capacity of the lungs for carbon monoxide was determined by the single-breath technique, in accordance with the ERS/ATS guidelines,[20] corrected by the hemoglobin value. Reference values were those of the European Community for Steel and Coal[21] and, for a group of patients (n = 201), we also tested the correlation of DLCO % predicted with the Global Lung Function Initiative (GLI) (e-Fig 1).[22,23] Arterial blood gases were measured with participants in the sitting position while breathing room air. The 6-min walk distance was measured according to the ATS guideline.[24] Dyspnea was evaluated with the modified Medical Research Council dyspnea scale. FEV_1, BMI, 6-min walk distance, and modified Medical Research Council values were integrated into the BODE (BMI, airflow obstruction, dyspnea, and exercise capacity) index.[25] The associated comorbidity load was determined with the Charlson index.[26] Hospitalizations and all-cause mortality were recorded, using information obtained from the family, and then confirmed by reviewing medical records as published previously.[18]

Statistical Analysis

Data are summarized as relative frequencies for categorical variables, mean (SD) for normally distributed variables, and median (10th-90th percentile) for nonnormal data. Comparisons were made between groups using Pearson χ^2 test, the Kruskal-Wallis H test, or the Mann-Whitney U test and one-way analysis of variance or Student t-test as appropriate. Correlations were estimated using Spearman or Pearson linear coefficients. Using all the patients in the study population, a random coefficients model (mixed-effects linear model) with random intercept and slope was applied to annual DLCO %, including COPD, sex, age, current smoker, pack-years, and FEV_1 % as covariates. Evaluation of the interactions of these variables over time allowed us to calculate the DLCO decline rate. In addition, models for patients with COPD and smokers without COPD were derived, using those covariates that had been significant. We performed a mortality Cox regression test including the main variables related to DLCO longitudinal analysis. We also performed a survival analysis, using a multivariate Cox proportional hazards regression model including the main variables related to DLCO longitudinal analysis, to evaluate the effect of DLCO on adjusted overall survival on relevant covariates such as sex.[27] A repeated-measures analysis of variance was applied to analyze the evolution of DLCO over the study period, including the time-by-sex interaction. In an effort to smooth the series and increase the number of individuals available throughout the study period, the definition of three periods of time (initial, intermediate, and final) was considered to be the moving average of two measurements in 2 years. In addition, the difference in FEV_1 % between the initial and final periods was included as a covariate to study the effect on the evolution of the DLCO %. Trend analysis was performed to estimate the individual slope of variables over time. A linear regression model with year as the explanatory variable was used to estimate the slope of the DLCO decline when at least three measurements were available. A significance level was established as a two-tailed P value < .05. Calculations were made with SPSS 25.0 (IBM).

Results

Characteristics of the Participants

The study population included 602 individuals (women, 33%). There were 506 (84%) with COPD, and 96 (16%) were smokers without COPD (control subjects). The classification of COPD vs control subject, using the lower limit of normal vs the FEV_1/FVC, would keep more than 95% of subjects in the same group and not influence the results. The baseline characteristics of the participants are shown in Table 1. The group of patients with COPD included more men; they were slightly older, had a greater pack-year history, but a lower proportion of current smokers. As expected, they had worse lung function, less exercise capacity, higher dyspnea and BODE index scores, more comorbidities, and higher hospitalizations and mortality. However, the two groups had similar hemoglobin levels and BMI values.

Longitudinal Changes in DLCO

The mean (± SE) rate of change in DLCO % over the 5 years in patients with COPD indicated a decline of 1.34% ± 0.015%/y and was higher compared with control subjects (0.04% ± 0.032%/y), that is, smokers without COPD ($P = .004$) (Fig 1). The rate of change was associated with the number of DLCO measurements for the COPD population ($P = .013$) but not for smokers without COPD ($P = .73$). These differences in the mean rate of decline were observed only for the group with one or two measurements

TABLE 1] Baseline Characteristics of Subjects Included in Study, Stratified by Presence of COPD and Number of D_{LCO} Assessments

Characteristic	COPD				Smokers Without COPD				
	Total (N = 506)	1-2 Period[a] (n = 201)	3-6 Period[a] (n = 305)	*P* Value	Total (N = 96)	1-2 Period (n = 27)	3-6 Period (n = 69)	*P* Value	*P* Value[b]
Sex (male)[c]	406 (80%)	149 (74%)	257 (84%)	.004	58 (60%)	19 (70%)	39 (56%)	.155	< .001
Age, y[d]	64 (8.9)	65 (9.0)	64 (8.8)	.542	55 (10.1)	56 (11.0)	55 (9.8)	.683	< .001
Pack-years[d]	59 (27)	60 (27)	58 (27)	.442	45 (24)	48 (23)	43 (24)	.337	< .001
Smokers active[c]	192 (38%)	87 (43%)	105 (34%)	.055	61 (64%)	19 (73%)	42 (61%)	.194	< .001
BMI, kg/m^2 [d]	27.4 (5.0)	27.6 (5.5)	27.3 (4.7)	.441	28.4 (4.9)	28.4 (5.7)	28.4 (4.6)	.954	.087
Hemoglobin, g/dL[d]	14.8 (1.32)	14.4 (1.41)	14.9 (1.25)	.003	15.3 (1.25)	15.8 (0.72)	15.1 (1.38)	.173	.065
CO-oximetry, ppm[e]	5.0 (2-19)	4.0 (2-17.4)	5.0 (2-20)	.103	10.0 (3-33)	12 (3-32.9)	10 (3-37)	.637	< .001
D_{LCO}, mmol/mL/kPa[d]	5.18 (1.98)	4.46 (2.02)	5.35 (1.94)	.016	7.86 (2.35)	7.46 (2.43)	7.95 (2.29)	.154	< .001
D_{LCO}, %[d]	65.0 (23.6)	62.8 (25.4)	66.3 (22.4)	.118	84.6 (19.3)	81.1 (17.9)	85.9 (19.7)	.291	< .001
K_{CO}, %[d]	73.4 (25.1)	70.8 (25.2)	75.2 (24.9)	.062	92.4 (20.6)	88.4 (18.2)	94.2 (21.5)	.226	< .001
FEV_1, L[d]	1.61 (0.63)	1.50 (0.60)	1.69 (0.64)	.001	2.88 (0.75)	2.90 (0.93)	2.87 (0.68)	.856	< .001
FEV_1, %[d]	57.7 (20.3)	56.0 (20.9)	58.7 (19.8)	.147	95.9 (13.8)	91.9 (18.3)	97.5 (11.3)	.147	< .001
FVC, L[d]	3.14 (0.90)	2.93 (0.85)	3.28 (0.91)	< .001	3.77 (1.00)	3.81 (1.21)	3.75 (0.92)	.816	< .001
FVC, %[d]	86.0 (21.1)	84.3 (21.5)	87.2 (20.8)	.128	100.1 (15.2)	96.4 (19.7)	101.6 (12.9)	.216	< .001
FVC_1/FVC, %[d]	51.2 (12.1)	50.9 (12.4)	51.4 (11.9)	.695	77.8 (6.0)	78.0 (6.8)	77.7 (5.6)	.794	< .001
6MWD, m[d]	471 (96)	445 (108)	488 (83)	< .001	534 (89)	538 (102)	533 (85)	.808	< .001
Charlson index[e]	0 (0-3)	0 (0-3)	0 (0-2.4)	.105	0 (0-1)	0 (0-3.9)	0 (0-0)	.055	.007
Dyspnea (mMRC)[e]	1 (0-3)	1 (0-3)	1 (0-2)	.248	0 (0-1.4)	0 (0-2)	0 (0-1)	.969	< .001
Pa_{O_2}, mm Hg[d]	70.0 (10.8)	69.1 (11.9)	70.8 (9.9)	.191	75.8 (13.1)	74.6 (14.1)	76.0 (13.1)	.795	.004
BODE index[e]	1 (0-4)	2 (0-6)	1 (0-4)	.005	0 (0-1)	0 (0-2.4)	0 (0-1)	.178	< .001
Hospitalization (at least one during the study period)[c]	137 (27%)	47 (23%)	90 (30%)	.078	13 (14%)	2 (8%)	11 (16%)	.247	.003
Hospitalization per patient-year[e]	0 (0-0.7)	0 (0-2)	0 (0-0.4)	.939	0 (0-0.3)	0 (0-1.5)	0 (0-0.3)	.628	.013
Respiratory mortality[c]	54 (11%)	30 (15%)	24 (8%)	.009	1 (1.0%)	1 (3.7%)	…	.281	.001
Global mortality[c]	130 (26%)	83 (41%)	47 (15%)	< .001	3 (3.1%)	3 (11.1%)	…	.020	< .001

6MWD = 6-minute walk distance; BODE = BMI, airflow obstruction, dyspnea, and exercise; D_{LCO} = diffusing capacity of the lungs for carbon monoxide; K_{CO} = CO transfer coefficient; mMRC = modified Medical Research Council.

[a]Subjects with fewer than three measurements (1-2 period) vs three or more measurements (3-6 period).

[b]Comparison between subjects with COPD and smokers without COPD.

[c]Data presented as number (percentage).

[d]Data presented as mean (SD).

[e]Data presented as median (10th percentile-90th percentile).

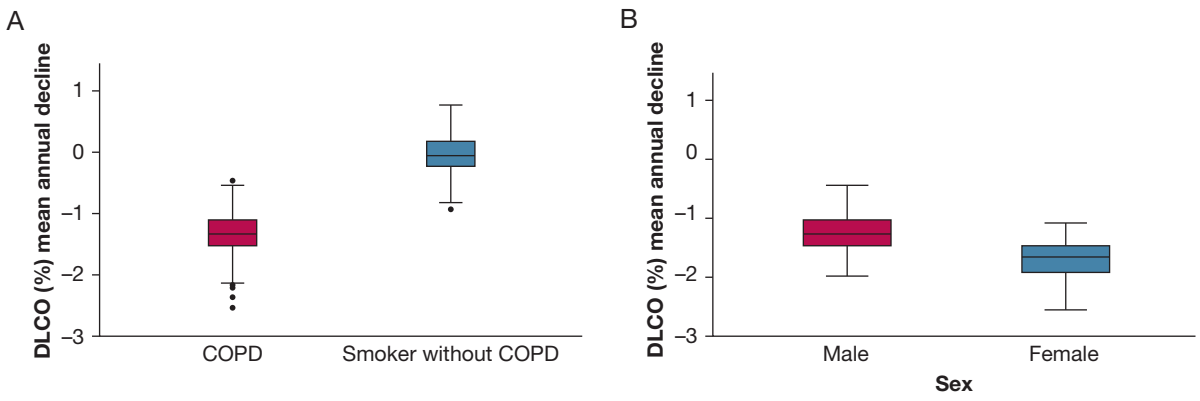

Figure 1 – *Values of DLCO (%) over 5 years. A, Values for all patients with COPD and smokers without COPD. B, Comparison of changes in DLCO (%) in men and women with COPD. DLCO = diffusing capacity of the lungs for carbon monoxide.*

(1.40% ± 0.027%/y; $P = .006$), and there were no differences between those with three (1.33% ± 0.037%/y) vs four to six measurements (1.31% ± 0.019%/y). Although 26% of the patients with COPD died during the study, the mean rates of change did not differ significantly from those who completed the study compared with those who did not (1.31% ± 0.026%/y vs 1.36% ± 0.018%/y; $P = .118$). Age, BMI, FEV_1 %, and presence of active smoking were not associated with differences in the longitudinal change in DLCO values in patients with COPD.

Being a woman was the only factor that related to the annual rate of change in DLCO (Table 2). Women with COPD had lower baseline DLCO values (–11.37% ± 2.27%; $P < .001$) than men with the disease in spite of a higher FEV_1 % than men (64.8% vs 55.9%; $P < .001$). Women exceeded the annual rate of DLCO decline by

TABLE 2] Effects of Patient Characteristics on Baseline DLCO and on Annual Rate of Change in DLCO

	Baseline DLCO		Annual Rate of Change in DLCO	
Characteristic	Effect on Baseline DLCO	*P* Value	Effect on Annual Rate of Change in DLCO	*P* Value
Total model				
COPD, yes vs no	−1.41 ± 2.50	.573	−1.19 ± 0.41	.004
Age, per y	−0.20 ± 0.09	.031	−0.01 ± 0.01	.647
Sex, female vs male	−10.40 ± 2.04	< .001	−0.59 ± 0.34	.096
BMI, per kg/cm^2	1.45 ± 0.16	< .001	−0.05 ± 0.03	.074
Smoking status				
Current smoker, yes vs no	−2.32 ± 1.70	.172	0.01 ± 0.30	.976
Pack-years, per pack-year	0.04 ± 0.03	.363	0.002 ± 0.005	.633
FEV_1 (%) baseline, per %	0.47 ± 0.04	< .001	0.01 ± 0.01	.207
COPD model				
Age, per y	−0.31 ± 0.10	.002	−0.01 ± 0.01	.401
Sex, female vs male	−11.37 ± 2.27	< .001	−0.89 ± 0.42	.039
BMI, per kg/cm^2	1.54 ± 0.17	< .001	−0.04 ± 0.03	.121
FEV_1 (%) baseline, per %	0.48 ± 0.04	< .001	0.004 ± 0.007	.558
Smoker without COPD model				
Age, per y	0.41 ± 0.16	.014	−0.01 ± 0.02	.514
Sex, female vs male	−10.67 ± 3.50	.003	−0.27 ± 0.50	.596
BMI, per kg/cm^2	1.40 ± 0.34	< .001	−0.10 ± 0.05	.065
FEV_1 (%) baseline, per %	0.46 ± 0.12	< .001	−0.01 ± 0.02	.459

Data are presented as mean ± SE. DLCO = diffusing capacity of the lungs for carbon monoxide.

TABLE 3] Evolution of D_{LCO} and Other Functional Variables in Patients With COPD and Smokers Without COPD Over Time: Patients With Three or More Measures of D_{LCO}

	COPD (n = 305)				Smokers Without COPD (n = 69)			
Variable	Initial	Intermediate	Final	*P* Value	Initial	Intermediate	Final	*P* Value
BMI, kg/m^2[a]	27.7 (4.4)	27.7 (4.5)	27.7 (4.7)	.898	28.6 (4.5)	28.7 (4.5)	28.9 (4.4)	.341
D_{LCO}, %[a]	64.2 (20.8)	59.9 (20.7)	57.4 (21.3)	< .001	83.1 (20.9)	80.6 (20.9)	80.8 (20.6)	.032
K_{CO}, %[a]	75.2 (24.7)	74.3 (24.4)	69.3 (25.3)	< .001	94.0 (20.9)	93.2 (20.9)	90.7 (21.6)	.019
Alveolar volume, L[a]	5.26 (1.07)	5.15 (1.11)	5.10 (1.14)	< .001	5.21 (0.96)	5.19 (0.90)	5.13 (0.99)	.406
FEV_1, L[a]	1.67 (0.63)	1.61 (0.62)	1.52 (0.64)	< .001	2.86 (0.75)	2.79 (0.74)	2.66 (0.78)	.007
FEV_1, %[a]	58.2 (19.0)	57.1 (19.0)	55.7 (18.9)	< .001	97.0 (11.7)	97.2 (12.3)	96.4 (13.6)	.519
FVC, L[a]	3.26 (0.90)	3.21 (0.89)	3.10 (0.90)	< .001	3.78 (0.95)	3.74 (1.00)	3.67 (1.02)	.005
FVC, %[a]	86.0 (19.9)	86.3 (20.4)	84.4 (21.4)	.023	102.1 (12.7)	101.3 (13.0)	101.2 (13.1)	.700
FVC_1/FVC, %[a]	51.6 (11.9)	50.3 (12.4)	50.0 (11.6)	< .001	76.6 (5.2)	74.9 (5.2)	74.6 (6.2)	.019
BODE index[b]	1.5 (0-4)	2 (0-4.5)	2 (0-5)	< .001	0 (0-1)	0 (0-1)	0 (0-1)	.206
Smokers active[c]	37.7%	34.1%	28.2%	.034	65.2%	58.8%	47.1%	.033

BODE = BMI, airflow obstruction, dyspnea, and exercise; D_{LCO} = diffusing capacity of the lungs for carbon monoxide; K_{CO} = CO transfer coefficient.
[a]Data are presented as mean (SD).
[b]Data are presented as median (10th percentile-90th percentile).
[c]Data are presented as number (percentage).

0.89% ± 0.42%/y ($P = .039$), compared with men. These differences were not explained by smoking habit (Table 2, e-Tables 1 and 2). There was no influence of center location on rate of DLCO decline (analysis not shown).

Analysis of Subgroups

We identified 305 patients with COPD and 69 smokers without COPD with at least three DLCO measurements over the 5 years (e-Fig 2). The patients with COPD with at least three DLCO measurements were similar to those with fewer than three DLCO measurements in terms of baseline DLCO, BMI, FEV_1 %, and Pao_2. However, they walked a greater distance in the 6-min walk test, had a lower BODE index, and lower mortality. There were no significant differences in the smokers without COPD (Table 1). Table 3 shows that in those patients with COPD, the DLCO %, FEV_1 %, and proportion of active smokers decreased over the 5 years of observation.

On the basis of the individual slope change, 50 patients with COPD (16.4%) (Fig 2) and three smokers without COPD (4.3%) showed a statistically significant yearly loss of DLCO %: –4.139 (95% CI, –4.622 to –3.622) and –4.440 (95% CI, –9.903 to 1.023), respectively (Table 4). In patients with COPD, more women (26%) than men (14%) were in the DLCO decliners group ($P = .005$).

Forty-seven patients with three DLCO measurements died during the follow-up period, and there was no significant difference in mortality between patients with COPD with and without slope DLCO decline ($P = .763$; e-Table 3). There were also no significant differences in hospitalization per patient-year ($P = .447$).

Discussion

This prospective observational study of patients with COPD attending pulmonary clinics has several important findings: First, over 5 years of observation, a proportion of patients with COPD (16%) had a statistically significant annual decline in DLCO. This proportion is four times higher than that of smokers without airflow limitation. Second, with better spirometric values at baseline and throughout the study, smoking women with and without COPD had a lower DLCO than men. Importantly, they also had a greater DLCO decline over the 5 years of observation. These results provide information about the testing frequency needed to use DLCO as a marker of COPD progression in clinical practice, as well as in trials of therapies aimed at improving emphysema. The results also suggest that compared with men, women have a different susceptibility to cigarette smoke in the alveolar or pulmonary vascular domains.

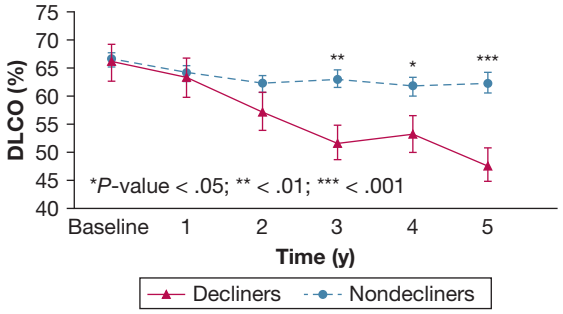

Figure 2 – *Evolution of the mean annual DLCO (%) for patients with COPD depending on its decline was statistically significant negative (decliners) vs the rest of the group (nondecliners).*

DLCO Over Time

Longitudinal studies with repeated measures of DLCO in respiratory diseases have been reported primarily in interstitial lung disease, with a decrease ≥ 15% over 6 to 12 months shown to be associated with increased mortality risk independent of other cross-sectional measures.[28] This has positioned the DLCO as an interstitial lung disease activity biomarker that could guide progression or response to treatment. In COPD, the prognostic information on DLCO has only been reported using single cross-sectional measurements.

To our knowledge, the current report represents the first observational study in patients with COPD compared with smokers without COPD, who served as control subjects. Our data on the mean annual decrease in DLCO in the patients with COPD were similar to those recently published in the multicenter observational study by Kang et al,[17] completed in a smaller number of patients with COPD (n = 155). That study had only nine women and, thus, they could not examine the influence of sex on DLCO progression.

The observed decline in DLCO confirms that COPD progresses relatively slowly, with 16% of the patients showing a statistically significant annual decline over the 5 years of observation. However, this proportion was four times higher than that of the group of smokers without COPD. To place these findings in a practical clinical context we have to relate our findings with those reported in the literature in two cross-sectional COPD studies.[13,29] Analysis of the COPDGene cohort[13] has shown that a 10% lower value of DLCO is associated with a significant impairment in exercise capacity and an increased risk of hospitalizations independent of FEV_1. In another study of a smaller cohort, a lower DLCO value was associated with a lower 6-min walking distance.[12] In our study, there was a numerical difference in the

TABLE 4] Slope Values of DLCO Change in Patients With Three or More Measurements

Slope	COPD (n = 305)					Smokers Without COPD (n = 69)				
	No.	Mean	95% CI		Mean	95% CI	No.	Mean	95% CI		Mean	95% CI
Significantly negative	50	−4.139	−4.622 to −3.657	]			3	−4.440	−9.903 to 1.023	]		
Nonsignificantly negative	180	−3.017	−3.418 to −2.616	}			49	−2.026	−2.579 to −1.474	}		
Nonsignificantly positive	71	1.552	1.221 to 1.882	]	−1.647	−2.044 to −1.251	17	1.548	0.950 to 2.146	]	−1.106	−1.684 to −0.527
Significantly positive	4	3.207	1.356 to 5.058				…	…	…			

Slope values provided according to their direction (positive for increase, negative for a decrease) and statistical significance. DLCO = diffusing capacity of the lungs for carbon monoxide.

number of hospitalizations in the DLCO decliners group, but it failed to reach statistical significance. Our findings, and those of the Korean study, suggest that patients with COPD do not need an annual follow-up measurement of DLCO and that perhaps this test can be performed every 3 to 4 years, even in the highest risk group such as women, as we discuss below.

DLCO in Women

The DLCO at baseline in our study was lower in women than in men with COPD, even though they had higher spirometric values at baseline. This has been reported previously, but has not been adequately discussed and has never been prospectively followed.[29,30] We show that women have a tendency to a more pronounced decrease in DLCO over time despite having a better FEV_1 than men, both at baseline and at the end of 5 years. This difference in DLCO needs to be added to other characteristics described for women with COPD. It is known that women report more dyspnea and worse health status than men,[31] and they have a marked tendency to develop some comorbidities such as anxiety, depression, malnutrition, lung adenocarcinoma, and osteoporosis.[32] Importantly, in studies using CT imaging, women with COPD show smaller emphysematous lesions than men.[33] We can only speculate about some potential reasons to explain the contradictory findings of our study (lower DLCO) and that of less emphysema by CT imaging in other studies.[33] One reasonable explanation is that women have a pulmonary vascular phenotype that may be related to the smoking habit. There may be a loss of the distal arterial capillaries (pruning) with relative preservation of the airways and alveoli.[34] It could also depend on the way smoke is inhaled in women[35] or on other hormonal (estrogenic) factors.[33] These pathophysiologic aspects were outside the scope of this study. However, some support for the potential vascular susceptibility to cigarette smoke in women is provided by the higher prevalence of pulmonary vascular hypertension in this sex.[36]

This study has some limitations. First, not all patients initially enrolled had all the annual measurements of their DLCO over the 5 years. Although the dropout of some subjects can affect the measurement of DLCO decline, we used a random coefficients model (mixed-effects linear model) to minimize this effect. In fact, the differences observed in patients with COPD with fewer measurements compared with those with more measurements were clinically irrelevant. Second, there

may be intrinsic variability in the instruments used to measure DLCO, an area that remains poorly studied. However, daily calibration and biological control subjects minimized this variability. Further, the observed differences in the proportion of rapid DLCO decliners in subjects with COPD vs smokers without obstruction, in a multicenter study, support its practical clinical use in different centers. Third, the current study does not include CT imaging of the chest, a test that would have provided insight into the contribution of factors, such as the behavior of the vascular compartment (vascular pruning), to the pathophysiologic explanation of our observations. This is an area that warrants further study in patients with COPD, but does not negate the importance of our findings. Finally, our results should be replicated in other populations and ethnic groups.

Interpretation

In summary, this longitudinal observational study shows that the decline in DLCO is on average more rapid in patients with COPD than in smoker control subjects. On average, 3 to 4 years is needed to observe a significant decline in DLCO. This information is relevant to help implement the use of this test in clinical practice and therapeutic trials. Importantly, we found that women with COPD have a lower DLCO than men, independent of airflow limitation, and appear to have a greater decline over time. This suggests a differential impact of sex among those factors influencing lung gas diffusion. Further studies in other populations should validate our results.

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Author contributions: All of the authors had full access to all the data in the study and accept responsibility for the submission of this work. All of the authors attest that they made substantial contributions to the conception and design of the study; to the acquisition, analysis, and interpretation of data; and to drafting of the article or critical revision for important intellectual content. All of the authors gave final approval of the version submitted for publication.

Role of sponsors: The sponsors had no role in the design of the study, the collection and analysis of the data, or the preparation of the manuscript.

Additional information: The e-Figures and e-Tables can be found in the Supplemental Materials section of the online article.

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Respiratory Research

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Telomere length dynamics over 10-years and related outcomes in patients with COPD

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Abstract

Background: Chronic obstructive pulmonary disease (COPD) has been proposed as a disease of accelerated aging. Several cross-sectional studies have related a shorter telomere length (TL), a marker of biological aging, with COPD outcomes. Whether accelerated telomere shortening over time relates to worse outcomes in COPD patients, is not known.

Methods: Relative telomere length (T/S) was determined by qPCR in DNA samples from peripheral blood in 263 patients at baseline and up to 10 years post enrolment. Yearly clinical and lung function data of 134 patients with at least two-time measures of T/S over this time were included in the analysis.

Results: At baseline, T/S inversely correlated with age ($r = -0.236$; $p < 0.001$), but there was no relationship between T/S and clinical and lung function variables ($p > 0.05$). Over 10 years of observation, there was a median shortening of TL of 183 bp/year for COPD patients. After adjusting for age, gender, active smoking and mean T/S, patients that shortened their telomeres the most over time, had worse gas exchange, more lung hyperinflation and extrapulmonary affection during the follow-up, (PaO_2 $p < 0.0001$; K_{CO} $p = 0.042$; IC/TLC $p < 0.0001$; 6MWD $p = 0.004$ and BODE index $p = 0.009$). Patients in the lowest tertile of T/S through the follow-up period had an increased risk of death [HR = 5.48, (1.23–24.42) $p = 0.026$].

Conclusions: This prospective study shows an association between accelerated telomere shortening and progressive worsening of pulmonary gas exchange, lung hyperinflation and extrapulmonary affection in COPD patients. Moreover, persistently shorter telomeres over this observation time increase the risk for all-cause mortality.

Keywords: Aging, COPD, Lung-function, Mortality, Telomeres

Background

Chronic obstructive pulmonary disease (COPD), one of the leading causes of morbidity and mortality worldwide, is a disease characterized by a persistent reduction of airflow that frequently progresses over time [1, 2]. In addition, patients with COPD develop 10 or 20 years earlier, comorbid diseases characteristically seen in elderly subjects without COPD [3, 4].

COPD has been described as a disease of accelerated aging and shorter telomere length as a surrogate marker of biological aging [5, 6]. In humans, telomeres consist of a repeating sequence of TAAGGG hexanucleotide located at the ends of chromosomes and have an important role in maintaining chromosome integrity and cell proliferation [7]. Telomeres shorten 30–100 base pairs during each cell division due to the end-replication problem of the DNA polymerase [8, 9]. Telomere shortening and telomere dysfunction may heavily influence the aging human lung [10]. It has been shown that patients with

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COPD exhibit shorter leucocyte telomeres when compared with smokers without COPD and healthy subjects [11–13]. Importantly, we have also shown that COPD patients experience accelerated telomere shortening over time when compared to smoking controls [13].

Shortening of telomere length may be a risk factor for all-cause or cause-specific mortality [14, 15]. The same appears to be true for patients with COPD, as shorter telomere length has been associated with worse lung function [16, 17], exacerbations and risk of death [18, 19]. However, these studies suggesting an association between telomere length and respiratory health were cross sectional in design. There is one recent study in the general population relating telomere length with longitudinal assessment of clinical data, but the study had only one measure of telomere length at baseline. In that study, smokers with short telomeres at baseline had accelerated lung function decline over time [20]. No long-term study of patients with COPD, has measured telomere length over time and explored the association between changes in telomere length and clinical and physiological variables of importance to those patients.

The aim of the present study was to test the hypothesis that telomere length shortening over time in patients with COPD is associated to clinical, lung function, and patient-related outcomes in 10 years of follow-up.

Methods

Subjects

A total of 263 were smokers with COPD diagnosis were screened for this study at the Hospital Universitario La Candelaria, Tenerife, Spain (Tenerife-cohort) that were followed annually as part of the BODE cohort [21, 22]. Inclusion criteria: age > 40 years, smoking history > 15 pack-years and post-bronchodilator FEV_1/FVC ratio < 0.70 clinically stable for at least 6 weeks at the time of evaluation. Spirometry, lung volumes and exercise capacity were measured according to ATS-ERS guidelines [23, 24]. Dyspnea, evaluated by mMRC scale [25], BODE Index [21] and Charlson index for comorbidities [26] were registered at every visit. Exclusion criteria: uncontrolled co-morbidities such as malignancy at baseline, asthma or other pulmonary conditions than COPD. Exacerbations were defined as a worsening of respiratory symptoms (dyspnea, cough or sputum) that required the use of antibiotics, systemic corticosteroids, or both or necessitated emergency room visit or hospital admission. All-cause mortality was recorded using information obtained from the family and then confirmed by reviewing medical records (Fig. 1).

Longitudinal study: included 134 patients from the overall cohort that were monitored through the 10 years of follow-up (413 observations). These patients presented at least two-time longitudinal measures of telomere length and a mean follow-up of 6 years. From these 42 reached 10 years of observation. As show in Additional file 1: Table S1, the clinical characteristics of these 42 patients were similar to that of the cohort as a whole, except for being slightly younger, presenting higher K_{CO} and able to walk more on the 6MWD test.

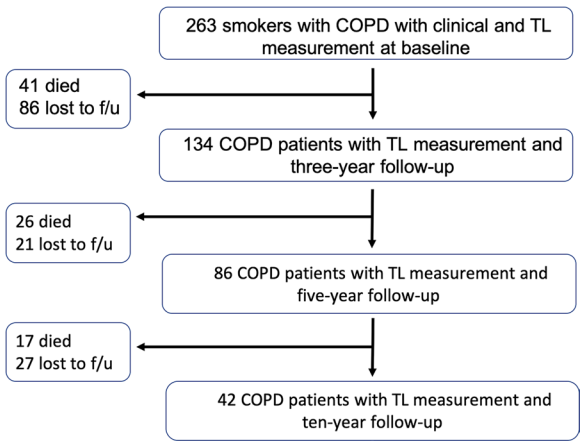

Fig. 1 Flow diagram representing individuals recruited for the present study

In each annual visit of the recruited participants peripheral blood sample was taken and all the clinical and functional parameters were recorded.

The study was approved by the institutional review board of HUNSC (PI14/12). All participants provided written informed consent.

Telomere length measurement

DNA was extracted from whole blood obtained at baseline, the 3rd-year, the 5th-year and at the 10th-year post enrolment. The QIAamp DNA Mini Kit (GE Healthcare) was used for this purpose and the resulting DNA samples were quantified using the Nanodrop lite spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Telomere length was measured in triplicate in each sample (20 ng of DNA) using a qPCR-based protocol as described in a previous publication of our group [13]. Also, calibrator samples were assayed in triplicate on each PCR plate to control for variation between plates. Intra-plate coefficients of variance (CV) were calculated between the replicates and samples with CV > 5% were excluded from further analysis. Two control DNA samples were assay per run as a normalizing factor. Inter-plate CV for the calibrator sample was calculated to be < 8.5%. Albumin, a single copy gene, was used as a reference gene.

Telomere length was calculated as a ratio of telomere to albumin where the T/S ratio for an experimental DNA sample is T, the number of nanograms of the standard DNA that matches the experimental sample for copy number of the telomere template, divided by S, the number of nanograms of the standard DNA that matches the experimental sample for copy number of the albumin single copy gene [27]. T/S was calculated using the "ΔΔCp with efficiency correction" calculation method [28].

TRF southern blot analysis

Telomere restriction fragment analysis [29] was performed by southern blot using the TeloTAGGG Telomere Length Assay Kit (Roche) according to the manufacturer instructions. The mean telomere length was calculated using the following: $TRF = \sum(ODi)/\sum(ODi/Li)$, where ODi is the chemiluminescent signal and ODi/Li is the length of the TRF at position. Conversion of T/S ratio to base pair was calculated for every subject based on the equation: $y = 1114.58 + 10{,}373.13 * x$ of the correlation analysis, where x is T/S ratio (Additional file 2: Figure S1).

Statistical analysis

Baseline characteristics and outcomes

The 263 patients with COPD were categorized in three groups by relative telomere length ratio (T/S) tertiles at baseline: shorter, medium or longer telomeres. T/S was inversely correlated with age, so all subsequent analyses were adjusted by this variable. Differences in means and proportions of baseline and follow-up characteristics between groups of patients were tested using t-Student, ANOVA, Chi^2, Fisher Exact, Kruskal–Wallis.

Telomere length shortening over time and outcomes

Longitudinal analysis was performed on each individual having at least two-time T/S measures during their follow-up over 10 years. A total of 134 patients were evaluated during follow-up until time of censoring (drop-out or death). In this analysis, we considered mortality as events that occurred during follow-up within the 3 years after the last clinical evaluation (n = 43). A linear regression mixed model for repeated measures was performed to test the association of telomere length dynamics over follow-up time and the clinical and pulmonary function variables. The effect of the change in relative telomere length through time was analysed in relation to pulmonary function variables measured during the observation time by the variable T/S_mCh: as the change in T/S with respect to its mean over time in each individual. The age, gender and the mean T/S of each individual were used as covariates.

To analyse the effect of telomere length on all-cause mortality we compared the risk of mortality across T/S in each individual of the entire cohort over the total follow-up period by using a Cox proportional hazards ratio (HR) regression model in multivariate analysis. T/S was measured four times; at baseline, at the third, the 5th and at the 10th-year post enrolment. Because every subject included had an annual evaluation of their clinical and lung function parameters, we used the last observation carried forward (LOCF) approach to manage the T/S measures registered at the four moments (baseline, the 3rd-year, the 5th-year and at the 10th-year post enrolment). Individual T/S values were analysed using the last observation (T/S) registered, and carried forward in order to construct the different models.

Mortality risk was tested in every subject included in the study throughout its follow-up and over the subsequent 12 months from the last clinical evaluation, taking into account that they had at least two-time longitudinal T/S measures. Kaplan–Meier estimator is used to illustrate the association between this time varying covariate and mortality as a clinical outcome. In the multivariate model, the following covariates were included: age, gender, smoking status (pack-years of smoking), active smoking (current or ex-smokers), FEV_1%, BODE index and 6MWD every year of follow-up.

SPSS 25.0 IBM Co and R software were used for all statistical analyses and two-tailed p-values < 0.05 were considered significant.

Results

Baseline analysis

The clinical characteristics and lung function data of 263 COPD patients at baseline distributed by tertiles of relative telomere length are summarized in Table 1. The range of airflow obstruction distributed by GOLD stages in COPD was as follows: I (16.7%), II (43%), III (30.8%) and IV (9.5%). Individuals with shorter telomeres were older and had a higher number of pack-years smoked ($p = 0.023$). There was no relationship between telomere length and clinical and lung function parameters ($p > 0.05$) cross-sectionally. Telomere length measured by the T/S ratio inversely correlated with age ($r = -0.236$; $p < 0.001$). The median TL of the patients` with an average of 64 years old, was 7.8 ± 2.7 kbp. Additional file 2: Figure S1 shows that on average, telomere length was shorter as age increased.

TFR by southern blot analysis

Telomere length was measured in forty COPD patients' DNA samples using southern blot. Relative telomere length (T/S) measured by qPCR in these same samples correlated with telomere length TL in base pairs measured by southern blot ($r = 0.502$, $p = 0.001$) (Additional file 3: Figure S2).

Table 1 Baseline clinical and lung function characteristics of COPD patients grouped by relative telomere length tertiles

Variable	Short T/S[d] N = 87	Medium T/S[d] N = 88	Long T/S[d] N = 88	p-value
T/S ratio[a]	0.40 ± 0.08	0.60 ± 0.05	0.92 ± 0.22	*< 0.001*
TL (bp)[a]	5248 ± 855	7346 ± 535	10,757 ± 2371	*< 0.001*
Age[a]	66 ± 9	64 ± 9	61 ± 10	*0.005*
Sex (male %)	76	80	66	0.104
BMI[a]	28 ± 6	28 ± 5	26 ± 5	0.068
Smoking habit (pack-year)[a,c]	69 ± 30	65 ± 26	58 ± 24	*0.023*
Active smoking (%)	43	43	40	0.888
FEV_1 (L)[a]	1.49 ± 0.64	1.60 ± 0.65	1.46 ± 0.66	0.326
FEV_1 (% pred)[a]	58 ± 21	59 ± 20	55 ± 23	0.544
FVC (% pred)[a]	87 ± 21	90 ± 23	85 ± 23	0.348
FEV_1/FVC (% pred)[a]	52 ± 13	51 ± 11	51 ± 13	0.930
PaO_2[a]	71 ± 12	73 ± 11	71 ± 12	0.605
K_{CO}[a]	79 ± 27	77 ± 24	69 ± 26	0.059
ICTLC (%)[a]	35 ± 8	35 ± 8	34 ± 10	0.875
6MWD (mts)[a]	477 ± 94	486 ± 102	480 ± 105	0.824
mMRC dysnea[b]	1 (0–2)	1 (0–2)	1 (0–2)	0.210
BODE index[b]	1 (0–2)	1 (0–3)	1 (0–3)	0.223
Charlson index[b]	0 (0–1)	0 (0–1)	0 (0–1)	0.769
Exacerbations[b]	0 (0–1)	1 (0–2)	0 (0–1)	0.146

T/S ratio relative telomere length, *TL* telomere length, *BMI* body mass index, *FEV_1* forced expiratory volume in 1 s, *FVC* forced vital capacity, *% pred* per cent predicted, *PaO_2* partial oxygen tension, *K_{CO}* transfer factor coefficient of the lung for carbon monoxide, which is DL_{CO}, *IC/TLC* inspiratory capacity to total lung capacity ratio, *6MWD* 6 min walking distance test. *p*-values < 0.05 are shown in italics

[a] Data are presented as mean ± SD

[b] Data are presented as median (25th–75th pc)

[c] Number of packs of cigarettes smoked per day × number of years smoking

[d] Groups defined by relative telomere length (T/S) tertiles: < 0.52, 0.52–0.71 and > 0.71

Longitudinal analysis

The longitudinal study was performed in the 134 patients followed annually over 10 years that presented at least two T/S measures over that time. The clinical and pulmonary function characteristics of these patients are shown in Table 2.

Table 3 shows the clinical and pulmonary function characteristics at baseline and after 10 years of follow up in the 42 patients that completed that period of observation. They were mostly men (67%) and had a medium age of 61 ± 8 at baseline. The telomere length was 7583 ± 2328 bp when first recruited and 5755 ± 1456 bp 10 years later. The medium loss in TL observed was 183 bp/year.

Telomere length shortening and pulmonary function

The effect of the change in T/S in relation to its mean value was analysed in each of the 134 patients included in the longitudinal study throughout their follow-up period. Overall patients that shortened the most their telomeres over that time, had worse pulmonary gas exchange measure by PaO_2, K_{CO}, worse static lung hyperinflation (IC/TLC) and extrapulmonary affection (BODE index), even after adjustment by age, gender, active smoking and the mean T/S of each subject (Table 4). Moreover, patients that died during the follow-up period had more telomere shortening in relation to the same clinical and pulmonary function variables.

Telomere length and mortality risk

During the follow-up period, 87 (33%) of the participants died (19.5% from cancer, 39.1% from a respiratory cause and 5.8% from a cardiovascular cause). Patients with COPD with shorter telomeres (T1 and T2 tertiles of T/S) showed a higher risk of all-cause mortality (Cox HR = 5.481, p = 0.026) (Table 5, Fig. 2). In the overall cohort, the individual variation of a decrement in 0.1 units of T/S over time increased the risk of mortality (HR = 1.446, p = 0.009).

Discussion

To our knowledge this is the first study to explore the relationship between telomere length change over 10 years and clinical outcomes, in a cohort of COPD

Table 2 Baseline characterization of COPD patients who achieved 10 years of follow-up and those who died

Variable	Total patients included (n = 134)	Alive after 10 year-follow-up (n = 42)‡	Died during 10 year-follow-up (n = 43)‡	p-value‡
Age	64 9	61 ± 8	69 ± 9	*0.001*
BMI[a]	27 ± 5	27 ± 6	28 ± 5	0.781
Smoking habit (pack-year)[a,c]	65 ± 26	61 ± 22	70 ± 31	0.139
Active smoking (%)	43	52	33	0.069
FEV_1 (L)[a]	1.52 ± 0.62	1.61 ± 0.61	1.34 ± 0.56	*0.038*
FEV_1 (% pred)[a]	58 ± 21	61 ± 19	51 ± 21	*0.027*
FVC (% pred)[a]	89 ± 24	90 ± 23	81 ± 25	*0.070*
FEV_1/FVC (% pred)[a]	51 ± 11	54 ± 10	49 ± 11	*0.024*
PaO_2[a]	72 ± 11	73 ± 10	68 ± 10	*0.015*
K_{CO}[a]	80 ± 26	90 ± 28	74 ± 23	*0.009*
IC/TLC (%)[a]	34 ± 8	35 ± 8	33 ± 8	0.143
6MWD (mts)[a]	495 ± 90	522 ± 83	474 ± 75	*0.008*
mMRC dysnea[b]	1 (0–2)	1 (0–1)	1 (0–2)	0.703
BODE index[b]	1 (0–3)	1 (0–2)	1 (0–3)	0.179
Charlson index[b]	0 (0–1)	0 (0–1)	1 (0–1)	0.740

Paired sample t test was used

BMI body mass index, *FEV_1* forced expiratory volume in 1 s, *FVC* forced vital capacity, *% pred* per cent predicted, *PaO_2* partial oxygen tension, *K_{CO}* transfer factor coefficient of the lung for carbon monoxide, which is DL_{CO}, *IC/TLC* inspiratory capacity to total lung capacity ratio, *SMWD* 6 min walking distance test

‡ p-value between compared groups

[a] Data are presented as mean ± SD

[b] Data are presented as median (25th–75th pc)

[c] Number of packs of cigarettes smoked per day × number of years smoking

Table 3 Characterization of patients with COPD (N = 42) at baseline and at 10 years of follow-up

Variable	Baseline	10 year-follow-up	p-value
T/S ratio[a]	0.62 ± 0.22	0.45 ± 0.14	*< 0.0001*
TL (bp)[a]	7583 ± 2328	5755 ± 1455	*< 0.0001*
BMI[a]	27 ± 6	27 ± 6	0.981
Active smoking (%)	52	40	*< 0.0001*
FEV_1 (L)[a]	1.61 ± 0.61	1.36 ± 0.57	*< 0.0001*
FEV_1 (% pred)[a]	61 ± 19	56 ± 18	*0.014*
FVC (% pred)[a]	90 ± 23	86 ± 24	*0.043*
FEV_1/FVC (% pred)[a]	54 ± 10	51 ± 10	*0.022*
PaO_2[a]	73 ± 10	68 ± 10	*< 0.0001*
K_{CO}[a]	90 ± 28	76 ± 24	*0.006*
IC/TLC (%)[a]	36 ± 8	31 ± 9	*0.001*
6MWD (mts)[a]	520 ± 84	448 ± 125	*< 0.0001*
mMRC dysnea[b]	1 (0–1)	1 (0–2)	0.209
BODE index[b]	1 (0–2)	1 (0–3)	*0.003*
Charlson index[b]	0 (0–1)	1 (0–1)	*< 0.0001*

Paired sample t test was used

T/S ratio relative telomere length, *TL* telomere length in base pairs, *BMI* body mass index, *FEV_1* forced expiratory volume in 1 s, *FVC* forced vital capacity, *% pred* per cent predicted, *PaO_2* partial oxygen tension, *K_{CO}* transfer factor coefficient of the lung for carbon monoxide, which is DL_{CO}, *IC/TLC* inspiratory capacity to total lung capacity ratio, *SMWD* 6 min walking distance test. *p*-values < 0.05 are shown in italics

[a] Data are presented as mean ± SD

[b] Data are presented as median (25th–75th pc)

patients. Those patients that shorten their telomeres the most during the follow-up period, showed worsening of alveolar gas exchange, lung hyperinflation and clinical outcomes compared with those whose telomeres did not shorten as much. Moreover, patients within the lowest telomere length presented a higher risk of all-cause mortality.

According to previous studies completed in general populations, leucocyte telomeres shorten 40–105 base pairs per year [14, 30]. We found that the mean telomere length in this cohort of COPD patients aged 64 years-old at time of recruitment was 7.6 kbp and it decreased to 5.7 kbp after 10 years, approximately 183 bp/year. In addition, the TL observed in COPD patients in this study corresponds to that observed by others in healthy subjects of similar age but 10 years older [14]. Rutten and colleagues also suggested an anticipated telomere attrition in patients with COPD corresponding to a biological age 7 years older [17]. Other studies using clinical observations but without telomere length determination, have suggested a relationship between COPD severity, and the development of diseases characteristically seen in the elderly [31–33]. Recently, Divo and co-workers [4] using comorbidities network analysis showed that patients with COPD developed a similar prevalence of diseases frequently seen in the elderly one or two decades

Table 4 Longitudinal association between decreased telomere length and lung function and clinical variables during 10 year-follow-up

	Total patients (n = 134)		Deaths during follow-up (n = 43)		Alive patients (n = 91)	
	β	p-value	β	p-value	β	p-value
FEV_1 (L)	0.13	*0.022*	0.17	0.112	0.11	0.104
FEV_1 (% pred)	0.56	0.784	2.91	0.501	0.59	0.798
FEV_1/FVC (% pred)	0.445	*0.0008*	0.579	*0.024*	0.377	*0.015*
PaO_2	0.771	*< 0.0001*	1.398	*0.0003*	0.443	*0.053*
IC/TLC (%)	0.006	*< 0.0001*	0.006	*0.021*	0.006	*0.0009*
K_{CO}	0.877	*0.042*	1.325	0.092	0.817	0.106
6MWD (mts)	4.655	*0.004*	10.53	*0.007*	1.769	0.299
BODE index	− 0.081	*0.009*	− 0.219	*0.002*	− 0.013	0.689

Linear regression of mixed models. β, coefficient

FEV_1 forced expiratory volume in 1 s, *FVC* forced vital capacity, *% pred* per cent predicted, *PaO_2* partial oxygen tension, *K_{CO}* transfer factor coefficient of the lung for carbon monoxide, which is DL_{CO}, *IC/TLC* inspiratory capacity to total lung capacity ratio, *6MWD* 6 min walking distance test. *p*-values < 0.05 are shown in italics

Table 5 Hazard ratio of all-cause mortality in patients with COPD grouped by tertiles of telomere length

	HR (95% CI)	p-value
Model 1		
Medium vs. long T/S[a]	4.803 (0.99–23.18)	0.051
Short vs. long T/S[a]	6.267 (1.32–29.82)	*0.021*
Model 2		
Short/medium vs. long T/S[a]	5.481 (1.23–24.42)	*0.026*
Model 3		
T/S (decrement 0.1 units)[a]	1.446 (1.10–1.91)	*0.009*

T/S relative telomere length ratio, *CI* confidence interval

[a] Cox HR analysis was adjusted by age, FEV_1% and active smoking (current and former smokers) as covariates. Long T/S was used as the reference level. *p*-value < 0.05 are shown in italics

earlier than in patients without COPD. These observations supporting accelerated aging as a potential mechanism in patients with COPD, should be associated with accelerated shortening of telomeres if this were a marker of aging. Indeed, we have previously shown that COPD patients shorten their telomeres over time at a higher rate than healthy individuals of the same age [13].

In this prospective study, COPD patients that shortened their telomeres the most over the 10 years of observation had significantly worse oxygenation (PaO_2), lower $K_{CO,}$ more hyperinflation (IC/TLC), lower BODE index and lower 6MWT than those patients with less telomere shortening. As shown in Table 3, these associations were stronger than that observed between telomere shortening and the change in FEV_1% predicted, suggesting that accelerated aging affects primarily the lung parenchyma over the airway tissue itself. In addition, the effects also seem to impact intensely in the extra-pulmonary components of the disease. In this way, patients with shorter telomeres over time score worse the BODE index, a good predictor of poor outcomes, compared to the FEV_1. Previously, one other study reported a correlation between shorter telomere length and worse oxygenation but not with lung function expressed by the FEV_1 [11]. Moreover, other authors proposed that telomere attrition may act as biomarkers of COPD severity [17, 34], impaired exercise capacity [35], health status (activity score domain of the SGRQ) and exacerbations [19, 35]. We expand on these findings by demonstrating for the first time a relationship between accelerated telomere shortening and worsening of alveolar gas exchange and clinical extrapulmonary variables in patients with COPD, thus supporting the concept that telomere shortening is a surrogate marker of the aging process in vivo [36].

Interestingly, individuals in the lowest tertile of telomere length through the follow-up were at an increased risk of mortality when compared to the highest tertile of TL, independent of age, active smoking and lung function. A telomere length ratio decrement of 0.1 units had a predictive risk value for all-cause mortality. Contradictory results have been reported from studies on general population exploring the relation between TL and mortality [15, 37, 38], but very few studies have been completed in patients with COPD. Our findings are in agreement with Lee and co-workers [18] who found that leucocyte telomere length was related to all-cause and cancer mortality in COPD patients followed a median of 7.5 years. Similarly, a recent study (MACRO study of azythromycin), reported an increased all-cause mortality risk for patients that exhibited shorter telomeres (lowest quartile of TL), although this was only observed in the placebo group [19]. COPD as a disease of accelerated aging is associated with earlier mortality. However, the exact mechanism remains unknown but certainly

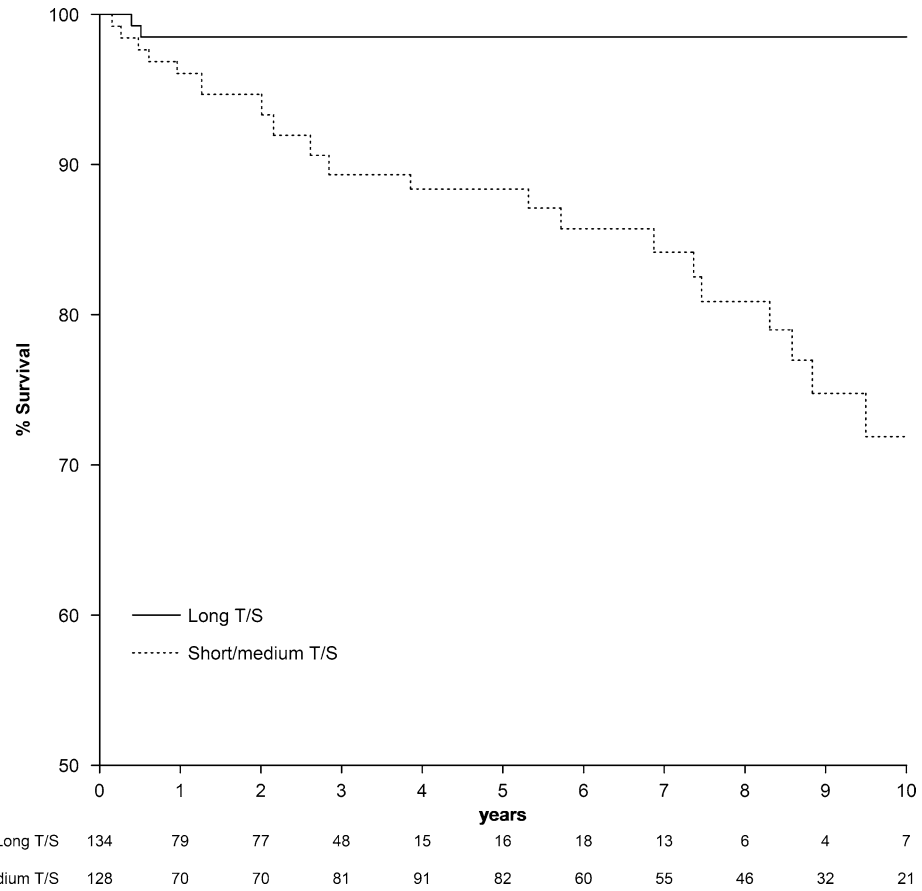

Fig. 2 Kaplan–Meier survival curves for COPD patients with different telomere length. Patients were divided into tertiles of T/S (relative telomere length ratio): T1 (long T/S), T2 (medium T/S) and T3 (short T/S)

inflammation plays a role, in consequence, some authors propose an "Inflammaging" process [39].

Interestingly, some authors have focused their research on certain molecules and existing drugs in an attempt to unravel how to control telomere attrition. SIRT1, an anti-aging protein, whose activation in mice has been reported to prevent inflammatory responses [40] and to be involved in the reduction of telomeric attrition [41]. On the other hand, telomerase activation has emerged as a potential treatment directed to cases with short telomeres and physiological aging [42]. Recently, metformin, the preferred first-line drug against type-2 diabetes is known to reduce oxidative damage accumulation, chronic inflammation, and increase overall lifespan in mice [43]. Recently, other studies have suggested that metformin use may reduce telomere shortening in adults [44, 45].

This study has several strengths. The most important is its prospective nature (first of its kind) and the excellent phenotypic characterization of the cohort and their outcomes registered annually through 10 years. It is also noteworthy that we were able to calculate the corresponding absolute telomere length data, as supported by the high correlation found between southern blot and the qPCR technique used. However, there are also some limitations: First, telomere length was measured in leucocyte cells and not in lung tissue. However, leukocytes remain the tissue of choice for TL measurement in large cohorts of individuals, because it is accessible and representative of distant tissues [46, 47]. Also, we cannot discard that the shortening of TL differs in different blood cells may vary through time. However, the samples were taken at similar times in all patients, thereby decreasing this potential bias, and TL was measured only if the patients presented blood leukocyte and differential counts values that were within the established normal ranges. Second, although 42 patients out of 263 reached 10 years of observation, their baseline clinical and physiological characteristics were similar to the group as a whole, supporting the validity of the results in these patients as a reflection of COPD as a whole. Furthermore, there were 134 patients having at least two measures of telomere

length with a minimum of 6 years follow-up included in the longitudinal analysis (413 observations of clinical and physiological variables) before they were censored or died. Their results provide further support to the conclusions here presented. Also, this is a single center study. A validation cohort would be required; however, this is difficult to achieve due to the complexity of the study design and the time required for monitoring. Another limitation of this study is the absence of histological or imaging data, but this does not detract from the results obtained. Finally, our sample size did not allow us to contrast specific causes of mortality such as cancer or cardiovascular disease, however this does not invalidate the overall findings as the multidimensional nature of the different variables measured moved in the same direction.

Conclusions

In conclusion, this longitudinal observational study showed that an accelerated telomere shortening over time is associated with worse alveolar gas exchange function, worse lung hyperinflation and extrapulmonary affection in patients with COPD. Moreover, having shorter telomeres is associated with all-cause mortality risk. Studies with larger cohorts with several time points of TL measurements, are needed to validate our findings.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12931-021-01616-z.

Additional file 1: Table S1. Baseline characteristics comparisons between patients with COPD (n = 42) that reached 10 years follow-up vs. the rest of the cohort (n = 221).

Additional file 2: Figure S1. Telomere length in patients with COPD distributed by range of age at baseline as follows: ≤ 59 (n = 83), 60–69 (n = 95) and ≥ 70 (n = 85) years old (p = 0.022).

Additional file 3: Figure S2. Correlation between telomere length measure by TFR and T/S ratio.

Abbreviations

COPD: Chronic obstructive pulmonary disease; T/S: Relative telomere length ratio; TL: Telomere length; qPCR: Quantitative Real Time Polymerase Chain Reaction; CV: Coefficients of variance; T/S_mCh: Change in T/S with respect to its mean over time; HR: Hazard ratio; GLIM: General Linear Modelling for repeated measures test; FEV1: Forced expiratory volume in 1 s; FVC: Forced vital capacity; BMI: Body Mass Index; PaO_2: Partial oxygen tension; KCO: Transfer factor coefficient of the lung for carbon monoxide, corrected by alveolar volume; IC/TLC: Inspiratory capacity to total lung capacity ratio; 6MWD: Six-minute walking distance test.

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Authors' contributions

ECL, SCR and CC participated in conception and design; analysis and interpretation and drafting the manuscript for important intellectual content and take responsibility for the integrity of the data and the accuracy of the data analysis. MAG participated in data analysis and interpretation. DM, FGH and JAP participated in data acquisition, BC participated in the interpretation of the results and helped draft the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Availability of data and materials

Data are available upon reasonable request. All data relevant to the study are included in the article or uploaded as additional information.

Ethics approval and consent to participate

Written informed consent was obtained from all participants. Ethical approval was obtained from Hospital Universitario La Candelaria, Tenerife, Spain (PI14/12).

Consent for publication

Not applicable.

Competing interests

Elizabeth Córdoba-Lanús, Sara Cazorla-Rivero, Miguel-Angel García-Bello, Delia Mayato, Francisca Gonzalvo-Hernández and Jessel Ayra-Plasencia declare not to have any financial or personal conflict of interests. Ciro Casanova Macario declares to have received lectures and / or scientific advice from Laboratorios Bial, Boehringer-Ingelheim, Gebropharma, GSK, Esteve, Menarini, Novartis and Rovi in the last 3 years. Bartolomé Celli declares to have received grants from Astra Zeneca; advisory board payments from Glaxo Smith Kline, Boehringer-Ingelheim, Astra Zeneca and Novartis, Pulmonix; and declares not to have shares or interest in any company, neither have received or had any relationship with tobacco money.

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Publisher's Note

ORIGINAL ARTICLE

Comparison of the 2017 and 2015 Global Initiative for Chronic Obstructive Lung Disease Reports

Impact on Grouping and Outcomes

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Abstract

Rationale: The Global Initiative for Chronic Obstructive Lung Disease (GOLD) document has modified the grading system directing pharmacotherapy, but how this relates to the previous one from 2015 and to comorbidities, hospitalizations, and mortality risk is unknown.

Objectives: The aim of this study was to evaluate the changes in the GOLD groups from 2015 to 2017 and to assess the impact on severity, comorbidities, and mortality within each group.

Methods: We prospectively enrolled and followed, for a mean of 5 years, 819 patients with chronic obstructive pulmonary disease (84% male) in clinics in Spain and the United States. We determined anthropometrics, lung function (FEV_1%), dyspnea score (modified Medical Research Council scale), ambulatory and hospital exacerbations, and the body mass index, obstruction, dyspnea, and exercise capacity (BODE) and Charlson indexes. We classified patients by the 2015 and 2017 GOLD ABCD system, and compared the differential realignment of the same patients. We related the effect of the reclassification in BODE and Charlson distribution as well as chronic obstructive pulmonary disease and all-cause mortality between the two classifications.

Measurements and Main Results: Compared with 2015, the 2017 grading decreased by half the proportion of patients in groups C and D (20.5% vs. 11.2% and 24.6% vs. 12.9%; $P < 0.001$). The distribution of Charlson also changed, whereas group D was higher than B in 2015, they become similar in the 2017 system. In 2017, the BODE index and risk of death were higher in B and D than in A and C. The mortality risk was better predicted by the 2015 than the 2017 system.

Conclusions: Compared with 2015, the GOLD ABCD 2017 classification significantly shifts patients from grades C and D to categories A and B. The new grading system equalizes the Charlson comorbidity score in all groups and minimizes the differences in BODE between groups B and D, making the risk of death similar between them.

Keywords: chronic obstructive pulmonary disease; Global Initiative for Chronic Obstructive Lung Disease; prevalence

Chronic obstructive pulmonary disease (COPD) has a great impact on morbidity and mortality worldwide (1). The Global Initiative for Chronic Obstructive Lung Disease (GOLD) document is the most used treatment guide, and its contents have been recently updated (2). The most important change for practicing clinicians is the one that modified the grading system suggested for the initial pharmacotherapy of patients from a schema that had three axes (perception of dyspnea or health status, degree of airflow limitation, and risk of exacerbations), in which patients were graded on an ABCD system (3), to one that

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At a Glance Commentary

Scientific Knowledge of the Subject: The Global Initiative for Chronic Obstructive Lung Disease (GOLD) document is the most widely accepted reference for the diagnosis and management of patients with chronic obstructive pulmonary disease. In 2017, a total revision of the document was completed. One important change in this version is the modification of the ABCD grading system directing pharmacotherapy. Whereas, in 2015, the degree of airflow limitation, frequency and intensity of exacerbations, and severity of compromise in dyspnea or health status provided the basis for the grading, in 2017, the degree of airflow limitation was excluded and only the other two domains were left to grade patient compromise. The relation to outcomes is not known.

What This Study Adds to the Field: Compared to the 2015 schema, the GOLD ABCD 2017 classification significantly shifts patients from the more severe grades C and D to categories A and B. The new grading system equalizes the Charlson comorbidity score among all groups. It also minimizes the differences in body mass index, obstruction, dyspnea, and exercise capacity index between groups B and D, making the risk of death similar between them. The 2015 grading relates better to risk of death than the 2017 system.

retains identical labeling but does not take into account the degree of airflow limitation measured with the FEV_1 % predicted. The new proposal includes only the history of exacerbations on the vertical domain and degree of impact of the disease on the perceptive domain to select the initial treatment for stable patients. While preserving the same alphabetical order, the new schema includes: group A, corresponding to patients with few symptoms and no exacerbations; group B, which includes patients with more symptoms and low exacerbations risk; group C, which includes those patients with few symptoms, but with exacerbations; and group D, which includes those patients with symptoms and exacerbation risk.

There is little evidence on how the new GOLD grades shift the proportion of patients that are grouped in each grade when compared with the 2015 classification and how the new grading relates to predictors of outcomes, such as the body mass index, obstruction, dyspnea, and exercise capacity (BODE) index (4), and the presence and severity of comorbidities measured with the Charlson index (5). Although the ABCD grading in the GOLD document is not meant to be a mortality-predictive tool, its implementation relates to disease severity as impacting on exacerbations and health status, both of which are associated with poor outcomes. Using this new grading system as it relates to the risk of exacerbations, hospitalizations, and death over time can inform future modifications of this multidimensional tool.

In this study, we tested the hypothesis that, by abolishing the degree of airflow limitation measured by the FEV_1% from the grading system, there would be significant changes in the proportion of patients in the different ABCD groups. We also evaluated whether the change would result in modifications in the BODE index and the Charlson comorbidity index in the different grades, both of which predict long-term outcomes. Finally, we also studied how the new grading system relates to risk of death over 5 years of observation.

Methods

The 819 patients included in this study are part of the BODE cohort consecutively recruited in three clinics in Spain and one in the United States between 1997 and 2016. The inclusion and exclusion criteria have been previously described (4). In brief, it is a prospective, multicentric, and ongoing cohort with an annual longitudinal follow-up in pulmonary clinics. In all patients, anthropometric variables and physical examination were recorded. Spirometry was performed using the standards provided by the American Thoracic Society and European Respiratory Society, and COPD diagnosis was made following international criteria (6, 7). All patients had at least a history of 10 pack-years and must have been stable for a minimum of 6 weeks. Exacerbations were defined as any episode of worsening of dyspnea, cough, or sputum requiring antibiotics or systemic corticosteroids or admission to the hospital due to COPD exacerbation. Exacerbations were recorded by direct assessment of the patients during or close to the episode when the patients were given a course of antibiotics or systemic corticosteroids, and confirmed by reviewing the electronic records. The exacerbations were classified as moderate if treated in an ambulatory environment or severe if they required hospitalization. Dyspnea was measured with the modified Medical Research Council scale (8). The BODE index was calculated as previously reported (4). The 6-mile walk distance test measured the best of two walks separated by at least 30 minutes (9). All-cause mortality was measured until February 2017. Cause-specific mortality was ascertained by each site investigator to the greatest detail possible, and then categorized in a systematic and masked fashion by four of the investigators (B.C., C.C.M., J.J.Z., and M.D.) as either death related to COPD, non-COPD respiratory cause, lung cancer, other causes, or unknown causes. Comorbidities were ascertained using self-reported diseases and reviewing the electronic records, and the Charlson comorbidity index was calculated (5). Detailed information on how comorbidities were assessed has been published previously (10). All participants received optimal medical treatment following international guidelines, and signed the informed-consent form previously approved by the ethics committees of each participating center.

GOLD 2015 versus GOLD 2017 and Outcomes

We stratified patients by the new and old GOLD classifications using data obtained at the time of enrollment and compared them to assess the changes in each group population. We compared the BODE and Charlson scores in each grading system, prospectively determined the yearly rate of exacerbations during the follow-up period, and compared this rate as well as mortality by groups using the GOLD 2015 and GOLD 2017 criteria.

Statistical Analysis

For demographics, ANOVA was used for continuous variables and chi-square test was performed for categorical variables. The log-rank test was used to compare the

difference in exacerbations, hospital admissions, and mortality within GOLD subgroups 1–4 and A–D. Finally, we tested the predictive accuracy of GOLD 2015 A–D compared with GOLD 2017 stratification for exacerbations and mortality. All analyses were performed with R version 2.13.1 (R Foundation for Statistical Computing) (11).

Results

The characteristics of the patients included in the study are shown in Table 1. The patients were primarily individuals with moderate, severe, or very severe COPD, but had a wide range of degree of airflow limitation, BODE, and Charlson scores. One-third of them were current smokers, but they all had an intense smoking history measured in pack-years. One-quarter of them reported having had exacerbations the year before enrollment, and 12% had been hospitalized for the episode. Over the 60 months of follow-up, 211 patients died, about half of them from COPD and respiratory failure.

Distribution and BODE Index: GOLD 2015 versus GOLD 2017

There was a substantial change in the prevalence of each grading group when classified by the 2017 compared with the 2015 GOLD criteria. The proportion and the change within each group are shown in Figure 1. The new classification significantly increased the proportion of patients in groups A and B, whereas it decreased the proportion of patients in groups C and D by one-half. The severity of the disease measured by the BODE index also changed between the classifications. Grade D in the 2015 GOLD grading had a significantly higher BODE index than the rest of the grades (4.22 ± 1.94; $P < 0.001$ compared with C, B, and A), followed by grade B (2.35 ± 1.27; $P < 0.001$ compared with grade A), grade C (1.86 ± 1.17; $P <$ 0.0 01 compared with grade A), and, finally, grade A (0.49 ± 0.65). The severity changes in the new GOLD 2017 classification resulted in groups D and B having similar values (3.9 ± 2.19 and 3.37 ± 1.78 respectively), both significantly higher than group C (1.36 + 1.19), which, in turn, was higher than group A (0.85 ± 1.02; $P = 0.008$) (Figure 2).

Table 1. Clinical Characteristics at Baseline of the Patients with Chronic Obstructive Pulmonary Disease Included in the Study

Characteristics	Value
N	819
Age, yr, mean ± SD	66 ± 9
Male, *n* (%)	686 (84%)
Current smokers, *n* (%)	271 (33)
Pack-years, mean ± SD	61 ± 30
FEV_1, %, mean ± SD	60 ± 20
GOLD 1, *n* (%)	141 (17)
GOLD 2, *n* (%)	401 (50)
GOLD 3, *n* (%)	213 (26)
GOLD 4, *n* (%)	55 (7)
BODE index	
Mean ± SD	1.9 ± 1.9
Quartile 1, *n* (%)	537 (69)
Quartile 2, *n* (%)	153 (20)
Quartile 3, *n* (%)	58 (8)
Quartile 4, *n* (%)	26 (3)
Charlson index, mean ± SD	2.8 ± 2.4
BMI, mean ± SD	27.4 ± 4.8
Dyspnea, mean ± SD*	1.31 ± 1.13
Dyspnea <2, *n* (%)	504 (62)
All exacerbations	680
Exacerbators, *n* (%)†	198 (24)
Ambulatory exacerbators, *n* (%)‡	118 (60)
Hospital exacerbators, *n* (%)§	80 (40)
All-cause mortality, *n* (%)	211 (26)
COPD mortality, *n* (%)	104 (13)
Follow-up, mo, mean ± SD	52 ± 33.96

Definition of abbreviations: BMI = body mass index; BODE = body mass index, obstruction, dyspnea, and exercise capacity; COPD = chronic obstructive pulmonary disease; GOLD = Global Initiative for Chronic Obstructive Lung Disease.

*Dyspnea measured by the modified Medical Research Council scale.

†Subjects with two or more exacerbations requiring systemic corticosteroids or antibiotics or at least one hospital exacerbation in the past year.

‡Subjects with at least two ambulatory exacerbations requiring systemic corticosteroids or antibiotics, but with no hospital exacerbations, in the past year.

§Subjects with at least one hospital exacerbation in the past year.

Exacerbations

The number of total exacerbation was 680, occurring in 198 (24%) of the patients from the whole cohort. There were more ambulatory than hospital exacerbations (118 vs. 80; $P = 0.05$). Patients with hospital exacerbations were younger (64 ± 9 vs. 67 ± 10 yr; $P = 0.05$) and had a lower FEV_1% (48 ± 17 vs. 55 ± 20; $P = 0.01$), but no differences in body mass index, sex, current smokers, or pack-years. Patients who were hospitalized for exacerbations had increased mortality (45% vs. 25%; $P =$ 0.003), whereas ambulatory exacerbations did not affect this end-point. If only dyspnea and hospital exacerbations were used to grade the patients (exacerbations with impact on mortality), grades C and D would include only 5.49% and 4.27% of the cohort population, respectively.

Comorbidities

In the 2015 document, group C had the highest numerical score (2.56 ± 2.26), followed by groups B, A, and D (2.32 ± 2.52, 2.19 ± 2.36, and 2.02 ± 2, respectively), with no statistical difference ($P = 0.11$) for all values among them. In the 2017 new classification, there were no significant differences among the groups either (2.21 ± 2.31, 2.18 ± 2.34, 2.77 ± 2.4, and 2.02 ± 1.91 for A, B, C, and D, respectively; $P = 0.08$). As has been shown, comorbidities measured by the Charlson index were a predictor of mortality ($P < 0.001$).

Mortality

The median time of follow-up in this cohort was 52 months. During the observation period, 211 (25%) patients died. The main cause of death was from COPD (29.3%), followed by lung cancer (17%) and cardiovascular diseases (sudden death

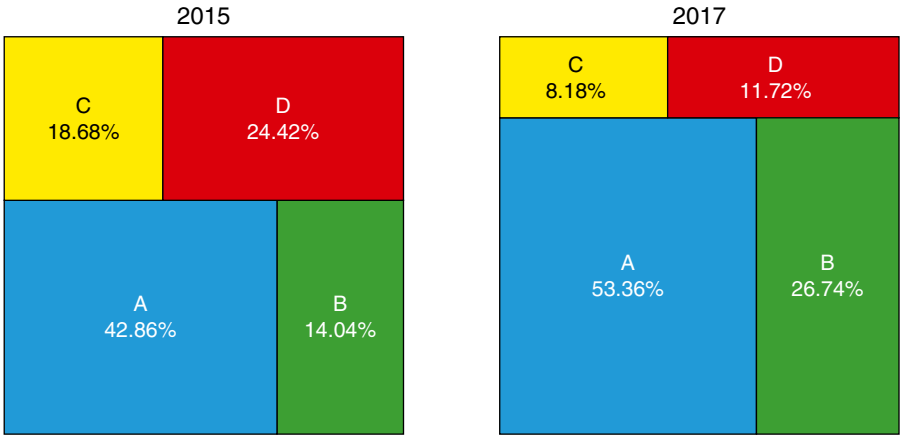

Figure 1. Distribution of the same patients with chronic obstructive pulmonary disease in the different ABCD Global Initiative for Chronic Obstructive Lung Disease grading groups using the 2015 version versus the new 2017 version.

included as a cardiovascular cause of death) (11.9%). Other causes of death included other malignant tumors, infections, and unknown causes. All-cause mortality at 5 years in the old 2015 GOLD was higher in grade D, followed by grades B, C, and A (Figure 3A). Grade A had a significantly lower mortality rate than B, C, and D, whereas grade D had significantly higher mortality than grade C ($P = 0.005$). Grades B and C had similar mortality, although there was a trend for grade B to be at a higher risk of death ($P = 0.06$). In the 2017 GOLD, grades B and D had similar mortality rates ($P = 0.98$), whereas grades C and A both had significantly lower mortality (A vs. B and D, $P < 0.001$ and C vs. B and D, $P = 0.02$ and 0.04, respectively) (Figure 3B). The degree of obstruction measured by FEV_1% affected mortality within each grade (Table 2). If the original grading was based only on dyspnea and hospitalized exacerbations, the mortality was similar between grades B, C, and D, with the group A patients showing a lower mortality rate ($P < 0.001$; Figure 3C). Mortality was also affected by FEV_1% in the cohort ($P < 0.001$), and had prognostic value in grades A, C, and D ($P < 0.001$, $P = 0.03$, and $P < 0.001$, respectively), but not in grade B ($P = 0.09$) (*see* Table E1 in online supplement). The GOLD 2015 model was a better predictor of mortality than the new 2017 model ($P = 0.019$; Figure E1).

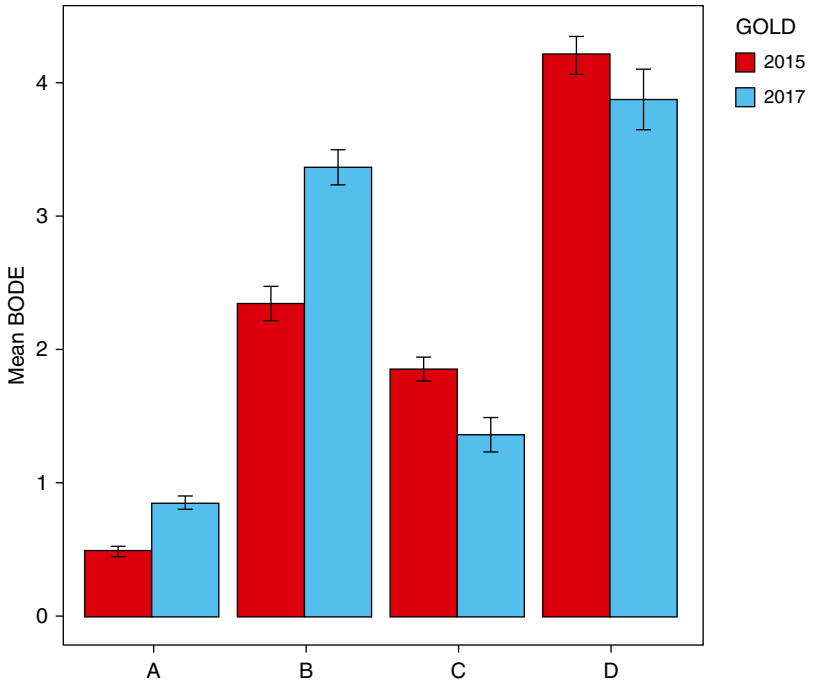

Figure 2. Changes in the BODE (body mass index, obstruction, dyspnea, and exercise capacity) index between the 2015 and 2017 Global Initiative for Chronic Obstructive Lung Disease (GOLD) documents (mean ± SE).

Discussion

Our study shows that there are important shifts in the proportion of patients in the ABCD grades between the 2015 and the 2017 GOLD classifications. Grades C and D now include half of the proportion they used to have, with significant increases in the number of patients in groups A and B. The BODE index has also shown changes, with groups A and C being similar and significantly lower than groups B and D, which, in turn, had equal scores. The new grading system decreases the ability to predict risk of death over 5 years. Using mortality as an outcome, the strongest driver is the information provided by the dyspnea scores in the new GOLD classification. The limited importance of exacerbations on mortality prediction is best explained by the low impact on mortality of the ambulatory exacerbations. The predictive power improves if only exacerbations leading to hospitalizations become the major determinant of the grading on the exacerbation axis.

The decision to leave out the pulmonary function in the new GOLD grading system that guides pharmacotherapy is clinically understandable, because drug treatment longitudinally has little effect on this outcome (12, 13). However, the practical consequences of this approach in the GOLD ABCD diagram have not been completely studied. The reclassification of the same patients in this study led to a migration from groups C and D to A and B, leaving groups C and D just for exacerbators. In this cohort, consisting of outpatients attending pulmonary clinics, groups C and D each account for only about 12% of the patients. This proportion is likely to be even lower in patients with COPD attending general medical clinics, as those patients tend to have milder

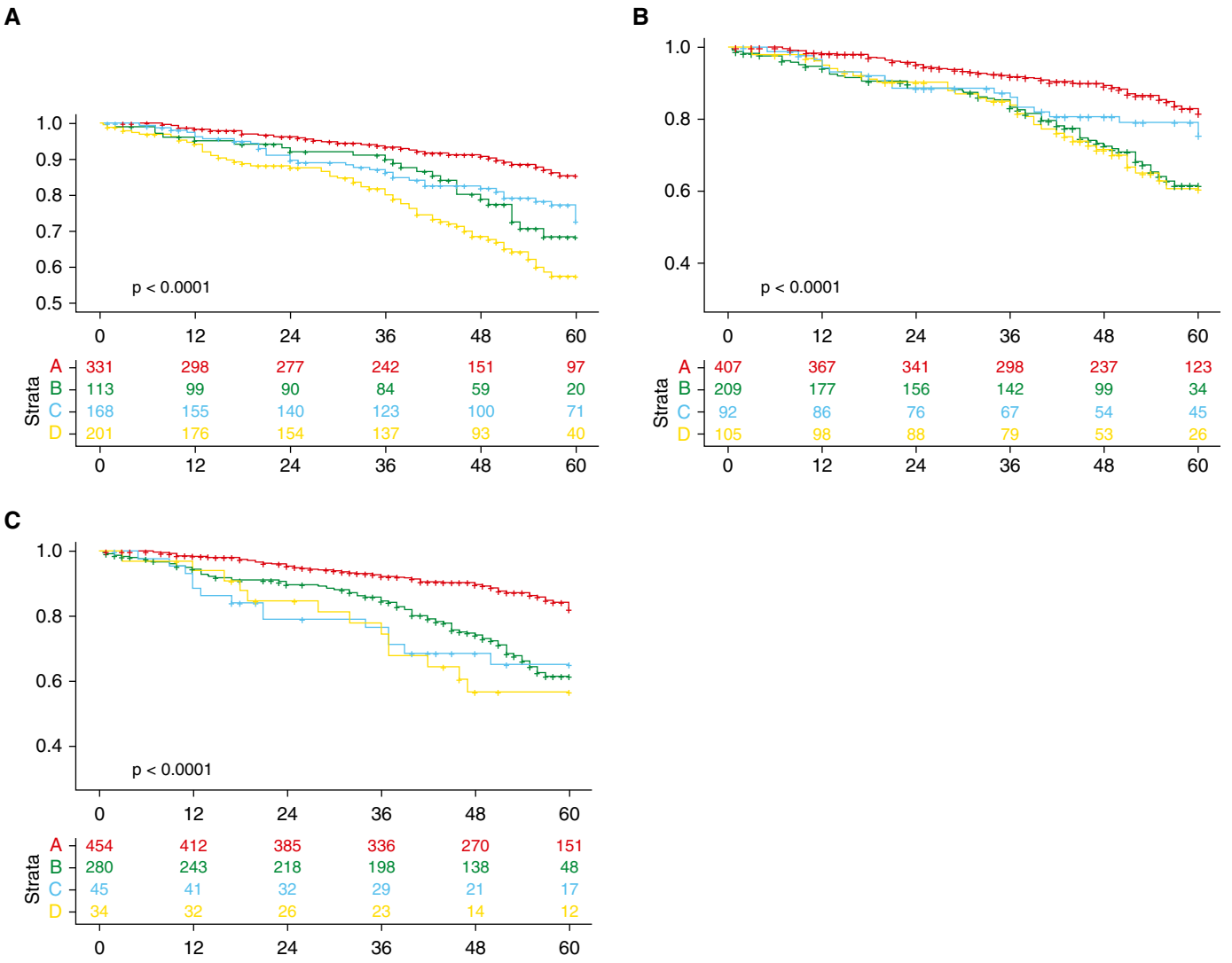

Figure 3. Kaplan-Meier curves for survival. (*A*) The Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2015 ABCD grading. (*B*) The new GOLD 2017 ABCD grading. (*C*) The 2017 ABCD grading, where only hospitalized, and not ambulatory, exacerbations were used to grade the patient.

severity of disease and lower exacerbation rates (14). This realignment will influence the pharmacotherapy approach compared with the 2015 version, because, in the vast majority of drug trials, patients were selected by the degree of FEV_1 impairment (12, 13, 15, 16). We fully recognize that a shift of patients to a lower grade is not wrong *per se*. However, the presence of groups containing a very small proportion of patients and with little relationship to outcomes suggests that further simplification is possible, thereby helping clinicians better adopt the grading.

Aside from the important changes in the realignment of the proportion of patients in the ABCD grouping, the new changes in the diagram also modify the severity of the population in each group. In the new GOLD classification, group B has the same BODE index as group D, and both of them are significantly higher than groups A and C. With this new approach, the differences between groups B and D and groups A and C become smaller, thereby decreasing the value of their separation. Particularly important is the scarce relevance of exacerbations (as defined today by the GOLD document) as a predictor of mortality, with the degree of dyspnea becoming the greatest determinant of the risk of death in this classification. This is probably caused by the definition of "frequent exacerbators." The association between exacerbations and mortality has been previously addressed, showing that mortality risk is strongly driven by exacerbations that lead to hospitalization (17). Our results support this finding, and extend it by showing that, if the exacerbation risk is stratified by the history of hospitalizations, the association with mortality in group C increases and reaches the same level as B and D groups, making exacerbations relevant for prognosis once again. We believe that exacerbations having no impact on mortality does not mean that one should refrain from treating or preventing them, because all exacerbations lower the quality of life and increase healthcare use (18, 19).

In this study, the degree of dyspnea is the variable that is best associated with mortality risk. Dyspnea is a powerful and well-known predictor of mortality in COPD (4, 20). It is remarkable that, in the new

Table 2. Severity of Spirometric Airflow Limitation within Each Group in the New 2017 Global Initiative for Chronic Obstructive Lung Disease Document

Group	Obstruction	*n* (%)
A	GOLD 1	104 (27)
	GOLD 2	217 (55)
	GOLD 3	65 (16)
	GOLD 4	6 (2)
B	GOLD 1	19 (10)
	GOLD 2	85 (44)
	GOLD 3	72 (37)
	GOLD 4	17 (9)
C	GOLD 1	10 (11)
	GOLD 2	46 (50)
	GOLD 3	28 (30)
	GOLD 4	8 (9)
D	GOLD 1	7 (7)
	GOLD 2	38 (36)
	GOLD 3	41 (39)
	GOLD 4	20 (18)

Definition of abbreviations: GOLD = Global Initiative for Chronic Obstructive Lung Disease; GOLD 1 = mild; GOLD 2 = moderate; GOLD 3 = severe; GOLD 4 = very severe.

GOLD classification, mortality is dichotomized by dyspnea severity. Groups B and D have the same mortality and are significantly higher than groups A and C (which also have the same mortality rate). A few studies have associated the higher symptoms score of patients in group B with the presence of more comorbidities in that group (21). However, very few studies have used a validated comorbidity score, such as the Charlson index, and most have centered on specific comorbidities, mostly of the cardiovascular system (22). In this study, although the Charlson score was associated with mortality, it did not seem to play an important role in the ABCD grouping, where there were no differences among groups in the new or old GOLD classifications. Based on these results, where groups B and D have the same BODE and Charlson scores and a similar mortality rate, both grades should start treatment with a similar therapeutic approach.

Our study has some limitations. We could not differentiate between cardiovascular comorbidities and their burden in the high–dyspnea score population. The proportion of cardiovascular comorbidities in these patients has previously been reported to be around 20% (21). This means that 80% of this group showed the same signal (dyspnea) independent of cardiovascular comorbidity. Intensity of dyspnea remains one of the best predictors of mortality in these patients, whether comorbidities are coexisting or not, and is the reason why it has been included in the majority of multidimensional indices attempting to stage the severity of disease (4, 23–26). Although every effort was made to determine the exact cause of death, local differences in the information provided in death certificates or given to the families and in the medical records in different areas of Spain and the United States make direct comparisons with other studies difficult to interpret. Another limitation of this study is the effect of treatment on exacerbations and mortality. As the inclusion time of our cohort is very long, patients had the optimal medical treatment initiated according to the guidelines valid at the time of enrollment. The treatment changed as the guidelines changed. The variation in treatment could have affected the rate or severity of exacerbations and the mortality rate. However, as the same patient was used to compare the effect of reclassification on outcomes, the comparisons are unlikely to have been affected by the treatment. An aditional limitation of the study is the small number of women in our cohort, making our results difficult to extrapolate to this population. However, there were 131 women, in whom the findings were very similar. Finally, we also measured dyspnea with the modified Medical Research Council scale, so our findings could vary if symptoms were classified by the COPD Assessment Test, and Clinical COPD Questionnaire score, as has been previously shown (27, 28).

In summary, moving from the 2015 grading system to this new GOLD classification changes the population within each group substantially. Groups C and D decrease by half, even though this is a cohort with a high number of frequent exacerbators. The change to the new GOLD document eliminates the differences between groups A and C and groups B and D in severity and mortality. Although intuitively attractive, the ABCD grading system could be simplified to better provide clinical guidance. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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