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Early View

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Symptomatic smokers without COPD have physiological changes heralding the development of COPD.

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Abstract

Background

Chronic Obstructive Pulmonary Disease (COPD) is a major health problem, mainly due to cigarette smoking. Most studies in COPD are dedicated to fully developed COPD in older subjects, even though development of COPD may start soon after smoking initiation. Therefore, there is a need to diagnose this “early disease” by detecting the initial events responsible for ultimate development of COPD.

Methods

Measurement of maximum mid expiratory flow between 25-75 of vital capacity (MMEF) in a routine spirometry, that detects small airways disease, was used to investigate if MMEF abnormalities in smokers without COPD (noCOPD) would relate to respiratory symptoms and identify smokers that might progress to COPD. For this purpose we studied 511 smokers, 302 COPD and 209 noCOPD, followed long term with spirometry including MMEF, CO diffusion capacity (DLCO), 6-minute walking test (6MWT), MRC Dyspnoea Scale, and COPD Assessment Test (CAT). Three spirometries V1,V2,V3 (5±2.5 and 10±4 years apart respectively from V1) were performed to assess functional decline and development of COPD.

Results

65 % of noCOPD had an abnormal MMEF (<80%) and 38% an abnormal DLCO. NoCOPD with MMEF<80% performed worse in the 6MWT (p=0.01), were more dyspnoeic (p=0.01) and had higher prevalence of chronic bronchitis than noCOPD with MMEF>80% (p=0.04). 21% of noCOPD with MMEF<80%, and 2.7% with MMEF>80% developed COPD by V3 (p=0.0004).

Conclusions

The MMEF, a functional test available in a routine spirometry, can detect early lung abnormalities and identify the subset of symptomatic smokers with pathologic changes that might lead to COPD.

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a major global public health problem, cigarette smoking being by far the chief etiological factor for its development. COPD can affect between 15 to 30% of smokers (1), and among those affected there is a large variation in the severity of the disease they could develop, indicating that a predisposing individual background, likely multifactorial, is the basis for both the development of the disease and its severity.

The great majority of studies in COPD have been so far dedicated to the investigation of severe COPD in older subjects, where the disease is fully developed. However, it is well established that the development of COPD may start soon after the beginning of smoking, which has emphasised the need to diagnose and define this “early disease”, in order to investigate the factors associated and possibly responsible for disease progression and eventual severity (2,3).

Ideally, early COPD would be defined by detecting the initial events responsible for ultimate development of pathology (2). It has been recently described that smokers could present with clinically significant pulmonary symptoms not reflected by spirometric airflow limitation (normal FEV1/FVC) (4), and respiratory symptoms are been entertained as a surrogated form of evidence for the definition of what it has been called “early disease” (2,3). A subset of symptomatic smokers probably already has pathologic changes in the lung that might or might not lead to COPD, but additional research is needed to identify that subgroup unambiguously (3).

If symptoms in smokers with normal FEV1/FVC were due to structural abnormalities, their identification by easily feasible tests would be essential for their diagnosis, validation of symptoms and the monitoring of their progression (5). Although airways abnormalities and early emphysema have been described by computed tomography (CT) in smokers with normal spirometry (6,7), CT does not properly visualize small airways and furthermore would not be adequate for large populations studies. However, detecting early small airways disease and assessing its progression might be accomplished using sensitive, but not readily available, tests such as single breath nitrogen washout and impedance oscillometry (8), or by simpler tests sensitive to small airways abnormalities available in a routine spirometry, like the Maximum Mid Expiratory Flow at 25-75% of FVC (MMEF) (also known as FEF25-75) and the transfer factor of the lung for carbon monoxide DLCO (9, 10). An abnormal MMEF is an early feature of lung disease in patients with α-1 antitrypsin deficiency associated with faster decline of FEV1 (11), and recently MMEF has been shown to be associated with emphysematous changes and airway abnormalities in a

cohort of smokers with and without COPD (12). However the value of MMEF in the detection of lung abnormalities and their possible progression in smokers without COPD (FEV1/FVC>70%) has never been investigated.

Based on those premisses we hypothesize that: a) MMEF abnormalities in smokers without COPD would relate to the respiratory symptoms; b) MMEF abnormalities might identify smokers that would eventually progress to COPD.

For this purpose, we used an ongoing cohort of smokers with and without COPD, free of significant comorbid conditions at recruitment, in which consecutive functional measurements over 10 years of follow up were available.

METHODS

Study population

Participants were recruited among smokers who first attended the Pulmonary Clinic at the Hospital Universitario Miguel Servet (Zaragoza, Spain) requesting to be included in a smoking cessation program or referred by other doctors to assess their respiratory health between October 2010 and April 2014. The inclusion criteria are detailed in Figure E1 in Supplementary Data.

At baseline, all subjects were clinically stable, free of major comorbidities, not having had any exacerbations for at least 8 weeks (details in Supplementary Data). Subjects with asthma or history of asthma, bronchiectasis, autoimmune diseases, haematological diseases, other respiratory diseases, or coexisting malignancy at recruitment were excluded. All subjects underwent a comprehensive clinical and functional examination including spirometry, Maximum Mid Expiratory Flow at 25-75% of FVC (MMEF), measurement of transfer factor of the lung for Carbon monoxide (DLCO), using the European CECA as predicted values (13). The 6-minute walking test (6MWT), modified Medical Research Council (mMRC) Dyspnoea Scale, and COPD Assessment Test (CAT) (14) were also obtained.

COPD was defined by FEV1/FVC <70% (1) and smokers without COPD (noCOPD) by FEV1/FVC>70% post-bronchodilator. In noCOPD subjects, the baseline visit spirometry (V1) was compared to a second (V2) and a third (V3) spirometry performed after 5±2.5 (V2) and 10±4 (V3) years of follow up to assess functional decline over time and the potential development of COPD. Patients with COPD at baseline had a second spirometry after 10±4 years of follow up to assess functional decline.

Chronic bronchitis was defined as the presence of cough and sputum production for at least 3 months in each of two consecutive years (1). Annual frequency and type of exacerbations were collected. The study was approved by human-research review board

(IRB.12/2010) and all patients provided informed written consent before any procedure was done.

Statistical analysis

Patient's characteristics were described using mean±SD or median (range) for continuous variables and counts and percentages for categorical variables. For continuous variables, normal distributions were tested using the Shapiro-Wilk test. Comparisons among groups were evaluated with Mann-Whitney U tests or Kruskal Wallis test as appropriated. Distributions of categorical variables were compared with the $\chi 2$-test. The Lower Limit of Normal (LLN), which represents the 5^{th} percentile and defined as -1.645 z-score value, was calculated for FEV1/FVC (15-17). Correlation coefficients were calculated using the nonparametric Spearman's rank method. In noCOPD patients we performed the repeated measurements analysis of variance (ANOVA) to evaluate the difference in FEV1 decline in the follow-up period.

A multivariate logistic regression was performed in smokers without COPD to detect possible significant predictors of COPD development and FEV1 decline at follow up.

All analyses were performed using SPSS (version 25.0.0.1 for Windows). Statistical significance was assumed for a p value <0.05.

RESULTS

Among the 511 smokers included in the study, 302 (59%) had COPD (COPD) and 209 (41%) did not (noCOPD), since FEV1/FVC was >70%, a value very similar to the 71% obtained by calculating the LLN using the z-score (16-18). Smokers with COPD were older, smoked more and had a higher prevalence of chronic bronchitis (CB) than noCOPD (45% vs 27%; p=0.001). The prevalence of CB was similar in all COPD GOLD stages. As expected, FEV1, MMEF and DLCO values were lower in COPD than in noCOPD (Table 1). The therapy received by the subjects in both groups are shown in table E1 of the online supplement. A higher proportion of COPD than noCOPD received treatment, and triple therapy was used significantly more in noCOPD with MMEF<80% than in those with MMEF>80%.

Functional changes in smokers without COPD.

The relation between FEV1/FVC and MMEF in the whole population at the first spirometry (Figure 1A) showed that 65% of the subjects with noCOPD had an abnormal MMEF (less than 80% predicted) (11,12,19), indicating airflow obstruction secondary to abnormalities of small airways or decrease in elastic recoil or both. When noCOPD were divided according to MMEF>80% (normal) or <80% (abnormal), those with MMEF<80% were

older, had a higher body mass index and had smoked more. The proportion of active smokers was similar in the two groups (Table 2).

The DLCO was abnormal (<80% predicted) in 38% of smokers with noCOPD (Figure 1B), further defining a significant and detectable lung abnormality in smokers without COPD. NoCOPD subjects with abnormal DLCO had a lower MMEF, a higher proportion of CAT score >10 (44% vs 27%; p=0.01) and a higher MRC dyspnoea score (1.34±1.17 vs 0.95±1.18; p=0.007) than noCOPD with normal DLCO (Table E2).

Relation of functional abnormalities to symptoms.

Clinically, noCOPD with MMEF<80% performed significantly worse in 6MWT (459±109 vs 519±114 meters; p=0.01), were more dyspnoeic (1.23±1.19 vs 0.85±1.15 dyspnoea score; p=0.01, Figure 2A), had a higher number of total exacerbations per year (0.56±1.12 vs 0.36±0.91; p=0.04) and a higher prevalence of CB than noCOPD with MEF>80% (31% vs 19%; Table 2). The CAT score was similar in noCOPD smokers regardless the MMEF (< or > 80%) and its value was influenced by the presence of CB: in the MMEF <80% population, those with CB had a higher CAT score than those without CB (13.95±6.63 vs 7.37±5.76; p=0.0001; Figure E2).

Subjects with noCOPD and MMEF<80% with CB had similar smoking history than those without CB, but more of them were active smokers (62% vs 39%; p=0.01). The proportion of subjects with MRC dyspnoea score >2 (55% vs 28%; p= 0.003), and the MRC dyspnoea score (1.69±1.19 vs 1.02±1.14; p=0.002; Figure 2A and B) were higher and the DLCO % predicted lower (78.45±16.5 vs 87.10±18.27; p=0.007) in the MMEF<80% group with CB than in those without. The total number of exacerbations per year were also higher in the MMEF<80% group with CB (0.87±1.44 vs 0.41±0.9; p=0.002).

FEV1 decline and COPD development.

Twenty one percent of noCOPD with MMEF<80%, halve of them younger than 50 years, developed COPD by the time of the third visit (V3), with a fall of FEV1/FVC from 73.1± 2.8% to 62.6±5.7% and a FEV1 decline of 52±23 ml/year, while only 2.7% of the noCOPD with MMEF>80% developed COPD at V3 (p=0.001). Of interest, 85% of the noCOPD with MMEF<80% who developed COPD at V3, had already developed COPD by the second visit (V2). The FEV1 decline in noCOPD with MMEF <80% who did not develop COPD was 24±34 ml/year, while in those who did develop COPD the decline was 52±23 ml/year (p=0.0001; Table 3). The proportion of symptomatic smokers and/or the score of symptoms, were not significantly different between noCOPD with MMEF<80% who developed COPD and those who did not develop it, except the MRC dyspnoea score that was higher in those who developed COPD.

Aknowledging that MMEF is a very sensitive but also highly variable test in the detection of small airway disfunction, we have also looked at the MMEF cut-off of 60% predicted, to provide a further insight into the interpretation of the data. As expected, the numbers of patients with MMEF <60% (26% of the 209 noCOPD) is lower but the percentage of those developing COPD by V3 is higher at 31% compared to the 21% developing COPD when the cut-off was at 80%. All these changes were seen while both the FEV1 (85%) and the FEV1/FVC (72%) were still within normal limits (Table E3 and E4). Furthermore, a logistic regression analysis in smokers without COPD showed that MMEF at V1 was the only factor associated with COPD development (p=0.001) and with lung function decline (p=0.03) at follow up, results that support the importance of the MMEF as a biomarker for disease progression (Table E5).

The FEV1 decline in the COPD group was very variable, variability accounted in part by the presence of CB, since COPD with CB decline more than COPD without CB (41±48 vs 22±53 ml/y, p<0.01) and in part by the smoking activity, since active smoking further accelerates FEV1 decline. In the presence of CB, ex-smokers declined less than active smokers (48.5±47.9 vs 31.6±45.1 ml/y, p<0.01; Figures E3).

DISCUSSION

In our population of smokers without COPD, 65% had an abnormal MMEF indicating airflow obstruction at the level of the small airways, and 38% had an abnormal DLCO, a manifestation of V/Q mismatching. The evident pathological abnormalities present in the lung before the spirometric diagnosis of COPD, were correlated with the clinical and symptomatic profile in smokers without COPD. Furthermore, 21% of smokers without COPD with an abnormal MMEF (halve of them younger than 50 years) developed COPD during the follow up, an important finding that alerts to a possible progression to COPD in these smokers.

Recent literature has underlined that smokers without COPD, or preserved pulmonary function, can present with significant respiratory symptoms (4,20), which it has been suggested could be defined as the initial events heralding the ultimate development of pathology before spirometry becomes abnormal (3,7). Yet a more comprehensive use of all the spirometric data could be helpful in this regard.

It is well accepted that the earliest lung abnormalities produced by cigarette smoking affect bronchioles less than 2 mm of diameter-the small airways- which contribute less than 30% to the flow resistance in normal lungs (9). Thus, small airways abnormalities could be present in smokers well before the FEV1/FVC% becomes abnormal (21,22), and could be detected by parameters available in a routine spirometry like the MMEF (9,22).

The significance of the pathological abnormalities reflected by the abnormal MMEF and DLCO, is underlined by the lower distance walked in the 6MWT, the higher dyspnoea score and the higher number of exacerbations in smokers without COPD.

How does small airways dysfunction fit into this scenario? The first evidence of the pathophysiological role of the small airways abnormalities was demonstrated by studies on the frequency-dependence of dynamic compliance by Woolcock and Macklem (23). Essentially the heterogeneous distribution of the small airways abnormalities throughout the lung, with some airways remaining more obstructed than others during the ventilatory phase, would result in some regions of the lung moving during the respiratory cycle out of phase with others. As a result, slow regions will have smaller tidal volumes than the fast ones which would result in significant abnormalities in ventilation distribution and gas exchange, especially as frequency of breathing increases (24).This would mean that, as requirement for ventilation increases, the volume of lung participating in ventilation decreases, with the consequent dynamic hyperinflation, which becomes the physiological basis for dyspnoea and decreasing exercise ability (25).

The abnormalities in DLCO at this stage of disease are not surprising, since the DLCO is influenced not only by the surface area for gas exchange, but also by ventilation distribution and ventilation/perfusion (mis)matching. Impaired perfusion in emphysema-free areas (26), by vascular compression in patchy areas of localized gas trapping due to small airway dysfunction, may decrease DLCO (27). A low DLCO signals high ventilation/perfusion (increased dead space) which underpins the excessive ventilation and dyspnoea described in subjects with low DLCO (28,29). Abnormalities in DLCO in smokers with normal spirometry and the increased risk of these patients to develop COPD have been described before (10).

The MMEF measures the flow between the 25 and 75% of the forced vital capacity, in which flow is determined by the resistance of the small airways and the elastic recoil pressure of the lung. Thus abnormalities in the MMEF, a test that has been shown to reflect these “initial” lung pathological abnormalities, could explain the symptomatic manifestations found in smokers with noCOPD (9,21,30,31). Small airways abnormalities in symptomatic smokers without COPD have been described by CT scans (3,6,9), and were significantly associated with low MMEF in another study (12). Furthermore, in alpha-1 antitrypsin deficient subjects, a reduction of MMEF, likely due in part to losses of elastic recoil and in part to small airways abnormalities (11,32,33), was associated with impaired health status and greater risk of disease progression (32). These results show how the MMEF might provide important insights into the underlying lung pathology before COPD is evident.

The important contribution of chronic bronchitis to the clinical presentation of smokers with noCOPD could be better understood by considering chronic bronchitis as part of the so called “muco-obstructive” disease (34), a disease characterized by abnormally raised mainly MUC5AC mucin concentrations (35), increased sputum production and mucus hyperconcentration that are central to the pathogenesis of chronic bronchitis (34-36). Accumulated mucus could form mucus plaques and plugs within airway lumens serving as the nidus for inflammation, intermittent infection and airflow obstruction (35-37). Luminal plugging has been identified by CT scan as a frequent finding significantly associated to chronic bronchitis, a finding that may play an important role in the pathophysiology of airflow obstruction in smokers, even without COPD (38).

The abnormal MMEF in smokers without COPD illustrates that smokers could and would develop small airways abnormalities not detected by the FEV1, and importantly that a significant percentage of noCOPD smokers with abnormal MMEF, halve of them jounger than 50 years, would develop COPD over time. We found a large variation in MMEF in the noCOPD subjects with normal FEV1/FVC and FEV1, a variability that has been defined as “noise” and has hence detracted from the use of the MMEF as a diagnostic tool for early lung abnormalities in smokers. However in our study we showed that noCOPD with MMEF <80% were more symptomatic and had lower FEV1/FVC, even if still within normal limits; than those with MMEF>80%. Furthermore 21% of noCOPD with abnormal MMEF did develop COPD at follow up, while only 2% of those with normal MMEF did. These results were confirmed using a MMEF<60% cut-off (at which 31% of subjects developed COPD at follow up) and with a logistic regression analysis that identified MMEF at baseline as the only factor associated with COPD development.These findings suggest that the variability of MMEF has an anatomical basis and hence ought to be considered “signal” rather than “noise”.

Since early small airways abnormalities detected by a lower MMEF do progress in a significant proportion of smokers to overt COPD, we believe that these patients ought to be carefully monitored. In our population of noCOPD smokers we could identify 3 groups using the MMEF: a group with MMEF>80%-no disease-, a group with MMEF<80%-abnormal lung pathology but no progression to COPD- and a group with MMEF<80%-abnormal lung pathology with progression to COPD-, which very likely represent 3 different susceptibility factors for the development of disease that could be investigated.

In our cohort of patients with COPD, having chronic bronchitis and being active smokers, as previously shown, (6,39-41) had important consequences in the disease progression. FEV1 decline in COPD patients with chronic bronchitis was about twice the decline in those without chronic bronchitis, and this was further accentuated when, besides having

chronic bronchitis, these patients were also active smokers (Fig E3 A-C). These data, by showing the important effects of actively smoking in the progression of the disease, underline the importance of the smoking cessation measures of these patients, as outlined in the ERS document (42). Contrary to the findings in smokers with COPD, neither chronic bronchitis nor active smoking could predict a faster fall in FEV1 over time and the eventual development of COPD in smokers without COPD, (Table E 5) underlining the importance of other factors governing susceptibility for the development of the disease in these subjects (43).

Being a single center study and a relatively small cohort are possible limitations of our study. Nonetheless, having a population of smokers with a mean age of 53 years carefully followed longitudinally by the same group of physicians for 10 years ensures an evenness in the data collection with protocols available to clinical practices. The lack of a replication cohort may detract from the value of the study, however since the original hypothesis was novel we thought it would be first necessary to "test" our point before a replication cohort could be done. Aknowledging that MMEF is a very sensitive but also highly variable test in the detection of small airway disfunction, besides the MMEF<80% we also looked at the MMEF cut-off of 60% predicted, to provide a further insight into the interpretation of the data, which solidified the main results of the study. MMEF<80% predicted was used to define abnormality in order to allow comparisons with other studies (11,12,19) and because it would be more practical since it is the way is reported in most laboratories.

In conclusion, our study shows that the analysis of MMEF, a simple and ancillary lung function test today considered obsolete, is an easy and important step to detect existing lung abnormalities and could be used as a biomarker to identify the subset of symptomatic individuals with pathologic changes that might lead to COPD. Furthermore, since the abnormality of this test reflects potentially reversible inflammatory changes in the small airways, it could be used for the follow up of possible treatment response.

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Statement Of Ethics: The study conformed to the Declaration of Helsinki. The study was approved by human-research review board (IRB.12/2010) and all patients provided informed written consent before any procedure was done.

Conflict Of Interest Statement: None declared.

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Author Contributions

E.B.; U.S.; J.M.M.; M.S.; M.G.C.: contributed to conception and design of the study. Drafting and editing the manuscript.

E.B.; U.S.; A.C.: performed experimental work and performed data analysis.

U.S.; M.T.; M.F.; P.C.; M.M-O; contributed to sample collection; undertook data collection and performed data analysis.

S.B.; G.T: data management and data interpretations.

All authors critically revised the manuscript for important intellectual content and approved the final version of the manuscript.

FIGURE LEGENDS

Figure 1. **A**. Relation between FEV1/FVC (%) and MMEF in the whole population at the first spirometry (V1). Normal values for FEV1/FVC (>70%) and MMEF (>80%) are outlined. 65 % of the subjects with noCOPD (FEV1/FVC >70%) (green) had an abnormal MMEF (<80% predicted) (COPD shown in red; r=0.95, p<0.01). **B.** Relation between FEV1/FVC (%) and DLCO (%) in the whole population at the first spirometry (V1). Normal values for FEV1/FVC (>70%) and DLCO (>80%) are outlined. 38% of subjects without COPD (green) had an abnormal DLCO (COPD shown in red; r=0.33, p=0.0001).

Figure 2. **A**. Mean MRC dyspnoea score in subjects without COPD with MMEF >80% (light green), MMEF <80% (dark green) and COPD (red) (Kruskal Wallis Test p=0.0001). **B.** The presence of chronic bronchitis (CB) in all groups significantly worsens the severity of the baseline dyspnoea score (Kruskal Wallis Test p=0.0001). The effect of CB in the deterioration of the dyspnoea is better understood by considering CB not only as sputum production but as part of the diffuse “muco-obstructive” disease that affects all airways (22,23). Histograms rapresent mean ± SD.

Table 1. Clinical and functional characteristics of all smokers, smokers without COPD (noCOPD) and with COPD (COPD).

	All smokers (n=511)	NoCOPD (n=209)	COPD (n=302)	p
Male n(%)	423(83%)	147(70%)	276(91%)	**0.001**
Age (years)	58±10	52±11	62±8	**0.001**
Smoking history (pack years)	43±24	35±19	49±25	**0.001**
FEV_1 post-bronchodilator (l)	2.34±0.85	2.88±0.78	1.96±0.67	**0.001**
FEV_1 post-bronchodilator (% pred)	79±22	95± 5	68±19	**0.001**
FEV_1/FVC post-bronchodilator (%)	64±15	78±5	54±11	**0.001**
MMEF 25-75 post-bronchodilator (% pred)	47±29	75±21	27± 3	**0.001**
DLCO (% pred)	80±21	86±17	76±22	**0.0001**
Decline of FEV_1 per year (ml/year)	32±46	33±37	31±52	N.S.
Subjects with chronic bronchitis, n(%)	193(38%)	56(27%)	137(45%)	**0.001**
Subjects with mMRC ≥2, n(%)	214(42%)	71(34%)	143(47%)	**0.006**
mMRC score	1.33±0.70	1.10±1.19	1.50±1.09	**0.0001**
CAT score	9.7±7.3	9.0±6.9	10.2±7.1	**0.042**
Distance at 6 minute walking test (m)	419±122	481±114	376±109	**0.001**
Number of total exacerbations per year	0.79±1.54	0.49±1.05	1.00±1.78	**0.001**
Number of severe exacerbations per year	0.05±0.14	0.03±0.09	0.06±0.17	**0.024**
Subjects who developed	418(82%)	160(77%)	258(85%)	**0.001**

comorbidities, n(%)				
GOLD 1, n(%)	-	-	81(27%)	-
GOLD 2, n(%)	-	-	169(56%)	-
GOLD 3-4, n(%)	-	-	52(17%)	-

Data are presented as number (%), mean ± SD or median (interquartile range),
p value refers to Mann-Whitney test or χ^2 test, for comparisons between noCOPD and COPD.
*Negative values mean gain.

Table 2. Subjects without COPD (noCOPD) according to MMEF above and below 80%.

	NoCOPD MMEF <80% (n=135)	NoCOPD MMEF >80% (n=74)	p
Age (years)	54.05±11.05	47.82±9.31	**0.01**
BMI	28.75±4.99	27.18±4.95	**0.01**
Smoking history (pack years)	45.17±25.57	38.10±20.92	**0.048**
Active smokers n(%)	61(45%)	33(45%)	N.S.
CAT score	9.42±6.84	8.24±6.94	N.S.
Subjects with CAT≥10 n(%)	47(35%)	23(31%)	N.S.
Distance at 6MWT (m)	459±109	519±114	**0.01**
Number of total exacerbations per year	0.56±1.12	0.36±0.91	**0.04**
Subjects with CB n(%)	42(31%)	14(19%)	**0.04**
Subjects with mMRC≥2 n(%)	49(36%)	22(30%)	N.S.
mMRC score	1.23±1.19	0.85±1.15	**0.01**
Subjects with DLCO <80%	56(41%)	24(32%)	N.S.
DLCO (% pred)	84± 18	89±17	N.S.
FEV1 (% pred)	90.74±12.74	105.11±12.74	**0.0001**
FEV1/FVC (%)	76.39±3.9	82.31±3.89	**0.0001**
Subjects who develop COPD at V3 n(%)	28(21%)	2(2.7%)	**0.001**

Data are presented as number (%), mean ± SD.
p value refers to Mann-Whitney test or χ^2 test.

Table 3. Subjects without COPD (noCOPD) with MMEF<80% with and without progession to COPD.

	NoCOPD MMEF<80% who did not develop COPD (n=107)	NoCOPD MMEF<80% who developed COPD (n=28)	p
Age (years)	54.2±11.4	53.3±9.9	N.S.
Smoking history (pack years)	35.9±19.6	36.3±14.8	N.S.
Active smokers n(%)	45(42%)	15(53%)	N.S.
mMRC score	1.1±1.1	1.8±1.2	**0.001**
FEV1 (% pred)	90.3±12.8	89.5±12.4	N.S.
FEV1/FVC (%)	77.2±3.1	73.1±2.8	**0.0001**
Decline of FEV1 per years (ml/year from V1 to V3)	24±34	52±23	**0.0001**

Data are presented as number (%) or mean ± SD.
p value refers to Mann-Whitney test or χ^2 test.

Fig. 1A

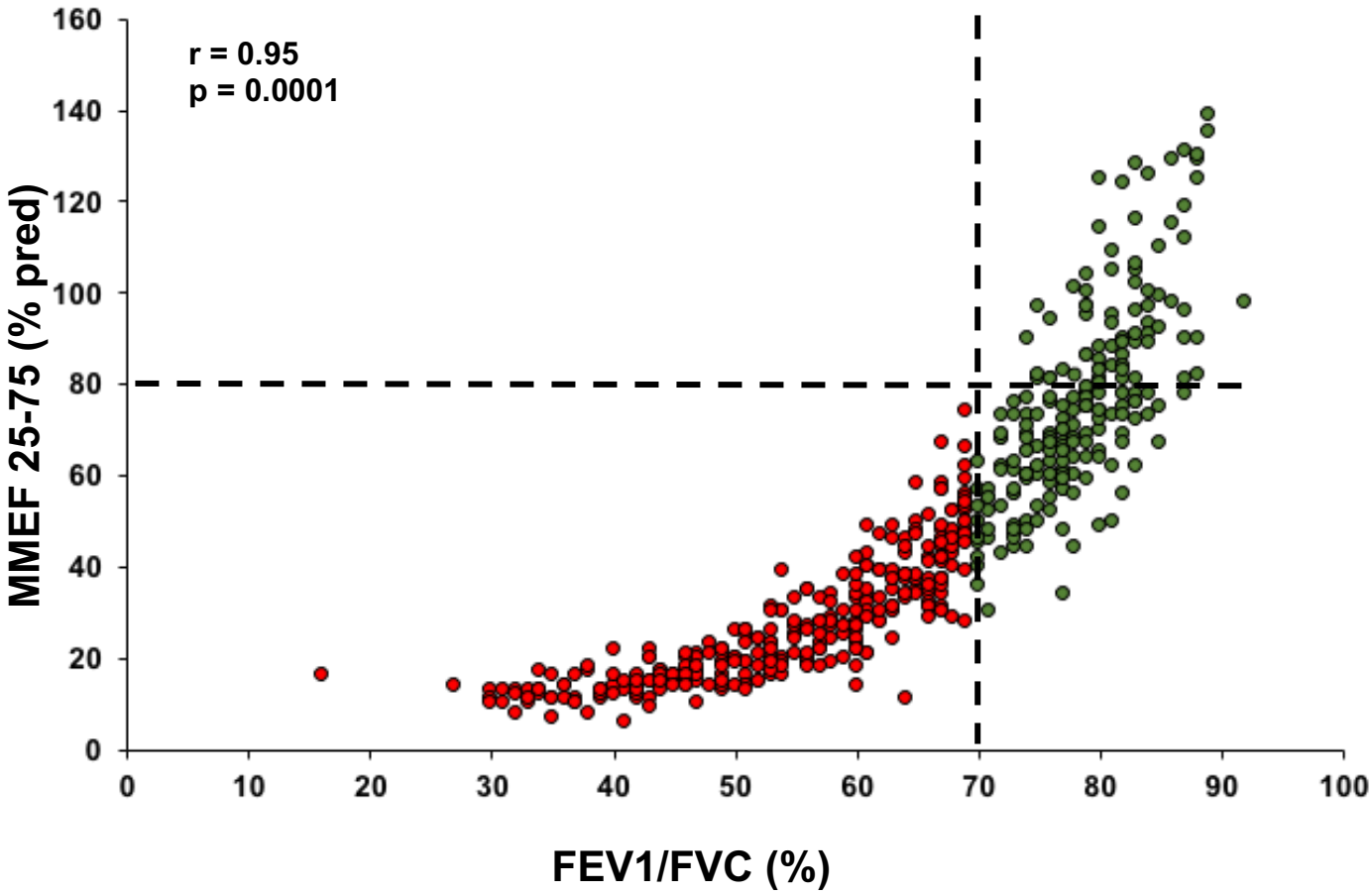

r = 0.95
p = 0.0001
MMEF 25-75 (% pred)
FEV1/FVC (%)
0
20
40
60
80
100
120
140
160
0
10
20
30
40
50
60
70
80
90
100

Fig. 1B

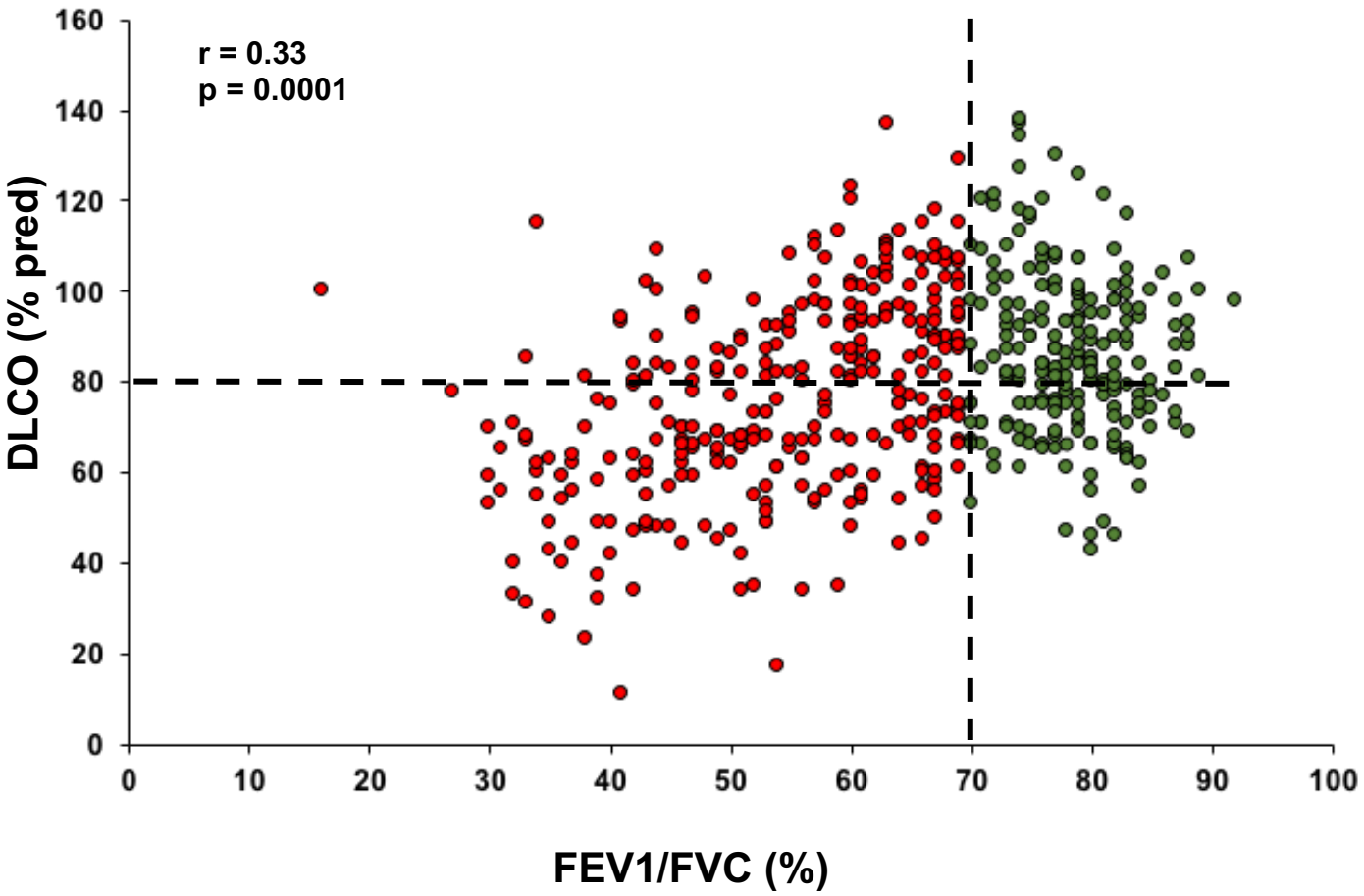

r = 0.33
p = 0.0001
DLCO (% pred)
FEV1/FVC (%)
0
20
40
60
80
100
120
140
160
0
10
20
30
40
50
60
70
80
90
100

Fig. 2A

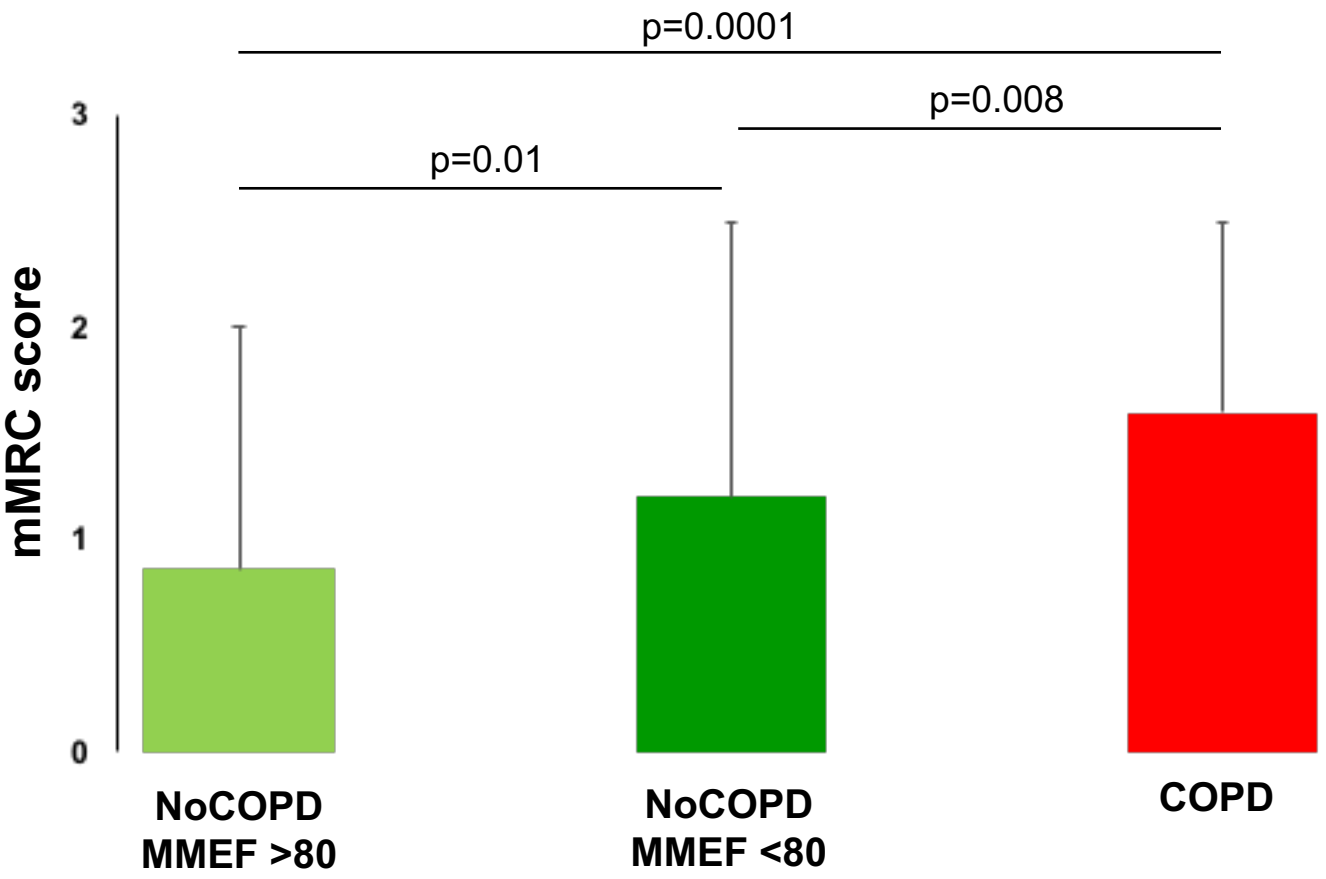
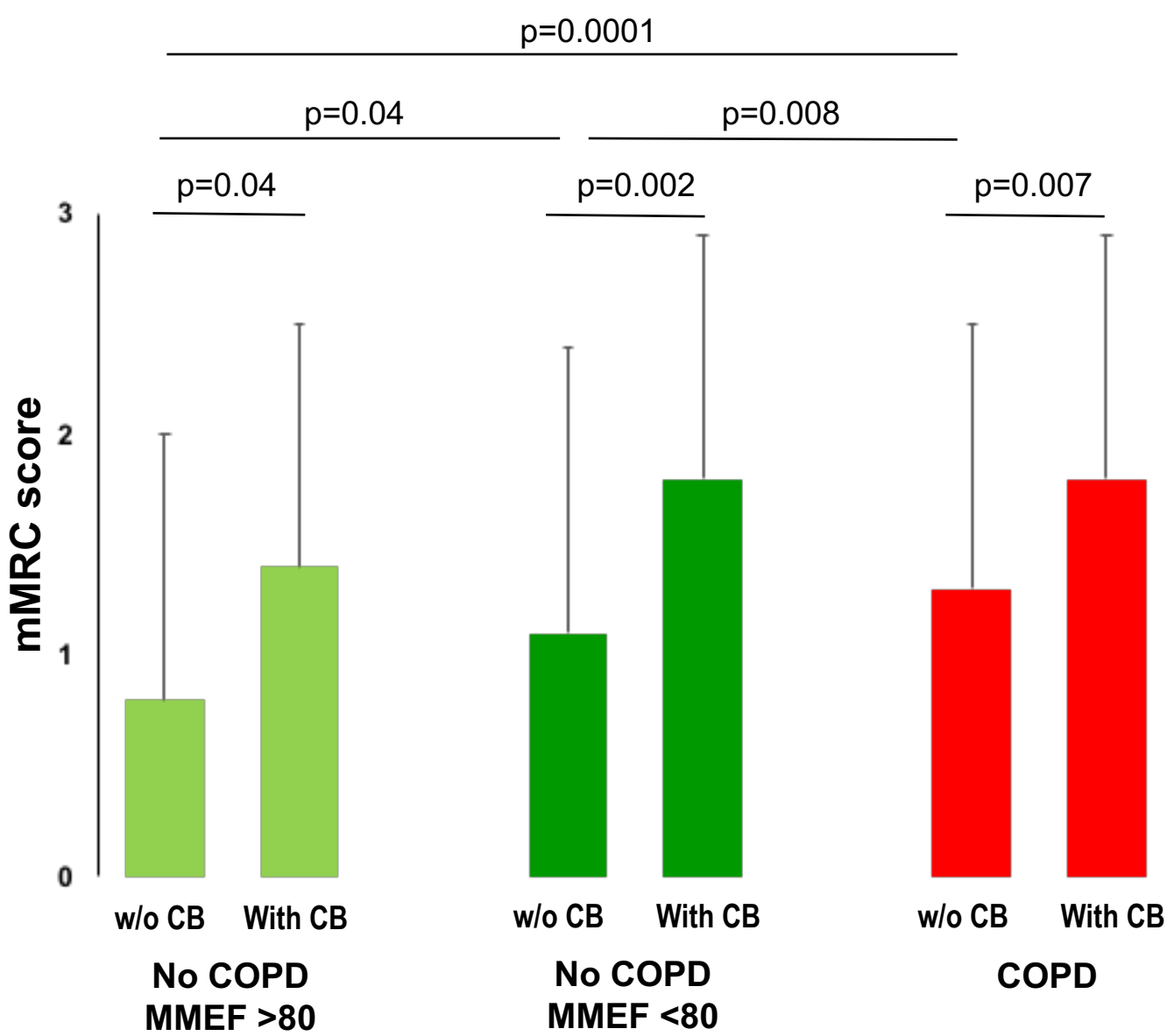

p=0.0001
p=0.008
p=0.01
mMRC score
3
2
1
0
NoCOPD
MMEF >80
NoCOPD
MMEF <80
COPD

Fig. 2B


p=0.0001
p=0.04
p=0.008
p=0.04
p=0.002
p=0.007
mMRC score
3
2
1
0
w/o CB
With CB
w/o CB
With CB
w/o CB
With CB
No COPD
MMEF >80
No COPD
MMEF <80
COPD

Supplement Data

Symptomatic smokers without COPD have physiological changes heralding the development of COPD.

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Methods

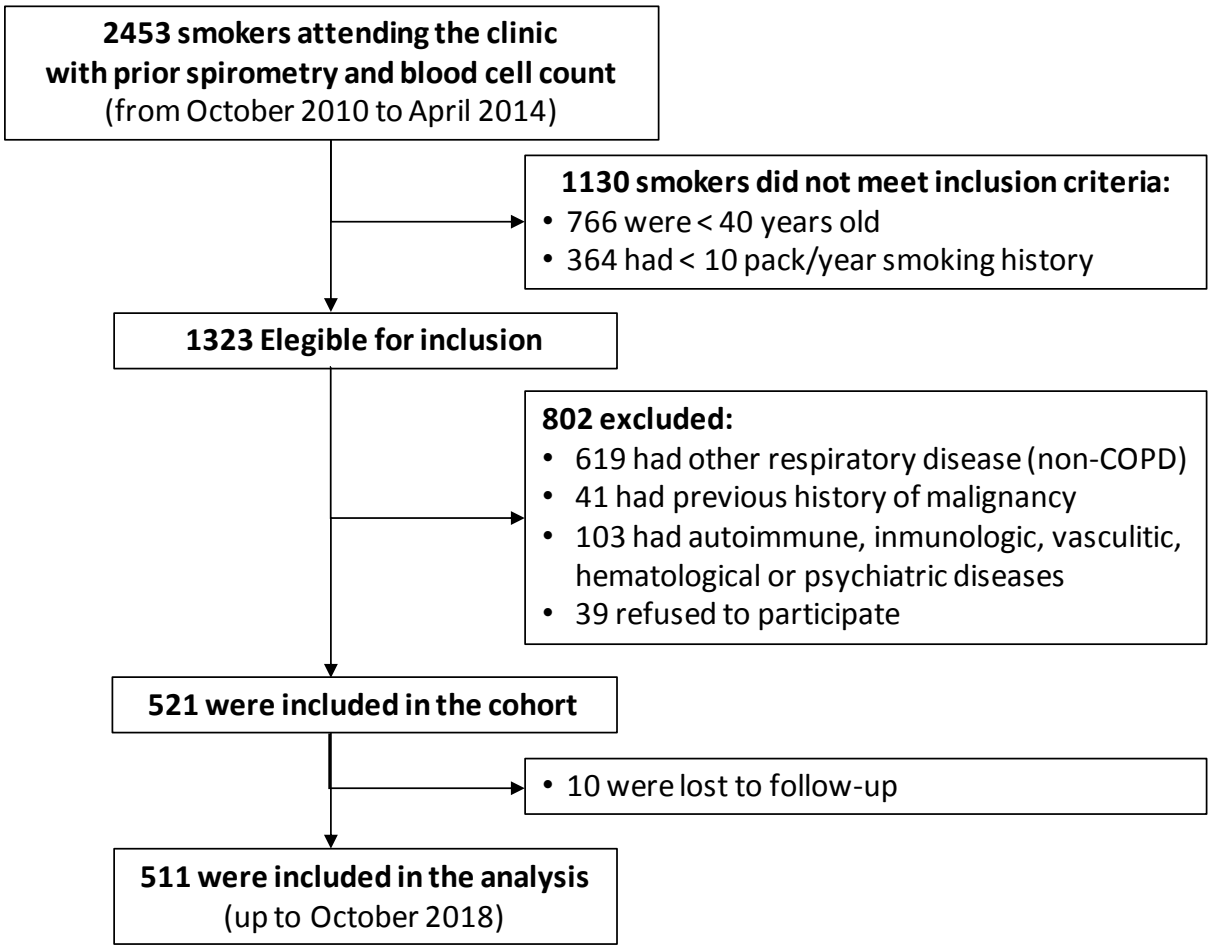
Patient population

Participants were recruited consecutively among smokers (>10 pack/years) who first attended the Pulmonary Clinic at the Hospital Universitario Miguel Servet (Zaragoza, Spain) between October 2010 and April 2014. The objective of constituting this cohort was to determine health related outcomes in smokers with and without COPD, free of major comorbidities (defined as chronic conditions that needed regular therapy: e.g, diabetes, hypertension, dyslipimia) at recruitment. Eligible individuals were >40 years old smokers who came to the clinic requesting to be included in our smoking cessation program or referred by other doctors to assess their respiratory health. At baseline, all subjects were clinically stable (free of exacerbations and not treated with oral corticosteroids and antibiotics for at least 8 weeks) and they were offered to be included in a smoking cessation program if they were active smokers. All COPD patients received standard treatment at first visit according with current guidelines [E1]. One lung function test and one CBCs done prior to study entry were available in all subjects. Patients who agreed to be included in the cohort signed an informed consent before any procedure was performed.

Of the 2453 smokers who came to the outpatient respiratory clinic during the recruiting period, 1130 subjects did not meet the inclusion criteria (>40 years, <10 pack/years), and 802 were excluded because of concomitant comorbidities. Among the remaining 521, 10 subjects were lost during follow-up, and 511 participants were included in the analysis; 302 with COPD and 209 without COPD (noCOPD) (Fig.E1).

All subjects underwent functional and clinical examination including pulmonary function tests, modified Medical Research Council (mMRC) dyspnoea and COPD Assessment Test (CAT) scores evaluation. Exacerbations were collected and defined as acute worsening of respiratory symptoms that required antibiotics and/or oral corticosteroids (moderate) by medical prescription, or hospitalization/visit at the emergency room (severe) [E1].

Figure E1: CONSORT diagram. Flow chart of the study design



Results

Table E1: Therapy in Subjects without COPD (noCOPD) and COPD (A) and subjects without COPD (noCOPD) according to MMEF above and below 80% (B).

A)

Therapy	***NoCOPD (n=209)***	***COPD (n=302)***	***P value***
ICS	44/209 (21%)	179/302 (80%)	**0.00001**
LAMA	13/209 (6%)	35/302 (11%)	**0.04**
LABA/LAMA	1/209 (5%)	10/302 (3%)	**0.03**
LAMA/ICS	23/209 (11%)	52/302 (17%)	**0.05**
Triple Therapy	20/209 (9%)	120/302 (40%)	**0.0001**

Data are presented as number (%).

B)

Therapy	***MEEF<80% (n=135)***	***MEEF>80% (n=74)***	***P value***
ICS	33/135 (24%)	11/74 (15%)	N.S.
LAMA	11/135 (8%)	2/74 (3%)	N.S.
LABA/LAMA	1/135 (0.07%)	0/74 (0%)	N.S.
LAMA/ICS	15/135 (11%)	7/74 (9%)	N.S.
Triple Therapy	18/135 (13%)	2/74 (3%)	**0.01**

Data are presented as number (%).

Table E2. Subjects without COPD (noCOPD), categorized according to DLCO above and below 80%.

	NoCOPD DLCO <80% (n=80)	NoCOPD DLCO >80% (n=129)	p
Age (years)	50.52±9.39	52.67±11.64	N.S.
BMI	27.28±4.98	28.77±4.97	**0.013**
Smoking history (pack years)	44.13±21.45	41.78±25.85	N.S.
Active smokers n(%)	40(50%)	54(42%)	N.S.
CAT score	10.69±7.94	7.96±5.95	**0.03**
Subjects with CAT≥10 n(%)	35(44%)	35(27%)	**0.01**
Distance at 6MWT (m)	464.95±107.87	490.73±117.50	N.S.
Number of total exacerbations per year	0.51±1.23	0.48±0.92	N.S.
Subjects with MMEF≤60% n(%)	28(35%)	26(20%)	**0.02**
MMEF 25-75 post-bronchodilator (% pred)	71±22	78±20	**0.008**
Subjects with CB n(%)	27(34%)	29(22%)	**0.053**
Subjects with mMRC ≥2 (%)	32(40%)	39(30%)	N.S.
mMRC score	1.34±1.17	0.95±1.18	**0.007**
FEV1 (% pred)	89.69±14.23	99.01±13.7	**0.0001**
FEV1/FVC (%)	78.6±4.7	78.41±4.9	N.S.

Data are presented as number (%), mean ± SD.
p value refers to Mann-Whitney test or χ^2 test.

Table E3. Subjects without COPD (noCOPD) according to MMEF above and below 60%.

	NoCOPD MMEF ≤60% (n=54)	NoCOPD MMEF >60% (n=155)	p
Age (years)	56±11	50±10	**0.001**
BMI	29.5±5.4	27.7±4.8	**0.03**
Smoking history (pack years)	41±21	32.5±16.9	**0.003**
Active smokers n(%)	24(44%)	84(54%)	N.S.
CAT score	10.1±6.7	8.6±6.9	N.S.
Subjects with CAT≥10 n(%)	15(28%)	44(28%)	N.S.
Distance at 6MWT (m)	485±105	488±117	N.S.
Number of total exacerbations per year	0.7±1.4	0.4±0.9	**0.04**
Subjects with CB n(%)	22(41%)	34(22%)	**0.007**
Subjects with mMRC≥2 n(%)	25(46%)	46(29%)	**0.02**
mMRC score	1.5±1.1	0.9±1.1	**0.002**
Subjects with DLCO <80%	28(52%)	52(33%)	**0.02**
DLCO (% pred)	79.4±16.9	88.2±17.6	N.S.
FEV1 (% pred)	85±10	99±14	**0.001**
FEV1/FVC (%)	74±3	80±4	**0.001**
Subjects who develop COPD at V3 n(%)	17(31%)	17(11%)	**0.001**

Data are presented as number (%), mean ± SD.
p value refers to Mann-Whitney test or χ^2 test.

Table E4. Subjects without COPD (noCOPD) with MMEF≤60% with and without progession to COPD.

	NoCOPD MMEF≤60% who did not develop COPD (n=37)	NoCOPD MMEF≤60% who developed COPD (n=17)	p
Age (years)	57±11	54±10	N.S.
Smoking history (pack years)	42.2±22.8	37.1±15.9	N.S.
Active smokers n(%)	13(35%)	11(64%)	**0.04**
mMRC score	1.3±1.1	1.9±1.2	N.S.
FEV1 (% pred)	85±11	85±10	N.S.
FEV1/FVC (%)	75±3	72±2	**0.003**
Decline of FEV1 per years (ml/year from V1 to V3)	12±30	51±23	**0.001**

Data are presented as number (%) or mean ± SD.
p value refers to Mann-Whitney test or χ^2 test.

Table E5. Logistic regression analysis in relation to COPD at follow-up (A) and to decline of FEV1 (>30 ml/years) (B).

A)

	Multivariate logistic regression analysis		
	OR	**95% CI**	**P**
Age (years)	0.97	0.93-1.01	N.S.
Gender (male)	3.19	1.07-9.46	0.04
Smoking history (pack years)	1.01	0.98-1.03	N.S.
Active smokers	1.79	0.73-4.24	N.S.
DLco (%pred)	0.99	0.97-1.02	N.S.
Presence of Chronic Bronchitis	1.29	0.53-3.13	N.S.
MMEF 25-75 post-bronchodilator (% pred)	0.94	0.92-0.97	0.001

CI Confidence interval; OR odds ratio;

B)

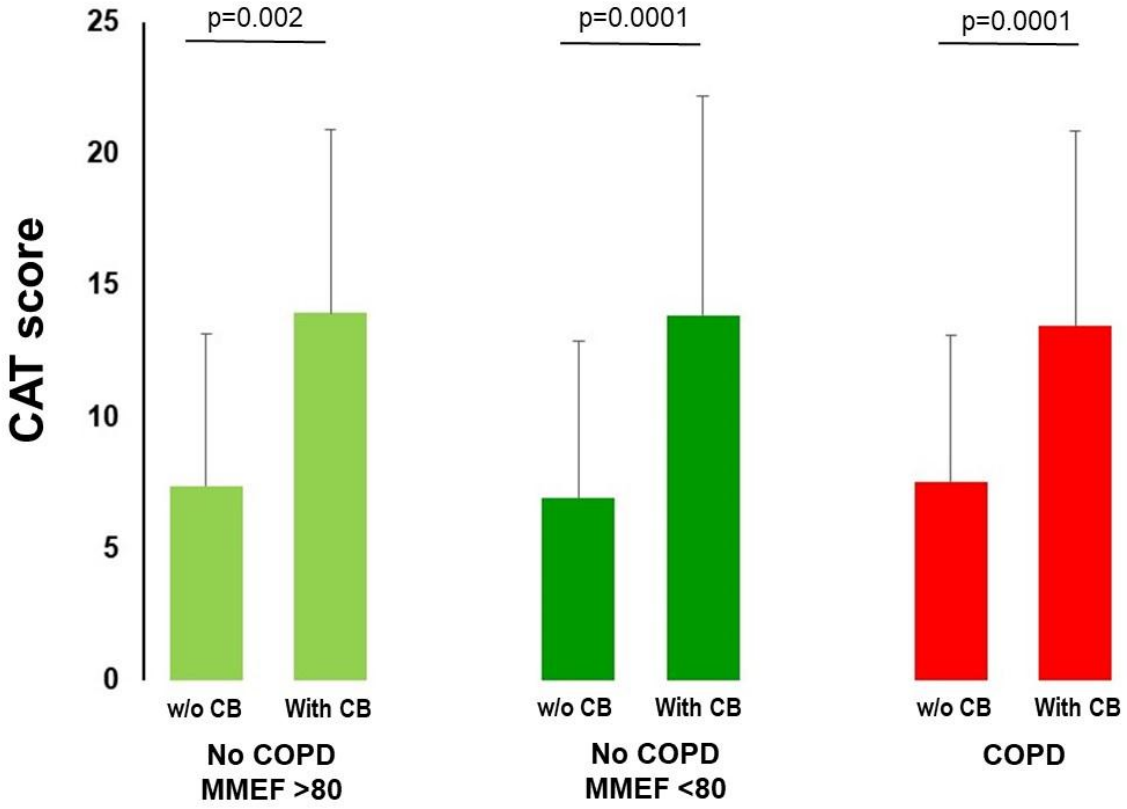
	Multivariate logistic regression analysis		
	OR	**95% CI**	**P**
Age (years)	0.99	0.96-1.02	N.S.
Gender (male)	1.36	0.69-2.67	N.S.
Smoking history (pack years)	1.00	0.98-1.02	N.S.
Active smokers	0.88	0.47-1.65	N.S.
DLco (%pred)	1.01	0.99-1.02	N.S.
Presence of Chronic Bronchitis	1.42	0.73-2.75	N.S.
MMEF 25-75 post-bronchodilator (% pred)	1.02	1.01-1.03	0.03

CI Confidence interval; OR odds ratio;

CAT score and chronic bronchitis (CB)

The CAT score is considered normal when the score is below 10, 40 being the maximum score (E2). In our population the CAT score was similar in noCOPD with normal or abnormal MMEF. However when separated by having or not chronic bronchitis, the CAT score was significantly higher in subjects with chronic bronchitis than in those without chronic bronchitis either with or without COPD. This finding is not surprising since the weight assigned to cough, 5 points, and sputum production, 5 points in the CAT score would easily add to 10 points in patients with chronic bronchitis.

Fig.E2: Effects of chronic bronchitis in the CAT score in smokers without COPD with MMEF < and > 80% and in smokers with COPD



Decline of FEV1 in COPD: Effects of CB and active smoking.

Figures E3 (panels A, B and C) show the effects of chronic bronchitis and actively smoking in the FEV1 decline in smokers with and without COPD.

In smokers without COPD neither chronic bronchitis nor actively smoking influence the rate of decline. On the contrary, in smokers with COPD the FEV1 decline in the COPD group was very variable, variability accounted in part by the presence of chronic bronchitis, since COPD with CB decline more than COPD without CB (41±48 vs 22±53 ml/y, p<0.01) and in part by the smoking activity, (active vs ex-smokers), since active smoking further accelerates FEV1 decline. In the presence of CB ex-smokers declined less than active smokers (48.5±47.9 vs 31.6±45.1 ml/y, p<0.01)

Fig E3-A. Effects of chronic bronchitis in the FEV1 decline in smokers with and without COPD.

Fig. 4A

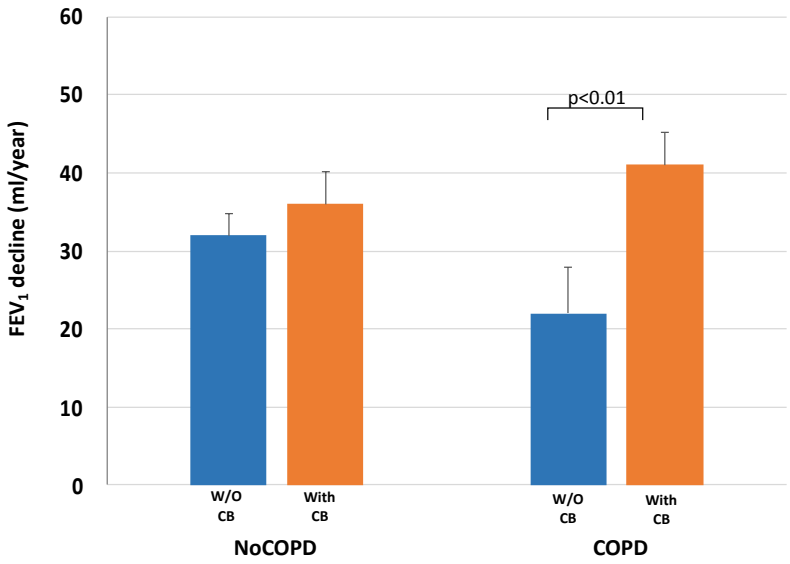


Fig E3-B. Effects of actively smoking on the FEV1 decline in smokers with and without COPD.

Fig. 4B

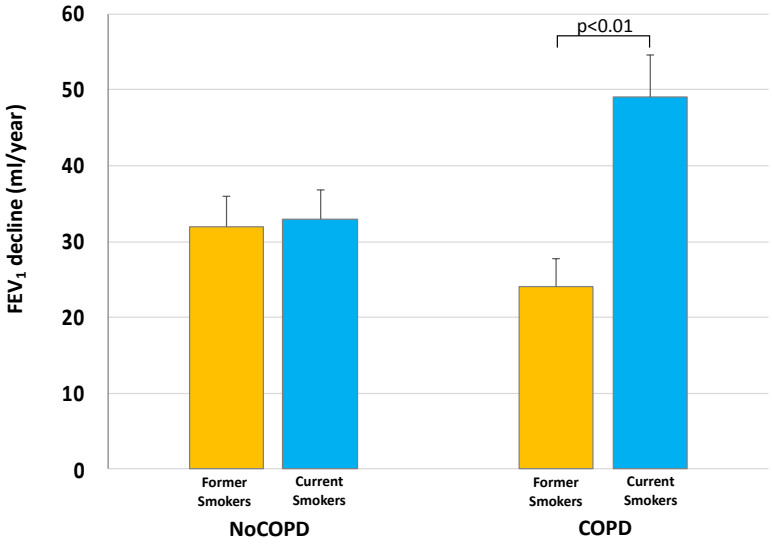
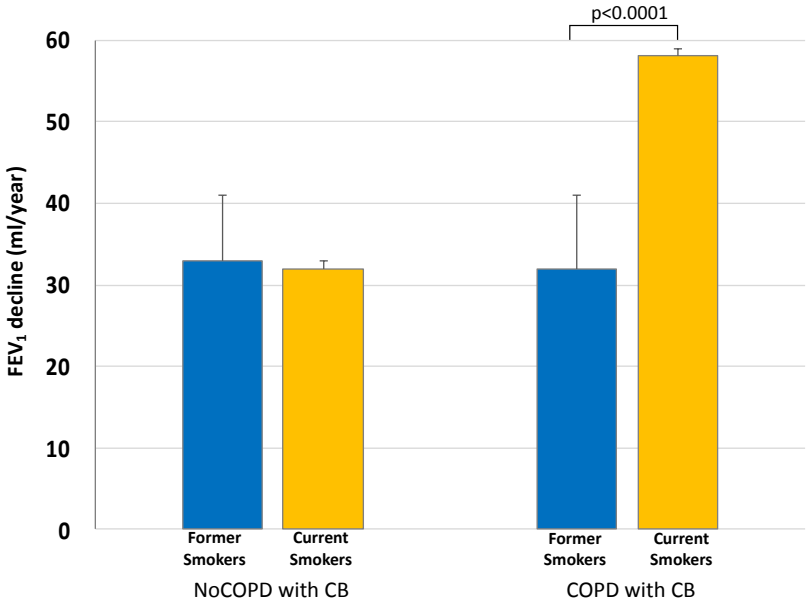


Fig E3-C. Effects of actively smoking in the FEV1 decline of smokers with chronic bronchitis with and without COPD.

Fig. 4C



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