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Philip E Silkoff[1], Dave Singh[2], J Mark FitzGerald[3], Andreas Eich[4], Andrea Ludwig-Sengpiel[5], Geoffrey C Chupp[6], Vibeke Backer[7], Celeste Porsbjerg[7], Pierre-Olivier Girodet[8], Mark T Dransfield[9,10], Frederic Baribaud[1], Vedrana S Susulic[1] and Matthew J Loza[1]

[1]Immunology, Janssen Research & Development, LLC, Spring House, PA, USA. [2]Medicines Evaluation Unit, University Hospital of South Manchester Foundation Trust, University of Manchester, Manchester, UK. [3]Centre for Heart and Lung Health, The Lung Centre, Vancouver General Hospital, Vancouver, BC, Canada. [4]IKF Pneumologie Frankfurt, Institut für klinische Forschung Pneumologie, Clinical Research Centre Respiratory Diseases, Frankfurt, Germany. [5]KLB Gesundheitsforschung Lübeck GmbH, Lübeck, Germany. [6]Pulmonary Critical Care and Sleep Medicine, Yale School of Medicine, New Haven, CT, USA. [7]Respiratory Research Unit, Department of Respiratory Medicine, Bispebjerg University Hospital, Copenhagen, Denmark. [8]Centre de Recherche Cardio-Thoracique de Bordeaux, Université de Bordeaux, Bordeaux, France. [9]Division of Pulmonary, Allergy & Critical Care Medicine, University of Alabama at Birmingham, Birmingham, AL, USA. [10]Pulmonary, Allergy, and Critical Care Medicine, Birmingham VA Medical Center, Birmingham, AL, USA.

ABSTRACT

RATIONALE: Chronic obstructive pulmonary disease (COPD) is a heterogeneous disease, and development of novel therapeutics requires an understanding of pathophysiologic phenotypes.

OBJECTIVES: The purpose of the Airways Disease Endotyping for Personalized Therapeutics (ADEPT) study was to correlate clinical features and biomarkers with molecular characteristics in a well-profiled COPD cohort.

METHODS: A total of 67 COPD subjects (forced expiratory volume in the first second of expiration [FEV_1]: 45%-80% predicted) and 63 healthy smoking and nonsmoking controls underwent multiple assessments including patient questionnaires, lung function, and clinical biomarkers including fractional exhaled nitric oxide (FENO), induced sputum, and blood.

MEASUREMENTS AND MAIN RESULTS: The impact of inhaled corticosteroids (ICSs), and to a lesser extent current smoking, was more associated with symptom control, exacerbation rates, and clinical biomarkers, than severity by FEV_1. The ICS-treated smoking subjects were most symptomatic, with significantly elevated scores on patient-reported outcomes and more annual exacerbations ($P < .05$). Inhaled corticosteroid users had greater airflow obstruction and air trapping compared with non-ICS users, regardless of smoking status. Smoking, regardless of ICS use, was associated with significantly lower FENO ($P < .05$). Smoking, in non-ICS users, was associated with an elevated proportion of sputum neutrophils and reduced sputum macrophages. Increased serum C-reactive protein was observed in smokers but not in ICS and nonsmoking ICS users ($P < .05$). In contrast, only air trapping and neutrophilic inflammation increased with severity, defined by postbronchodilator FEV_1.

CONCLUSIONS: Compared with COPD severity by FEV_1, ICS use and current smoking were better determinants of clinical characteristics and biomarkers. Use of the ADEPT COPD data promises to prove useful in defining biological phenotypes to facilitate personalized therapeutic approaches.

KEYWORDS: COPD, severity, phenotypes, profiling, personalized

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CORRESPONDING AUTHOR: Philip E Silkoff, Janssen Research & Development, LLC, 1400 McKean RD., Spring House, PA 19477, USA. Email: philsilkoff@gmail.com

Background

Chronic obstructive pulmonary disease (COPD) is a highly prevalent heterogeneous disease characterized by progressive airflow obstruction, chronic bronchitis, and emphysema. The predominant cause of COPD is tobacco smoking, but other exposures including air pollution and indoor exposures to biomass fuels may be contributory in some. Prominent symptoms include cough, sputum, and dyspnoea.[1] Patients are also at risk of acute exacerbations which are associated with increased health care utilization, morbidity, and death, whereas multiple factors can predict the risk for these events.[2]

The clinical manifestations and pathophysiologic abnormalities of COPD can vary greatly between patients.[3] Consequently, the prognosis is highly variable, ranging from stable disease with little functional impact, to severe,

progressive, and ultimately fatal disease. There is a need for better understanding of the specific disease mechanisms that drive COPD heterogeneity and to better understand how to match therapies to phenotypes.[4] On a deeper level, linking phenotypes to specific pathogenic mechanisms, ie, endotypes, may allow more targeted approaches as recently reviewed.[5]

The primary objective of the Airways Disease Endotyping for Personalized Therapeutics (ADEPT) study was to assess molecular and cellular profiles in multiple matrices including induced sputum (IS), bronchial tissue, and blood, to enable the correlation of molecular subtyping with clinical characteristics first in asthma,[6] followed by COPD.

Other programs have extensively characterized COPD. The ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points) study was a 3-year longitudinal study that evaluated pulmonary function, chest computed tomography (CT), biomarker measurement, health outcomes, body impedance, resting oxygen saturation, and 6-minute walking distance.[7] Unlike ADEPT, this study did not obtain bronchial tissue but included CT imaging and followed phenotypes over 3 years. SPIROMICS (Subpopulations and Intermediate Outcomes in COPD Study) aims to enroll 3200 subjects in 4 strata, with the principal objective being to identify phenotypes to guide personalized therapy.[8] SPIROMICS includes imaging, biomarker assessments, substudies with bronchoscopy sampling, exacerbation evaluation, and multiyear follow-up. COPDGene (COPD gene study) is a 10 000-patient study evaluating smokers and COPD patients using imaging, and genetic characterization with genome-wide association studies, and includes long-term follow-up to quantify disease progression.[9]

This report focuses on the differential impact of disease severity measured by lung function, current smoking status, and inhaled corticosteroid (ICS) use on subject characteristics and biomarkers.

Methods

Design

The ADEPT COPD study was a cross-sectional, observational noninterventional study of patients with COPD and healthy subjects run in North America, Germany, United Kingdom, Romania, and Denmark from 2012 to 2014. The study received institutional ethics approval at all sites. All subjects provided written informed consent (genomic testing was optional). The clinicaltrials.gov identifier is NCT01274507. The full study protocol is linked to this report.

Men and women, aged 40 to 65 years, produced a valid sample of IS at screening and were fit for bronchoscopy, with a body mass index (BMI) <32 kg/m^2. Important inclusion and exclusion criteria are described below, and all criteria are in the protocol linked to this report.

Healthy subjects. Recruitment of 30 healthy nonsmokers and 30 healthy asymptomatic smokers was planned, with no clinically significant abnormalities in medical history, vital signs, physical examination, routine laboratory analyses, and an electrocardiogram. Subjects with significant bronchodilator reversibility (BDR) of ≥12% and ≥200 mL or a forced expiratory volume in the first second of expiration (FEV_1) value <85% of predicted normal were excluded. Nonsmokers were required not to have smoked in the previous year and have a ≤10 pack-year history of smoking. A positive urinary cotinine was exclusionary. Smokers were required to be currently smoking with a history of ≥20 pack-years. Subjects who had respiratory symptoms or a FEV_1/forced vital capacity ratio <0.7 were excluded.

COPD subjects. The COPD subjects were enrolled into 2 severity cells to ensure a range of severity. These were as follows: (1) "moderate COPD"-postbronchodilator (post-BD) FEV_1 between 60% and <80% predicted and (2) "severe COPD"-post-BD FEV_1 between 45% and <60% predicted (adapted from COPD guidelines[1]). All participants had stable COPD for 3 months prior to screening, were active or previous smokers with ≥10 pack-year history, and were on ≥1 COPD medication in the 4 weeks prior to screening. Exclusion criteria included the following: a history of asthma, other chronic lung diseases, life-threatening COPD exacerbations, right heart failure, a pulse oximetry <90% on room air, and long-term oxygen therapy at rest.

Study Design and Visits

All participants underwent screening visits, followed within ~14 days by a baseline biomarker assessment visit, and then ~14 days later, a bronchoscopy visit. The complete study procedures and detailed methodology are in the protocol linked to this report. The study procedures were as follows:

1. Spirometry before and after a short-acting bronchodilator;
2. Postbronchodilator body plethysmography was performed in COPD subjects only for residual volume (RV), total lung capacity (TLC), functional residual capacity (FRC);
3. Diffusing capacity of lung for carbon monoxide in COPD subjects only;
4. In COPD subjects, patient-reported outcomes (PROs): the St. George's Respiratory Questionnaire for COPD (SGRQc)[10] and a novel symptom score, the EXACT (EXAcerbations of Chronic Obstructive Pulmonary Disease Tool) respiratory symptoms scale (E-RS).[11] The E-RS involves the use of 11 of the 14 EXACT[12] items and a new scoring algorithm to assess the severity of respiratory symptoms in patients with COPD. A purpose-built novel COPD history questionnaire (CHQ) was also administered;
5. Fractional exhaled nitric oxide (FENO), airway mucosal biopsy and brushings, IS, nasal brushings, whole blood, serum, urine, and genomic samples were also collected.

Biomarker Assessments

These are described in detail in the online supplement.

Safety

Investigators were instructed to capture adverse events (AEs) that were attributed to study procedures, eg, sputum induction and bronchoscopy. Emergent AEs that were unrelated to procedures were captured at the investigators' discretion.

Statistical considerations

Based on a priori hypotheses that current smoking status and ICS use would be major extrinsic factors affecting both clinical and molecular phenotypes, the primary analyses included comparisons among 4 COPD groups defined by smoking and ICS status and these groups to respective healthy control nonsmoker and currently smoking cohorts. The 4 COPD groups were as follows: (1) ICS-treated smoking (ICS/SM) (n = 26), (2) ICS-treated nonsmoking (ICS/NSM) (n = 15), (3) ICS-nontreated smoking (ICS-NO/SM) (n = 20), and (4) ICS-nontreated nonsmoking (ICS-NO/NSM) (n = 6). Active smoking was based on the medical records and/or a positive urinary cotinine at any study visit. Inhaled corticosteroid use was based on medications captured in the case report form.

Statistical analyses of clinical and biomarker data used OmicSoft Array Studio v8 (Cary, NC, USA). No imputation was performed for missing data. For data with lognormal distributions (eg, FENO, blood differential counts), logarithm transformations were performed. Significance of differences among groups, and interactions between groups, was evaluated using General Linear Model analyses. To test for significance of difference in proportions between categorical variables, Fisher exact tests were used. Correlations among variables were tested using Spearman correlation tests that do not require assumptions of normality and linearity. A P value of <.05 was considered to be statistically significant. In light of the exploratory hypothesis-generating nature of the study, no correction for multiple comparisons was performed.

Results

Study population

Healthy controls. In total, 63 healthy subjects (31 nonsmokers and 32 smokers) were enrolled. Of the 63 healthy subjects, 3 (4.8%) subjects terminated study participation prematurely, 1 due to withdrawal of consent, and 2 for "other" reasons. Healthy nonsmokers were slightly younger than the healthy smokers and both groups were significantly younger than COPD participants (mean ± SD: 49 ± 7 vs 58 ± 5; $P < 10^{-6}$). Healthy nonsmokers were predominantly women, but the smokers were approximately balanced for sex. Demographic characteristics are shown in Table 1.

Impact of smoking on lung function in healthy controls

Despite requirements for normal lung function, healthy control smokers had numerically worse values for all spirometric values compared with healthy nonsmokers, but only prebronchodilator (pre-BD) FEV_1 %predicted was significantly lower ($P = .015$), although still in the normal range (Figure 1, panel A).

COPD subjects

Demographics and disposition for COPD subjects. A total of 67 COPD subjects were enrolled; of whom 62 (92.5%) completed the study, whereas 5 (7.5%) subjects terminated the study participation prematurely. Most of the COPD subjects were men (60%) and white (91.0%). About 61% of COPD subjects were on ICS and 69% were current smokers. Within ex-smokers and current smokers, 71% and 57% were on ICS, respectively. A key finding from the purpose-built COPD questionnaire (CHQ) was a high burden of comorbid conditions, with a high prevalence of gastroesophageal reflux disease and hypertension in COPD as previously reported.[13] About 68% of subjects continued to smoke, and the prevalence of continuing second-hand smoke exposure at home (50%) and at work (25%) was disturbingly high. About 26% reported ≥3 exacerbations per year, with a modest correlation of exacerbation rate FEV_1, in agreement with previous reports that exacerbations rise with increasing severity, although they also occur in milder disease and even smokers with no airflow obstruction.[14] Of note, despite asthma being an exclusion criterion, 9% of severe COPD subjects self-reported a history of asthma before they developed COPD.

Subgrouping by smoking status and use of inhaled steroids. Division of the COPD population into 4 groups based on use of ICS and smoking status (as described in the "Methods" section) was found to be associated with significantly more COPD clinical characteristics and biomarkers than analysis by FEV_1 severity and is therefore the focus of this report. The 4 COPD groups were as follows: (1) ICS-treated smoking (ICS/SM) (n = 26), (2) ICS-treated nonsmoking (ICS/NSM) (n = 15), (3) ICS-nontreated smoking (ICS-NO/SM) (n = 20), and (4) ICS-nontreated nonsmoking (ICS-NO/NSM) (n = 6).

Demographic characteristics are shown in Table 1 for COPD subjects by ICS use and smoking. Demographic parameters did not differ within the 4 COPD groups, but the COPD subjects were significantly older than the healthy controls. Use of long-acting β_2 agonists tracked with ICS in ICS users only.

There were no significant demographic factors associated with the 4 smoking/ICS groups that could potentially influence the observed differences in lung capacity measurements and PROs. Specifically, there were no significant differences in

Table 1. Summary of demographics at baseline visit.

	HEALTHY		COPD					*P* VALUES[a]		
	NSM	SM	TOTAL	ICS-NO/ NSM	ICS-NO/SM	ICS/NSM	ICS/SM	HEALTHY: SM VS NSM	COPD VS HEALTHY	WITHIN COPD
Enrolled subjects (N)	31	32	67	6	20	15	26			
Age, y, mean (SD)	48.9±6.9	49.1±7.1	57.9±5.4	57.5±6.2	57.2±6.2	60.5±4.2	57.0±5.1	.93	$<10^{-4}$	.22
Sex, No. (%)								.32	.16	.42
Male	12 (38.7)	17 (53.1)	40 (60)	2 (33)	12 (60)	11 (73)	15 (58)			
Female	19 (61.3)	15 (46.9)	27 (40)	4 (67)	8 (40)	4 (27)	11 (42)			
Race, No. (%)								.15	.42	.50
White	29 (93.5)	25 (78.1)	61 (91)	6 (100)	17 (85)	15 (100)	23 (88)			
Black or African American	2 (6.5)	7 (21.9)	6 (9)	0 (0)	3 (15)	0 (0)	3 (12)			
Body mass index, kg/m^2, mean (SD)	25.9±3.0	26.6±3.3	25.7±4.1	26.1±3.6	24.6±4.0	27.9±2.6	25.2±4.5	.35	.38	.094
ICS use=yes, No. (%)	NA	NA	41 (61)	0 (0)	0 (0)	15 (100)	26 (100)	NA	NA	NA
Long-acting β_2 agonist=yes, No. (%)	NA	NA	34 (51)	0 (0)	0 (0)	13 (87)	21 (81)	NA	NA	$<10^{-4}$
Long-acting muscarinic antagonist=yes, No. (%)	NA	NA	43 (64)	4 (67)	13 (65)	12 (80)	14 (54)	NA	NA	.41
OCS=yes, No. (%)	NA	NA	2 (3)	0 (0)	1 (5)	0 (0)	1 (4)	NA	NA	$<10^{-4}$
Actively smoking=yes, No. (%)	0 (0)	32 (100)	46 (69)	0 (0)	20 (100)	0 (0)	26 (100)	NA	NA	NA

Abbreviations: COPD, chronic obstructive pulmonary disease; ICS, inhaled corticosteroid; ICS/NSM, ICS-treated nonsmoking; ICS/SM, ICS-treated smoking; ICS-NO/NSM, ICS-nontreated nonsmoking; ICS-NO/SM, ICS-nontreated smoking; NA, not applicable; NSM, nonsmoking; OCS, oral corticosteroid; SM, smoking.

[a]*P* values from General Linear Models, respectively, for healthy current smokers vs healthy nonsmokers, healthy groups combined vs COPD groups combined, between the 4 groups (from *F* test statistic).

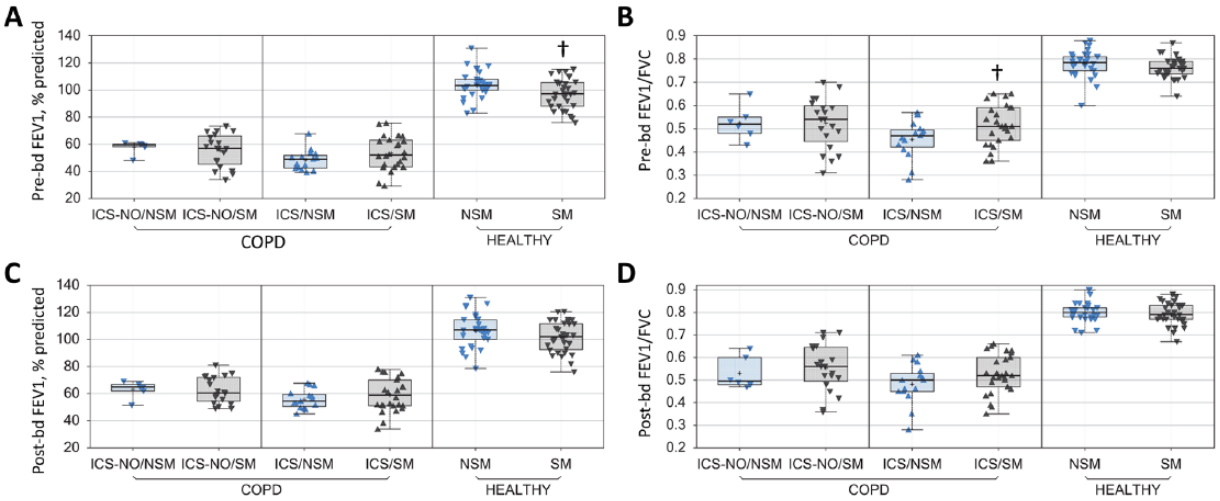

Figure 1. Airflow obstruction associated with smoking prebronchodilator in healthy controls but not COPD subjects: (A) prebronchodilator and (B) postbronchodilator FEV_1 expressed as % predicted normal and (C) prebronchodilator and (D) postbronchodilator FEV_1/FVC ratio, stratified by smoking and ICS use status, are presented as symbols representing individual participants and summarized by box (interquartile range and median) and whiskers (range), with "+" indicating the mean. †$P < .05$ for current (SM) vs ex-smokers (NSM) within indicated ICS status/disease group. COPD indicates chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in the first second of expiration; FVC, forced vital capacity; ICS, inhaled corticosteroid; ICS/NSM, ICS-treated nonsmoking; ICS/SM, ICS-treated smoking; ICS-NO/NSM, ICS-nontreated nonsmoking; ICS-NO/SM, ICS-nontreated smoking; NSM, nonsmoking; SM, smoking.

age, sex, and race among the 4 groups. However, there was a nonsignificant trend for modestly lower BMI in active smokers, as expected. Demographic variables also were not correlated with % predicted post-BD FEV_1 values, with only a nonsignificant trend higher age correlating with lower % predicted FEV_1 measurements ($r = -.21$, $P = .091$; data not shown).

Impact of active smoking and/or ICS use on COPD clinical characteristics

Disease characteristics with significant smoking-ICS interactions or between-group differences are presented in Table 2 for each of the 4 groups (ICS/SM, ICS/NSM, ICS-NO/SM, and ICS-NO/NSM). The *F* test relates to the significant impact of ICS use and/or active smoking. The full data sets are presented in supplemental Table E1 (spirometry, COPD PROs, and plethysmography parameters) and Tables E2 and E3 (*P* values for between-group comparisons). In general, ICS use was associated with more differences for clinical than biomarker parameters, whereas smoking affected less parameters overall and more biomarkers than clinical parameters.

Use of ICS, regardless of smoking status (ICS/SM and ICS/NSM), had greater airflow obstruction in general based on spirometry compared with non-ICS users (ICS-NO/NSM and ICS-NO/SM) (Figure 1 and Table 2).

The ICS/SM group was the most symptomatic, with significantly elevated EXACT scores compared with the other 3 groups and SGRQc scores compared with ICS/NSM and ICS-NO/NSM groups ($P < .05$) (Figure 2 and supplemental Table E3). In addition, this group had a significantly greater annual exacerbation rate compared with the ICS-NO/SM and ICS-NO/NSM groups ($P < .05$), associated with ICS use.

The ICS-treated groups regardless of smoking status had significantly greater FRC values compared with ICS-nontreated subjects. Similar patterns pertained to TLC, RV, and RV/TLC (Table 2, Figure 3, and supplemental Tables E1 and E3).

Impact of active smoking and/or ICS use on biomarkers

Supplemental Tables E4 and E5 present all the evaluated biomarkers for the 4 COPD groups. Table 2 and Figure 4 present a subset of these biomarkers that differed by active smoking or ICS use. Inhaled corticosteroid use and smoking affected a similar number of biomarkers. There was suppression of FENO in smokers regardless of ICS use as previously reported.[15] Inhaled corticosteroid use was associated with an elevated absolute number of sputum neutrophils, a reduced absolute number of sputum macrophages, and increased serum C-reactive protein (CRP) levels particularly in the context of current nonsmoking status ($P < .05$).

Correlations of disease characteristics with FEV_1 severity

Postbronchodilator FEV_1 % predicted was used as the indicator of severity (see Table 3, reporting significant correlations and supplement Table E6 for the full variable list).

There were several correlations of spirometric parameters with post-BD FEV_1 as expected and not called out here. Regarding post-BD plethysmography, RV/TLC (% predicted) moderately ($r = -.43$) and FRC (% predicted) modestly ($r = -.29$) were inversely correlated with post-BD FEV_1. Diffusing capacity of lung for carbon monoxide (% predicted) was modestly inversely

Table 2. COPD characteristics that were significantly affected by active smoking, ICS, or both factors combined.

	ICS-NO/NSM	ICS-NO/SM	ICS-YES/NSM	ICS-YES/SM	*P* VALUES[A]			
	N = 6	N = 20	N = 15	N = 26	*F* TEST	SM	ICS	SM × ICS
Pre-BD FEV_1 (L)	1.62 ± 0.16 (6)[b]	1.67 ± 0.60 (20)	1.50 ± 0.42 (15)	1.59 ± 0.53 (26)	.8144	**<10⁴**	.8669	.4057
Post-BD FEV_1 (L)	1.77 ± 0.18 (6)	1.87 ± 0.58 (20)	1.71 ± 0.39 (15)	1.75 ± 0.51 (26)	.7692	**<10⁴**	.8897	.5841
Pre-BD FEV_1 (% predicted)	57.7 ± 4.8 (6)	55.5 ± 12.1 (20)	48.3 ± 7.5 (15)	53.4 ± 12.3 (26)	.1843	**<10⁴**	.2475	.4167
Post-BD FEV_1 (% predicted)	63.2 ± 6.2 (6)	62.6 ± 9.7 (20)	55.8 ± 7.0 (15)	58.9 ± 12.3 (26)	.2020	**<10⁴**	.2341	.8799
Pre-BD FEV_1/FVC	0.53 ± 0.07 (6)	0.52 ± 0.11 (20)	0.45 ± 0.08 (15)	0.51 ± 0.09 (26)	.1202	**<10⁴**	.4277	.8291
Post-BD FEV_1/FVC	0.53 ± 0.07 (6)	0.55 ± 0.10 (20)	0.48 ± 0.09 (15)	0.53 ± 0.09 (26)	.1637	**<10⁴**	.4519	.9368
Post-BD FRC (L)	3.01 ± 1.13 (6)	3.60 ± 0.95 (20)	4.40 ± 1.30 (14)	4.59 ± 1.15 (25)	**.0031**	.5983	**.0030**	.7787
Post-BD residual volume (L)	2.91 ± 0.90 (6)	3.02 ± 1.12 (20)	3.84 ± 1.05 (14)	3.66 ± 1.09 (25)	.0681	.8580	**.0353**	.9015
Post-BD FRC (% predicted)	102.7 ± 42.1 (6)	114.3 ± 30.8 (20)	135.0 ± 38.7 (14)	144.5 ± 26.2 (25)	**.0044**	.3196	**.0035**	.5108
EXACT daily diary score	24.00 ± 8.39 (6)	28.95 ± 12.04 (20)	23.73 ± 13.83 (15)	36.23 ± 11.41 (26)	**.0084**	.0527	.0633	.8437
SGRQc, overall respiratory score	26.81 ± 15.40 (6)	39.96 ± 24.73 (20)	33.27 ± 20.29 (15)	51.29 ± 19.01 (26)	**.0163**	.3089	.0607	.4766
Annual exacerbation history (CHQ)	0.70 ± 1.30 (5)	0.82 ± 0.87 (14)	1.12 ± 1.18 (13)	1.80 ± 1.13 (22)	**.0389**	.2420	**.0321**	.5943
FENO, ppb	19.4 + 8.7/–6.0 (6)	10.4 + 8.7/–4.7 (20)	13.5 + 5.2/–3.7 (13)	10.2 + 9.0/–4.8 (25)	**.0516**	.874	.2827	.1015
Sputum neutrophils, ×1000/mm^3 (GM)	57.8 + 127.7/–39.8 (3)	19.6 + 85.2/–15.9 (9)	115.6 + 120.4/–59 (12)	58.9 + 71.7/–32.3 (17)	**.0057**	.8203	.0590	.2459
Sputum neutrophils, % of leukocytes	91.1 ± 3.6 (3)	51.8 ± 26.5 (13)	79.3 ± 22.2 (13)	75.6 ± 20.3 (18)	**.0054**	.0719	.4959	**.0055**
Sputum macrophages, % of leukocytes	8.0 ± 4.0 (3)	45.4 ± 27.5 (13)	16.2 ± 18.2 (13)	23.1 ± 20.4 (18)	**.0038**	.1580	.3684	**.0113**
Serum CRP, RFU (GM)	1.03 +0.70/–0.42 (6)	1.85 +1.68/–0.88 (20)	2.08 +0.61/–0.47 (15)	1.76 +1.14/–0.69 (26)	**.0472**	.6613	**.0202**	**.0016**

Abbreviations: CHQ, COPD history questionnaire; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in the first second of expiration; FRC, functional residual capacity; FVC, forced vital capacity; GM, geometric mean; ICS, inhaled corticosteroids; post-BD, postbronchodilator; pre-BD, prebronchodilator; RFU, radiofrequency unit; SGRQc, St. George's Respiratory Questionnaire for COPD; SM, smoking.
[a]In COPD population, General Linear Model test modeling smoking + ICS + smoking × ICS interaction term, with *P* values reported for each model term (bolded when $P<0.05$).
[b]In COPD population, mean ± SD (sample size) reported for each smoking × ICS group indicated.

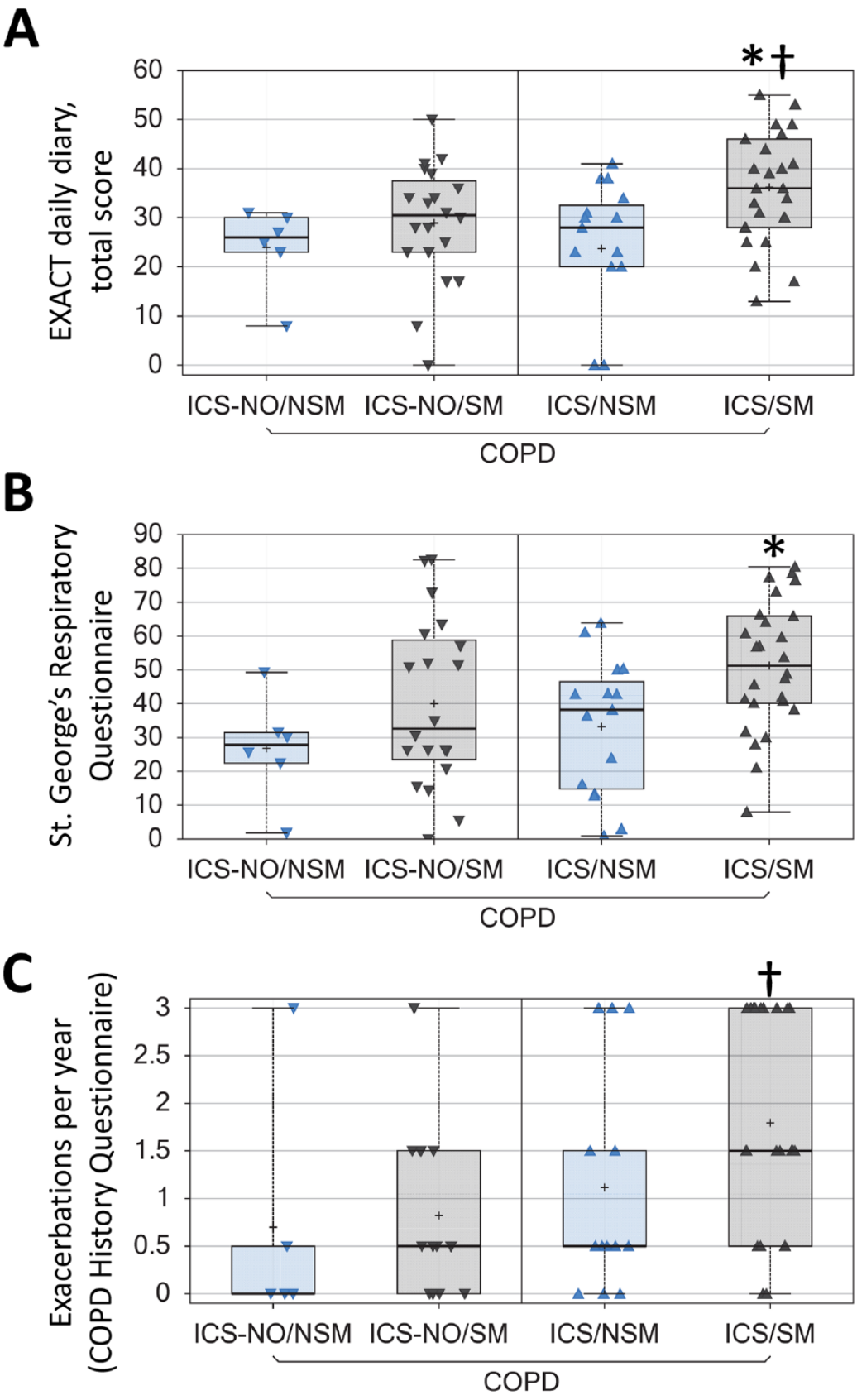

Figure 2. Patient-reported outcomes worst in currently smoking COPD subjects on ICS. (A) EXACT scores, (B) SGRQ total score, and (C) exacerbation history of COPD, stratified by smoking and ICS use status, is presented as symbols representing individual participants and summarized by box (interquartile range and median) and whiskers (range), with "+" indicating the mean. *$P < .05$ for current (SM) vs ex-smokers (NSM) within indicated ICS status group; †$P < .05$ for ICS vs ICS-NO within indicated smoking status group. COPD indicates chronic obstructive pulmonary disease; EXACT, EXAcerbations of Chronic Obstructive Pulmonary Disease Tool; ICS, inhaled corticosteroid; ICS/NSM, ICS-treated nonsmoking; ICS/SM, ICS-treated smoking; ICS-NO/NSM, ICS-nontreated nonsmoking; ICS-NO/SM, ICS-nontreated smoking; NSM, nonsmoking; SGRQ, St. George's Respiratory Questionnaire; SM, smoking.

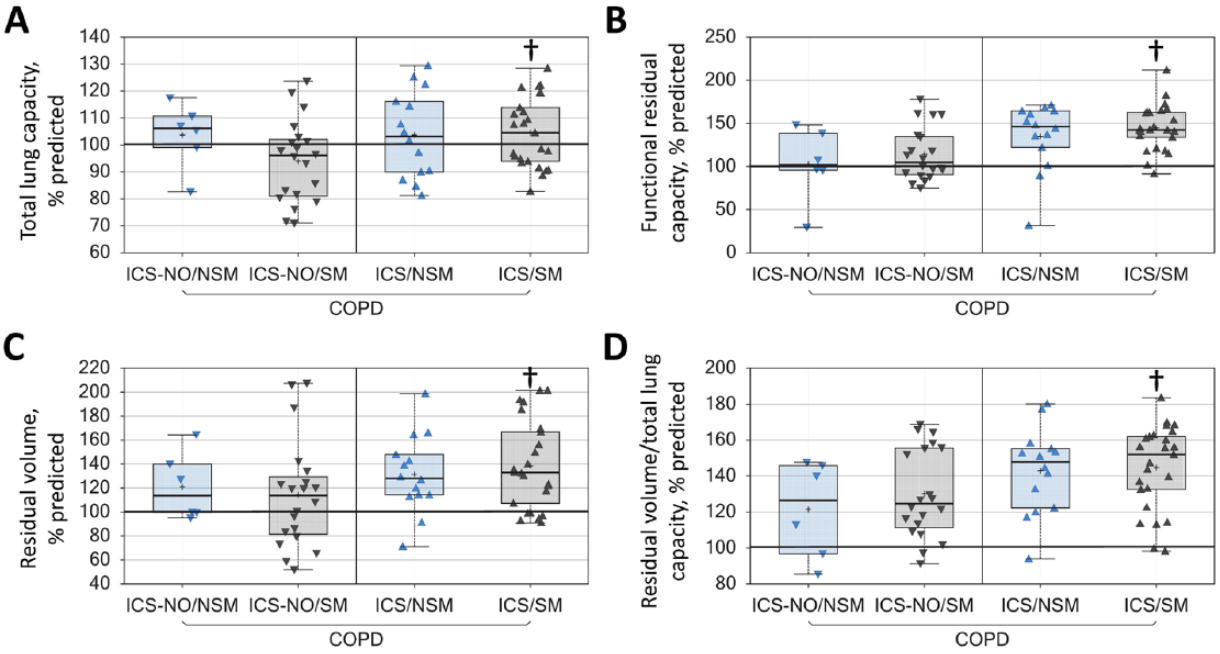

Figure 3. Plethysmography measurements were worst in currently smoking COPD subjects on ICS. % predicted normal values for (A) total lung capacity (TLC), (B) functional residual capacity, (C) residual capacity, and (D) the ratio of residual volume over TLC, stratified by smoking and ICS use status, are presented as symbols representing individual participants and summarized by box (interquartile range and median) and whiskers (range), with "+" indicating the mean. †$P < .05$ for ICS vs ICS-NO within indicated smoking status/disease group. COPD indicates chronic obstructive pulmonary disease; ICS, inhaled corticosteroid; ICS/NSM, ICS-treated nonsmoking; ICS/SM, ICS-treated smoking; ICS-NO/NSM, ICS-nontreated nonsmoking; ICS-NO/SM, ICS-nontreated smoking.

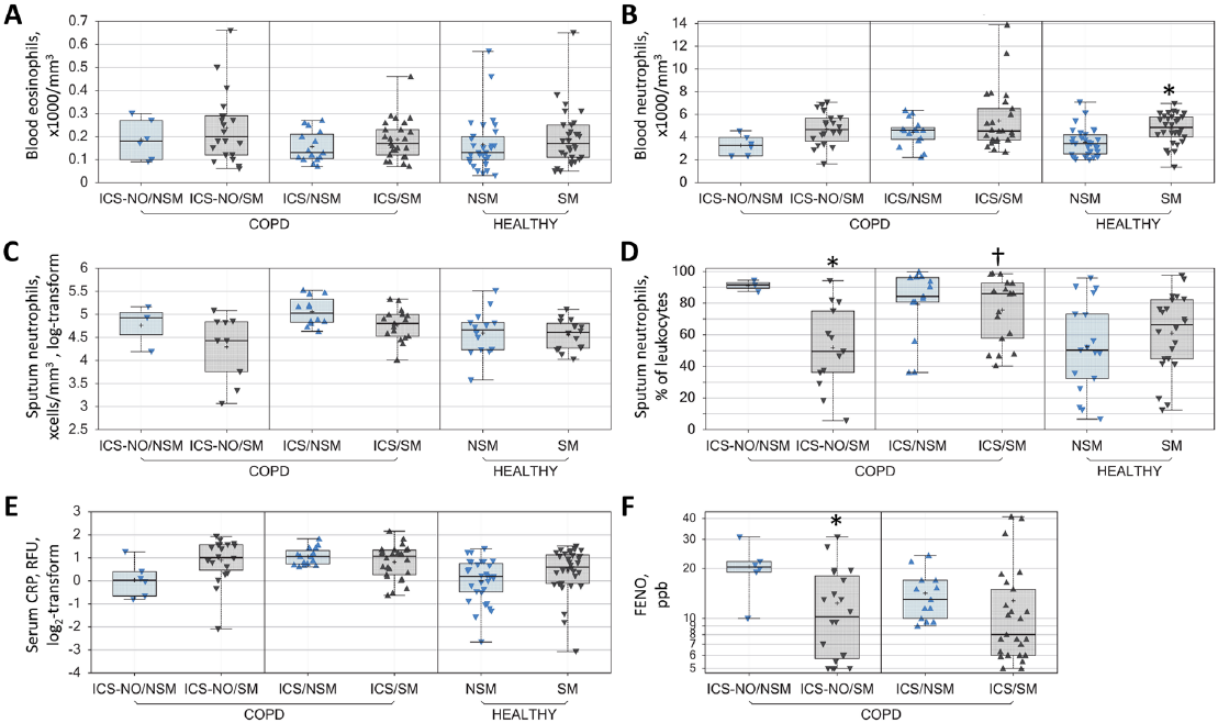

Figure 4. Biomarkers in healthy controls and COPD subjects impacted by smoking and ICS. Density of blood (A) eosinophils and (B) neutrophils, (C) density and (D) proportion of leukocytes for sputum neutrophils, (E) serum C-reactive protein concentration, and (F) FENO, stratified by smoking and ICS use status, are presented as symbols representing individual participants and summarized by box (interquartile range and median) and whiskers (range), with "+" indicating the mean. *$P < .05$ for current (SM) vs ex-smokers (NSM) within indicated ICS status/disease group; †$P < .05$ for ICS vs ICS-NO within indicated smoking status/disease group. COPD indicates chronic obstructive pulmonary disease; FENO, fractional exhaled nitric oxide; ICS, inhaled corticosteroid; ICS/NSM, ICS-treated nonsmoking; ICS/SM, ICS-treated smoking; ICS-NO/NSM, ICS-nontreated nonsmoking; ICS-NO/SM, ICS-nontreated smoking; NSM, nonsmoking; SM, smoking.

Table 3. Correlations of clinical and biomarker variables with post-BD FEV_1 (% predicted).

VARIABLE[a,b]	UNADJUSTED[a]		ADJUSTED[a]		N
	R_{SP}	*P* VALUE	R_{SP}	*P* VALUE	
Post-BD FEV_1, % predicted	1.00	$<10^{-6}$	1.00	$<10^{-6}$	67
Pre-BD FEV_1, % predicted	0.88	$<10^{-6}$	0.89	$<10^{-6}$	67
Post-BD FVC, % predicted	0.70	$<10^{-6}$	0.69	$<10^{-6}$	67
Pre-BD FVC, % predicted	0.68	$<10^{-6}$	0.68	$<10^{-6}$	67
Post-BD FEV_1 (L)	0.61	$<10^{-6}$	0.62	$<10^{-6}$	67
Pre-BD FEV_1 (L)	0.60	$<10^{-6}$	0.61	$<10^{-6}$	67
Post-BD RV/TLC, % predicted	−0.43	.0004	−0.38	.0019	65
Pre-BD FVC, % predicted	0.39	.0011	0.42	.0004	67
Post-BD FVC, % predicted	0.38	.0017	0.40	.0009	67
spMAC %	0.37	.0102	0.34	.0189	47
Post-BD RV (L)	−0.37	.0026	−0.34	.0059	65
spLYM %	0.34	.0214	0.28	.0565	47
bNEU (×10E9/L)	−0.33	.0068	−0.34	.0053	66
spNEU %	−0.33	.0252	−0.30	.0396	47
DLCO, % predicted	0.32	.0115	0.37	.0032	61
Post-BD FRC, % predicted	−0.29	.0184	−0.22	.0830	65
Pre-BD FVC (L)	0.29	.0191	0.31	.0111	67
Post-BD RV, % predicted	−0.26	.0366	−0.25	.0417	65
Post-BD FRC (L)	−0.32	.0102	−0.21	.0987	65
Annual rate exacerbations (CHQ)	−0.28	.0383	−0.18	.2035	54

Abbreviations: bNEU, blood neutrophils; CHQ, COPD history questionnaire; DLCO, diffusing capacity of lung for carbon monoxide; FEV_1, forced expiratory volume at 1 second; FRC, functional residual capacity; FVC, forced vital capacity; post-BD, postbronchodilator; spLYM, sputum lymphocytes; spMAC, sputum macrophages; spNEU, sputum neutrophils.
[a]Spearman correlation tests were performed for the indicated variables vs baseline post-BD FEV_1 % predicted for the actual (unadjusted) values for all patients with COPD or after analysis of variance–based adjustment of the data set for smoking status and ICS use.
[b]Variables displayed are for those that had $P < .05$ for the nonadjusted data set; 2 variables in bold had $P < .05$ for the unadjusted but not adjusted data set.

correlated with post-BD FEV_1 ($r = .31$). Annual exacerbation rates also increased with severity, with a modest inverse correlation to post-BD FEV_1 ($r = -.26$). Notably, post-BD FEV_1 was not significantly correlated with BDR or disease activity questionnaires (SGRQ, EXACT scores), as shown in Supplemental Table E6.

Correlations of biomarkers with FEV_1 severity

Blood neutrophil density and sputum neutrophil percentages were similarly inversely correlated with post-BD FEV_1 ($r = -.33$), increasing with severity, whereas sputum macrophage and lymphocyte percentages similarly decreased with severity ($r = .34$-$.37$). Postbronchodilator FEV_1 was not significantly correlated with serum CRP. Sputum eosinophils were not correlated with severity, nor did they differ from healthy control groups, with both groups having 13% of participants with >3% sputum eosinophils (data not shown).

Because current smoking and ICS use could also directly affect these variables and confound the interpretation, all correlations were also tested after adjusting the data set for smoking status and ICS use. Despite this adjustment, all the above correlations with post-BD FEV_1 held, except for the correlation with annual exacerbation rate (which went from significant, $r = -.28$, $P = .038$ to insignificant, $r = -.18$, $P = .20$), consistent with ICS reducing exacerbation rates.

Safety

There were no serious AEs related to the study procedures.

Discussion

The major finding in a well-characterized cohort of COPD is that active smoking and/or ICS use were of greater impact in driving differences in PROs, lung volumes, and biomarkers than severity by post-BD FEV_1.

Of note, the healthy control cohort was significantly younger than the COPD subjects, and age was a significant covariate in multiple biomarker analyses (data not shown). The healthy smokers had a significantly lower pre-BD FEV_1 % predicted (albeit within the normal range) compared with healthy control nonsmokers and significantly elevated blood neutrophil density. The possibility remains that this cohort included subjects with early COPD in keeping with Regan and colleagues,[14] who described acute exacerbations in smokers without COPD.

The CHQ provided a more comprehensive insight into COPD history than the SGRQ and E-RS. Even though this questionnaire was created for ADEPT and has not been formally validated, important insights regarding the COPD disease experience were gained.

In contrast to severity defined by post-BD FEV_1 (discussed below), active smoking and/or ICS therapy were confounding factors for comparisons between multiple clinical and biomarker parameters. The ICS/SM group was the most severe by EXACT and SGRQ scores, a greater annual exacerbation rate, worse airflow obstruction, and hyperinflation. In general, ICS negatively affected these parameters in the presence or absence of active smoking. It is not possible to establish cause and effect, but ICS use is probably a marker of greater severity driven by clinical practice guidelines, which recommend adding ICS for those experiencing exacerbations, although ICS use is associated with increased risk for pneumonia.[16]

Active smoking was associated with changes in inflammatory markers including lower FENO values, elevated blood leukocyte and neutrophil densities, and sputum neutrophil proportions. The ICS/SM group had a significantly higher blood neutrophil density compared with the ICS/NSM group. Inhaled corticosteroid use was associated with increased sputum neutrophils and elevated CRP.

Severity definitions based on post-BD FEV_1 predictably resulted in significant correlations to most spirometric parameters but provided an opportunity to evaluate how the global initiative in chronic obstructive lung disease (GOLD [Global Initiative for Chronic Obstructive Lung Disease]) severity[1] drove differences in other demographic, clinical characteristics and biomarkers. Only a few parameters, including RV/TLC (air trapping), and sputum neutrophils and macrophages segregated by severity. Thus, post-BD FEV_1 was associated with increased neutrophilic inflammation in sputum. Although the reasons for this are unclear, airflow obstruction progression may relate to a change and/or increase in lower airway microbiota, caused by reduced airway clearance, which may link to the increased risk of exacerbation. In a meta-analysis, Franciosi et al[17] also reported a rise in sputum neutrophils, as well as sputum interleukin 8 and serum CRP with COPD severity. However, in this study, serum CRP was associated with smoking and ICS use but not with airway obstruction per se.

The prevalence of eosinophilic sputum inflammation was low in the COPD cohorts (13% of patients at cutoffs of 3% and 2%). These prevalences are lower than others have reported[18,19] and this is topical in light of the recent interest in an asthma and COPD overlap syndrome (ACOS) which is characterized by BDR and also type 2 inflammation (eosinophilia and elevated FENO).[20] In a report by Alshabanat et al,[21] ACOS comprised 27% to 28% of the COPD population, whereas in that of Barrecheguren et al,[22] up to 20% of COPD had ACOS. However, while approximately 1/3 of the ADEPT cohort showed reversible airflow obstruction, only 3% had an elevated FENO (>35 ppb), 13% had sputum eosinophilic inflammation, or 9% had high blood eosinophils (>300 cells/mm^3). The proportion of COPD subjects with sputum eosinophils >3% was higher in a report from Zanini et al[19] (31%). Singh et al[18] reported that 31% of COPD subjects had persistent blood eosinophilia (≥2%), but in a small subset with repeated sputum measures, only a minority (n = 138, 4%) had persistent sputum eosinophils ≥2%. Pascoe et al[23] and Pavord et al[24] both reported a greater prevalence of COPD with elevated blood eosinophils, which predicted the reduction in exacerbation frequency to ICS.

The number of subjects who had quality sputum samples available for analysis was approximately 2/3 for the screening and/or baseline visits despite considerable efforts to use experienced research sites and to train sites and subjects. The sputum success rate at the multiple sites in ADEPT was understandably lower than that achievable in highly specialized single centres.[25,26] Based on our results here, together with a similar sputum success rate in the ADEPT asthma study,[6] it is unlikely that profiling COPD with sputum feasible in the routine clinical setting.

Limitations of the ADEPT population are predominantly due to protocol-mandated restrictions. The healthy control cohort was significantly younger than the COPD subjects and this affected multiple biomarkers (data not shown). We set the lower limit for post-BD FEV_1 at 45% predicted due to the invasive procedures, thus not including very severe subjects. For similar reasons, COPD subjects were also required to be free of recent exacerbations, to have no history of life-threatening COPD exacerbations or right heart failure, and BMI was limited to <32 kg/m^2. For these reasons, the study population included only a minority of subjects with frequent exacerbations. When subsetting by active smoking and/or ICS use, which drove more differences in parameters than FEV_1, the sample sizes are small. Unlike other programs, we did not include imaging, which would undoubtedly have added an important dimension to understanding phenotypes. Subjects were not followed up for a longer period as this was deemed to be too high a burden on these elderly symptomatic subjects. One gap, in light of the recent interest in the role of the microbiome,[27] is the lack of microbial sampling. Finally, in retrospect and due to the profound impact of active smoking and ICS use, the study was relatively underpowered for analysis of high-dimensional biomarker data sets (eg, gene expression

microarray, proteomics), limiting such future analyses to exploratory, hypothesis-generating results.

Conclusions

In summary, ADEPT recruited a COPD cohort that underwent extensive profiling with comparison with smoking and nonsmoking healthy controls. We report that active smoking status and ICS use were more impactful than severity based on post-BD FEV_1 in determining clinical characteristics and biomarkers. Studies that profile COPD should take into account ICS use and smoking status, in addition to severity of airflow obstruction when assessing phenotypes and endotypes.

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[1]Arensia Exploratory Medicine, Sos. Viilor 90, Bucharest 050159, Romania. Email: irina.strambu@arensia-em.com

[2]Institut Universitaire de Cardiologie et Pneumologie de Québec (IUCPQ), 2725, Chemin Ste-Foy, Québec, QC G1V 4G5, Canada. Email: Michel.Laviolette@fmed.ulaval.ca

[3]Centre for Heart and Lung Health, The Lung Centre, 7th Floor, Gordon and Leslie Diamond Health Care Centre, 2775 Laurel Street, Vancouver, BC V5Z 1M9, Canada. Email: slam2@vch.ca

[4]Centre de Recherche Cardio-Thoracique de Bordeaux, Université de Bordeaux, U1045, CIC 1401, F-33000 Bordeaux, France. Email: patrick.berger@u-bordeaux.fr

[5]Cumming School of Medicine, University of Calgary, 3280 Hospital Drive NW, Calgary, AB T2N 4Z6, Canada. Email: rleigh@ucalgary.ca

[6]Division of Pulmonary, Critical Care, and Occupational Medicine, University of Iowa, W219B GH UIHC, 200 Hawkins Drive, Iowa City, IA 52242, USA. Email: joel-kline@uiowa.edu

[7]4.116 John Sealy Annex, University of Texas Medical Branch, 301 University Blvd, Galveston, TX 77555-0568, USA. Email: William.Calhoun@utmb.edu

[8]Parexel International, Shelton Simmons (MD), 3001 S Hanover St #7, Brooklyn, MD 21225, USA. Email: Azra.Hussaini@parexel.com

[9]Department of Pulmonary and Critical Care, Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195, USA. Email:khatris@ccf.org

[10]Pneumologie, Aix Marseille University, APHM/INSERM U1067, Chemin des Bourellys, 13015 Marseille, France. Email: Pascal.chanez@univ-amu.fr

[11]Institut Universitaire de Cardiologie et Pneumologie de Québec (IUCPQ), 2725, Chemin Ste-Foy, Québec, QC G1V 4G5, Canada. Email: Francois.Maltais@fmed.ulaval.ca

[12]Temple University Hospital, 3401 N. Broad Street, Philadelphia, PA 19140, USA. Email: Steven.Kelsen@tuhs.temple.edu

The following Janssen personnel contributed significantly to the success of ADEPT: Francisco Leon, Keith Lasher, Jennifer Campos, Debra Alvarez, Robert Gordon, Hongjuan Liu, Dipti Shah, Jennifer Montello, Filza Potapova.

Author Contributions

MJL, VSS, FB, and PES contributed to the conception and design. The ADEPT investigators acquired the data. MJL, FB, and PES analyzed and interpreted the data. MJL and PES contributed to the drafting of the manuscript for important intellectual content. All authors approved the final version.

Availability of Data and Material

The data sets used and/or analyzed during the ADEPT COPD study are available from the corresponding author on reasonable request.

Ethical Approval

The study was approved by institutional review boards/ethics committees at each individual site. A list is available on request. All subjects signed an informed consent after being given a detailed explanation of the study procedures and risks.

REFERENCES

1. From the Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2015. http://www.goldcopd.org/.2015.
2. Han MK, Muellerova H, Curran-Everett D, et al. GOLD 2011 disease severity classification in COPDGene: a prospective cohort study. *Lancet Respir Med*. 2013;1:43–50.
3. Turner AM, Tamasi L, Schleich F, et al. Clinically relevant subgroups in COPD and asthma. *Eur Respir Rev*. 2015;24:283–298.
4. Barnes PJ. Therapeutic approaches to asthma-chronic obstructive pulmonary disease overlap syndromes. *J Allergy Clin Immunol*. 2015;136:531–545.
5. Woodruff PG, Agusti A, Roche N, Singh D, Martinez FJ. Current concepts in targeting chronic obstructive pulmonary disease pharmacotherapy: making progress towards personalised management. *Lancet*. 2015;385:1789–1798.
6. Silkoff PE, Strambu I, Laviolette M, et al. Asthma characteristics and biomarkers from the Airways Disease Endotyping for Personalized Therapeutics (ADEPT) longitudinal profiling study. *Respir Res*. 2015;16:142.
7. Vestbo J, Anderson W, Coxson HO, et al. Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE). *Eur Respir J*. 2008;31:869–873.
8. Couper D, LaVange LM, Han M, et al. Design of the Subpopulations and Intermediate Outcomes in COPD Study (SPIROMICS). *Thorax*. 2014;69:491–494.
9. Regan EA, Hokanson JE, Murphy JR, et al. Genetic epidemiology of COPD (COPDGene) study design. *COPD*. 2010;7:32–43.
10. Meguro M, Barley EA, Spencer S, Jones PW. Development and validation of an improved, COPD-specific version of the St. George Respiratory Questionnaire. *Chest*. 2007;132:456–463.
11. Leidy NK, Murray LT. Patient-reported outcome (PRO) measures for clinical trials of COPD: the EXACT and E-RS. *COPD*. 2013;10:393–398.
12. Leidy NK, Wilcox TK, Jones PW, et al. Development of the EXAcerbations of Chronic Obstructive Pulmonary Disease Tool (EXACT): a patient-reported outcome (PRO) measure. *Value Health*. 2010;13:965–975.
13. Chen W, Thomas J, Sadatsafavi M, FitzGerald JM. Risk of cardiovascular comorbidity in patients with chronic obstructive pulmonary disease: a systematic review and meta-analysis. *Lancet Respir Med*. 2015;3:631–639.
14. Bowler RP, Kim V, Regan E, et al. Prediction of acute respiratory disease in current and former smokers with and without COPD. *Chest*. 2014;146:941–950.
15. Corradi M, Majori M, Cacciani GC, Consigli GF, de'Munari E, Pesci A. Increased exhaled nitric oxide in patients with stable chronic obstructive pulmonary disease. *Thorax*. 1999;54:572–575.
16. Crim C, Dransfield MT, Bourbeau J, et al. Pneumonia risk with inhaled fluticasone furoate and vilanterol compared with vilanterol alone in patients with COPD. *Ann Am Thorac Soc*. 2015;12:27–34.

17. Franciosi LG, Page CP, Celli BR, et al. Markers of disease severity in chronic obstructive pulmonary disease. *Pulm Pharmacol Ther*. 2006;19:189–199.
18. Singh D, Kolsum U, Brightling CE, et al. Eosinophilic inflammation in COPD: prevalence and clinical characteristics. *Eur Respir J*. 2014;44:1697–1700.
19. Zanini A, Cherubino F, Zampogna E, Croce S, Pignatti P, Spanevello A. Bronchial hyperresponsiveness, airway inflammation, and reversibility in patients with chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis*. 2015;10:1155–1161.
20. Postma DS, Rabe KF. The Asthma-COPD overlap syndrome. *N Engl J Med*. 2015;373:1241–1249.
21. Alshabanat A, Zafari Z, Albanyan O, et al. A systematic review and meta analysis. *PLoS ONE*. 2015;10:e0136065.
22. Barrecheguren M, Esquinas C, Miravitlles M. The asthma-chronic obstructive pulmonary disease overlap syndrome (ACOS): opportunities and challenges. *Curr Opin Pulm Med*. 2015;21:74–79.
23. Pascoe S, Locantore N, Dransfield MT, Barnes NC, Pavord ID. Blood eosinophil counts, exacerbations, and response to the addition of inhaled fluticasone furoate to vilanterol in patients with chronic obstructive pulmonary disease: a secondary analysis of data from two parallel randomised controlled trials. *Lancet Respir Med*. 2015;3:435–442.
24. Pavord ID, Lettis S, Locantore N, et al. Blood eosinophils and inhaled corticosteroid/long-acting β-2 agonist efficacy in COPD. *Thorax*. 2016;71:118–125.
25. Vlachos-Mayer H, Leigh R, Sharon RF, Hussack P, Hargreave FE. Success and safety of sputum induction in the clinical setting. *Eur Respir J*. 2000;16:997–1000.
26. Simpson JL, McElduff P, Gibson PG. Assessment and reproducibility of non-eosinophilic asthma using induced sputum. *Respiration*. 2010;79:147–151.
27. Wang Z, Bafadhel M, Haldar K, et al. Lung microbiome dynamics in chronic obstructive pulmonary disease exacerbations [published online ahead of print February 25, 2016]. *Eur Respir J*. doi:10.1183/13993003.01406-2015.