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ORIGINAL ARTICLE

Mucus Plugs and Emphysema in the Pathophysiology of Airflow Obstruction and Hypoxemia in Smokers

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Abstract

Rationale: The relative roles of mucus plugs and emphysema in mechanisms of airflow limitation and hypoxemia in smokers with chronic obstructive pulmonary disease (COPD) are uncertain.

Objectives: To relate image-based measures of mucus plugs and emphysema to measures of airflow obstruction and oxygenation in patients with COPD.

Methods: We analyzed computed tomographic (CT) lung images and lung function in participants in the Subpopulations and Intermediate Outcome Measures in COPD Study. Radiologists scored mucus plugs on CT lung images, and imaging software automatically quantified emphysema percentage. Unadjusted and adjusted relationships between mucus plug score, emphysema percentage, and lung function were determined using regression.

Measurements and Main Results: Among 400 smokers, 229 (57%) had mucus plugs and 207 (52%) had emphysema, and subgroups could be identified with mucus-dominant and emphysema-dominant disease. Only 33% of smokers with high mucus plug scores had mucus symptoms. Mucus plug score and emphysema percentage were independently associated with lower values for FEV_1 and peripheral oxygen saturation ($P < 0.001$). The relationships between mucus plug score and lung function outcomes were strongest in smokers with limited emphysema ($P < 0.001$). Compared with smokers with low mucus plug scores, those with high scores had worse COPD Assessment Test scores (17.4 ± 7.7 vs. 14.4 ± 13.3), more frequent annual exacerbations (0.75 ± 1.1 vs. 0.43 ± 0.85), and shorter 6-minute-walk distance (329 ± 115 vs. 392 ± 117 m) ($P < 0.001$).

Conclusions: Symptomatically silent mucus plugs are highly prevalent in smokers and independently associate with lung function outcomes. These data provide rationale for targeting patients with mucus-high/emphysema-low COPD in clinical trials of mucoactive treatments.

Clinical trial registered with www.clinicaltrials.gov (NCT01969344).

Keywords: COPD; computed tomography; FEV_1; mucus plugs; emphysema

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At a Glance Commentary

Scientific Knowledge on the Subject: Mucus plugs and emphysema are mechanisms of lung dysfunction in chronic obstructive pulmonary disease, but the relative role of mucus plugs is not well established because of limitations in quantifying them.

What This Study Adds to the Field: Radiologists scored mucus plugs on computed tomographic lung images, and imaging software automatically quantified percentage emphysema to demonstrate that symptomatically silent mucus plugs are highly prevalent in smokers and associate with lung function outcomes independently of emphysema.

Smoking-related chronic obstructive pulmonary disease (COPD) is associated with airflow limitation and hypoxemia (1). Lung pathology in COPD includes emphysema and intraluminal mucus plugs (2), but the prevalence and clinical importance of mucus plugs in COPD are poorly understood because of methodologic difficulties quantifying them. Mucus symptoms are often absent in patients with COPD who have pathologically proven mucus plugs (3). A recently developed method for quantifying mucus plugs involves scoring mucus plugs in computed tomographic (CT) lung images (4). Coupled with longstanding methods for automated quantification of emphysema using imaging software (5–7), it is now possible to explore how measures of mucus plugs and emphysema associate with airflow obstruction and hypoxemia in smokers. SPIROMICS (Subpopulations and Intermediate Outcome Measures in COPD Study) is a multicenter observational cohort study that includes thoracic CT imaging and measures of airflow and oxygenation (8). We set out here to relate image-based measures of mucus plugs and emphysema to measures of airflow obstruction and oxygenation.

Methods

SPIROMICS

A total of 2,973 participants have been recruited into the following four strata: never-smokers of cigarettes (stratum 1), current or former smokers without airflow obstruction (stratum 2), never-smokers of cigarettes with airflow obstruction (stratum 3) and current or former smokers with airflow obstruction (detailed in the online supplement). For the study reported here, 20 baseline multidetector CT (MDCT) lung scans were randomly selected from participants in stratum 1, 100 baseline scans were randomly selected from participants in each of strata 2 and 3, and 200 baseline scans were randomly selected from participants in stratum 4. The online supplement details methods for measuring peripheral oxygen saturation (Sp_{O_2}) using pulse oximetry, the 6-minute-walk test (6MWT), the COPD Assessment Test (CAT) (9), mucus-related questions (10, 11), and sputum analysis.

Emphysema Percentage

SPIROMICS previously reported on quantitative CT lung assessment system (12) and methods for measuring emphysema and airway wall thickness (8). Emphysema percentage was calculated as the percentage of lung voxels below −950 Hounsfield units. To account for normal variation in emphysema percentage, derived reference equations for emphysema percentage were used that included terms for age; sex, race, and ethnicity; height; and body mass index category, as previously described (7). In this way, a binary variable of emphysema was generated in which "emphysema present" is an emphysema percentage value above the upper limit of normal and "emphysema absent" is an emphysema percentage value below the lower limit of normal. Additional details are provided in the online supplement.

MDCT Mucus Plug Score

For the study reported here, four radiologists (B.M.E, T.H., D.G., and M.L.S.) with subspecialty training in thoracic radiology scored mucus plugs on the MDCT scans using a scoring system based on bronchopulmonary segment anatomy, as described previously by a subset of the authors (4) and further detailed in the online supplement. Briefly, mucus plugs were identified as areas of opacification within the airway lumen, contiguous with patent airway lumen across sequential transverse CT slices. These opacities were less radiodense than adjacent blood vessels, and mucus plugs were defined as complete occlusion of a bronchus, irrespective of generation or size. When parallel to the scan plane, mucus plugs were recognized as

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Author Contributions: Conception and design: E.M.D., B.M.E., T.H., D.S.G., M.L.S., P.G.W., and J.V.F. Acquisition of data for the work: E.M.D., B.M.E., T.H., D.S.G., M.L.S., W.A., I.B., R.G.B., E.R.B., R.C.B., R.B., S.A.C., A.C., C.B.C., D.C., G.J.C., M.D., C.M.D., M.B.D., N.N.H., M.K.H., A.T.H., E.A.H., J.A.K., S.C.L., F.J.M., W.K.O'N., V.E.O., R.P., S.P., J.D.S., P.G.W., and J.V.F. Analysis and interpretation: E.M.D., B.M.E., T.H., D.S.G., M.L.S., D.C., J.A.K., C.E.M., P.G.W., and J.V.F. Drafting the manuscript for important intellectual content: E.M.D., B.M.E., T.H., D.S.G., M.L.S., D.C., J.A.K., C.E.M., P.G.W., and J.V.F.

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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.

Table 1. Characteristics of Mucus Plugging Study Compared with the Entire SPIROMICS Cohort at Baseline

Variable	Mucus Plugging Study						Entire SPIROMICS Cohort (*N* = 2,770)
	Control Subjects Who Never Smoked (*n* = 20)	Current or Former Smokers					
		Preserved Lung Function (*n* = 101)	GOLD Stage 1 (*n* = 40)	GOLD Stage 2 (*n* = 61)	GOLD Stage 3 (*n* = 153)	GOLD Stage 4 (*n* = 45)	
Age, yr*	58.9 ± 9.5	60.7 ± 9.6	64.7 ± 8.8	64.3 ± 7.9	64.7 ± 7.6	60.6 ± 8.3	63.0 ± 9.3
Sex, M, *n* (%)	5 (25.0)	54 (53.5)	28 (70.0)	35 (57.4)	82 (53.6)	29 (64.4)	1,449 (52)
BMI, kg/m^2*	27.7 ± 5.6	29.5 ± 5.6	28.4 ± 4.6	28.6 ± 5.8	26.9 ± 5.2	26.0 ± 5.2	28 ± 5.2
White, *n* (%)	16 (80.0)	65 (64.4)	32 (80.0)	47 (77.0)	125 (81.7)	34 (75.6)	2,102 (75.9)
Current smoking, *n* (%)*	0 (0.0)	51 (50.5)	14 (35.0)	22 (36.1)	46 (30.1)	8 (17.8)	1,055 (38)
Smoking pack-years*	0 ± 0	42.4 ± 19.0	49.2 ± 21.1	46.6 ± 17.5	51.4 ± 22.0	49.1 ± 19.5	45.5 ± 29
Current asthma, *n* (%)	1 (5.0)	12 (11.9)	6 (15.0)	17 (27.9)	37 (24.2)	10 (22.2)	401/2,638 (15.2)
Inhaled steroid use, *n* (%)	0 (0.0)	7 (6.9)	10 (25.0)	28 (45.9)	109 (71.2)	36 (80.0)	972 (35.1)
Inhaled bronchodilator use, *n* (%)	1 (5.0)	22 (21.8)	16 (40.0)	41 (67.2)	139 (90.8)	40 (88.9)	1,440 (52.0)
FEV_1% predicted*	95.2 ± 12.5	91.9 ± 15.4	83.7 ± 12.1	59.5 ± 11.0	33.7 ± 6.7	21.5 ± 4.7	78.4 ± 23.9
FVC% predicted*	97.8 ± 8.8	96.1 ± 14.2	103.1 ± 12.5	83.7 ± 13.2	66.7 ± 15.0	56.3 ± 14.6	93.7 ± 6.4
Supplemental oxygen use, *n* (%)	0 (0)	2 (2.0)	4 (10.3)	8 (13.1)	64 (42.1)	32 (74.4)	110 (26.5)
Resting Sp_{O_2}, %*	97.0 ± 2.2	96.3 ± 2.1	95.9 ± 2.6	94.7 ± 2.7	93.6 ± 3.0	93.3 ± 2.4	94.7 ± 2.9
Postexercise Sp_{O_2}, %*	97.4 ± 1.8	95.0 ± 3.4	93.9 ± 5.4	92.1 ± 4.8	88.1 ± 6.5	87.1 ± 4.8	91.4 ± 6.1
Emphysema, %*	0.9 (0.6–1.3)	1.1 (0.5–2.3)	3.1 (1.5–7.0)	4.2 (1.5–11.1)	13.4 (5.8–24.6)	22.6 (14.4–31.9)	5.3 (1.3–16.7)
Airway wall thickness, mm†	3.68 ± 0.09	3.72 ± 0.08	3.70 ± 0.09	3.72 ± 0.07	3.74 ± 0.06	3.74 ± 0.10	3.72 ± 0.08

Definition of abbreviations: BMI = body mass index; GOLD = Global Initiative for Chronic Obstructive Lung Disease; SPIROMICS = Subpopulations and Intermediate Outcome Measures in Chronic Obstructive Pulmonary Disease Study; Sp_{O_2} = oxygen saturation as measured by pulse oximetry.
Data are shown as mean ± SD or median (interquartile range) unless otherwise indicated.
*Significant difference between groups, $P < 0.001$.
†Significant difference between groups, $P < 0.01$.

tubular densities with or without branching. When oriented obliquely or perpendicularly to the scan plane, they were identified as oval or rounded opacities seen on sequential slices and were differentiated from blood vessels by their continuity with patent portions of the bronchial lumen and their position relative to adjacent blood vessels (*see* Supplemental Video 1 in Reference 4). The segments of each lobe were systematically examined for the presence or absence of mucus plugs and given a score of 1 or 0, accordingly. This generated a segment score ranging from 0 to 20 for each patient. The radiologists also examined the scans for the presence of bronchiectasis, as defined by a broncho:arteriole ratio of >1.5.

The 420 scans were randomly assigned to the four radiologists to be scored. The mucus plug score for each patient's scan was generated by an individual radiologist. Radiologist scores were not combined or averaged in this paper. The radiologists analyzing the scans were blinded to any clinical details of the subjects and entered their mucus score data in real-time into Research Electronic Data Capture, a secure online study survey instrument. Interrater agreement was assessed in a subset of 100 scans. Each scan in the subset was assigned to be independently rescored by a second radiologist randomly selected from the three radiologists who did not score the initial scan. All radiologists participated in this concordance analysis with a relatively even representation of each radiologist pair in the analysis.

The SPIROMICS protocol was approved by the institutional review board at each participating institution, and all the participants provided written informed consent. The University of California, San Francisco, developed protocol for mucus plug scoring on MDCT lung scan analysis was reviewed and approved by the University of California, San Francisco, institutional review board.

Statistics

Study analysis details are in the online supplement. Linear regression models were informed by directed acyclic graphs (DAGs), and minimal adjustment sets required for regression modeling were identified using the web-based "DAGitty" platform (13). A negative binomial regression was used to model the association between mucus plugs and exacerbations adjusted for covariates. Wald tests were performed to assess the statistical significance of interaction terms, and statistically significant interactions were displayed using marginal-effect estimation (14). Marginal effects were calculated using the margins command in STATA (StataCorp). Emphysema was divided into tertiles for the interaction term, as it simplifies the model of interaction, allowing the use of the margins command (which does not accept noninteger values for factor variables) and making it easier to interpret and graph the interaction. Receiver operator characteristic (ROC) curve analyses with comparisons of the areas under multiple ROC curves were performed using the roccomp command in STATA. Statistical significance was accepted for two-sided *P* values of less than 0.05.

Results

Human Subjects

Table 1 shows the clinical characteristics of the 300 patients with COPD, the 100 smokers without airflow obstruction, and the 20 nonsmoking subjects.

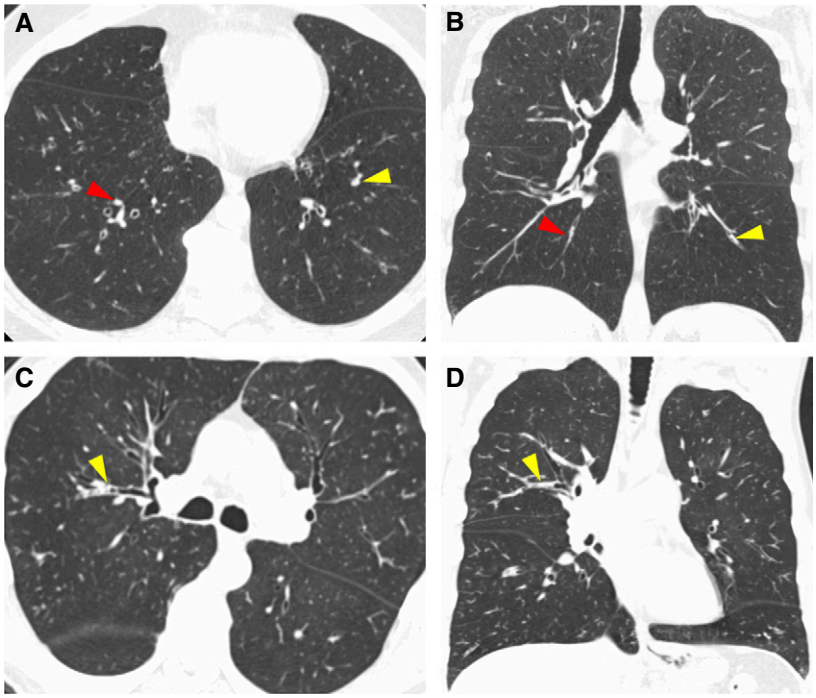


Figure 1. Airway mucus plugs in smokers with chronic obstructive pulmonary disease. (*A*) An example of two mucus plugs occluding subsegmental airways in the lower lobes identified as tubular opacifications in the axial plane (yellow and red arrowheads). (*B*) The mucus plugs in the coronal plane (the yellow and red arrowheads indicating the same mucus plugs as in *A*), revealing that the plugs extend for several millimeters and branch. (*C*) An example of a branching mucus plug occluding a segmental airway in the right upper lobe (yellow arrowhead), as visualized in the axial plane. (*D*) The same plug in the coronal plane (yellow arrowhead).

Airway Mucus Plugs and Emphysema Are Prevalent in Smokers

Mucus plugs were highly prevalent in smokers and appeared as focal or branching opacities, usually seen in subsegmental airways in the absence of bronchial dilatation. Examples of plugs occluding large and small airways are shown in Figures 1 and 2. The intraluminal plugs were only scored if they completely occluded the lumen; partial occlusions were not scored, as previously explained (4). Radiographically apparent intraluminal plugs comprise a mixture of mucins, plasma proteins, and inflammatory cells, and the term "mucus plugs" that we use here does not infer a predominance of any one of these components over the other. The intraclass correlation coefficient for interrater agreement in mucus plug score was 0.80.

Among the 400 ever-smokers, 229 (57%) had mucus plugs and 207 (52%) had emphysema. The median mucus plug score was 0 in the nonsmoking healthy control subjects, 0 in the ever-smokers with preserved lung function, and 3 in the ever-smokers with airflow obstruction (post-bronchodilator FEV_1/FVC of less than 0.70) (patients with COPD) (Figure 3A). Five of the 20 scans from the nonsmoking healthy control subjects had mucus scores between 1 and 5, a higher prevalence of mucus plugging in health than we have previously reported (4). However, the previous healthy control subjects we studied were nearly 30 years younger than the healthy control subjects studied here, and we suspect (minor) mucus plugging associated with older age. The 95% percentile value of the mucus plug scores in the nonsmokers was 5, a value used as the mucus plug high/low cutoff in subsequent analyses. Among all patients with COPD, 67% had a mucus plug score higher than 0 (Figure 3B), and this prevalence was similar in former smokers and current smokers (70% vs. 64%; $P = 0.96$). A diagnosis of asthma occurred with similar frequency in the low and high mucus subgroups in the ever-smoker subgroups (*see* Table E1 in the online supplement).

Among the current or former smokers, 48 of 400 (12%) had bronchiectasis, and the prevalence of bronchiectasis was higher in current or former smokers with a high mucus plug score than in those with a low score (19% vs. 9%; $P < 0.001$).

Mucus Plug Scores, Emphysema Percentage, and Airflow Obstruction

The Global Initiative for Chronic Obstructive Lung Disease (GOLD) system categorizes airflow limitation in COPD on the basis of post-bronchodilator FEV_1. An FEV_1 >80% predicted is GOLD stage 1, an FEV_1 between 50% and 80% is GOLD 2, an FEV_1 between 30% and 50% is GOLD 3, and an FEV_1 <30% is GOLD 4. We found that the mucus plug score was significantly higher in GOLD 3 and 4 patients than in smokers with preserved lung function (Figure 4A) and that 60% of GOLD 4 patients had a high mucus plug score (Figure 4B). We also found that the emphysema percentage was significantly higher in GOLD 2, 3, and 4 patients than in smokers with preserved lung function (Figure 4C) and that 96% of the GOLD 4 patients had emphysema percentage values higher than the upper limit of normal (Figure 4D). The relationship between mucus plug scores and emphysema percentage values in smokers was relatively weak ($r_s = 0.42$; $P < 0.001$) (Figure 4E), making it possible to explore the independent effects of these pathologies on airflow obstruction. A DAG identified emphysema, airway wall thickness expressed as the square root of wall area of a 10-mm lumen perimeter (Pi10), and smoking pack-years as the minimal sufficient adjustment set of covariates for estimating the total effect of mucus plug score on FEV_1 (Figure 4F). In linear regression models adjusting for these covariates, we found that both mucus plug score and emphysema percentage were independently associated with post-bronchodilator FEV_1 and FVC in smokers (Table 2). However, emphysema had a strong modifying effect on the relationship between mucus plug scores and FEV_1 in smokers, as revealed by an interaction term constructed between mucus plug score and tertiles of emphysema and the Wald test for interaction ($P < 0.001$) (Figure 5A). The slope of the line describing the inverse relationship between mucus plug score and FEV_1 was steeper in smokers in the lowest tertile of emphysema percentage values (emphysema percentage <2.4) than in the highest tertile (emphysema percentage

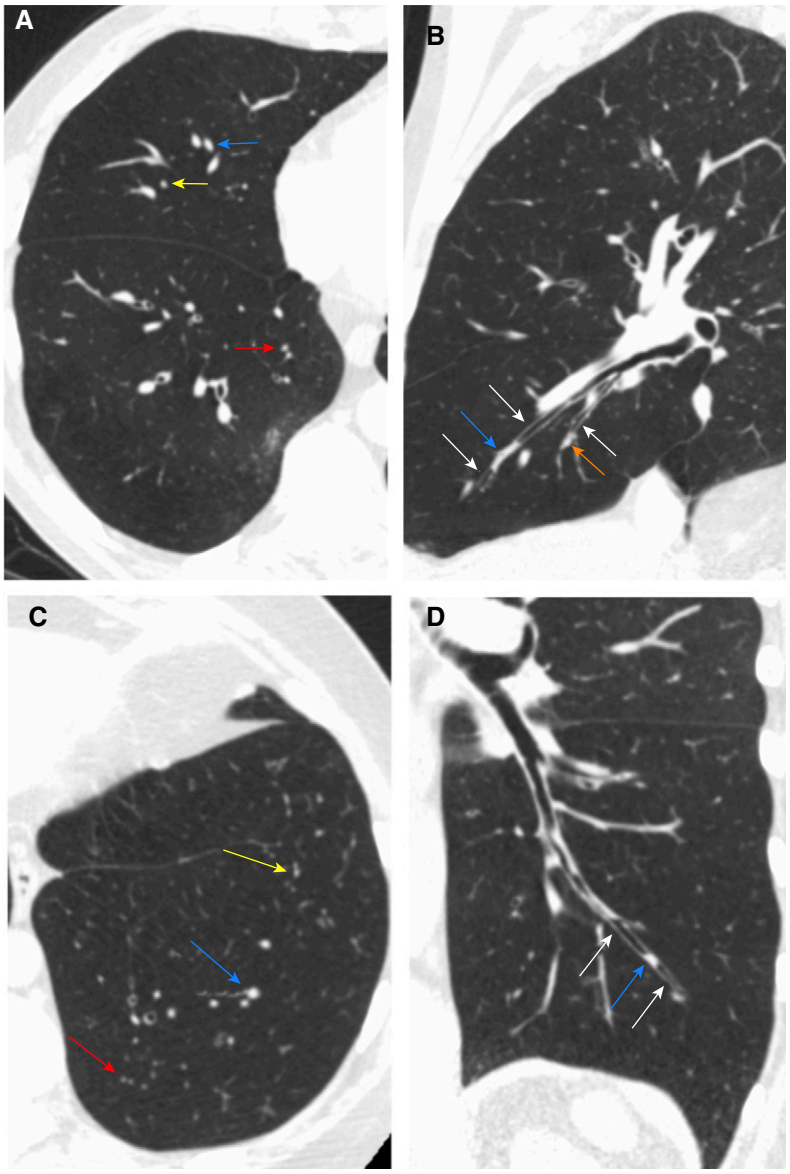


Figure 2. Large and small plugs are identifiable on computed tomographic scans of the lungs. (*A* and *B*) Axial (*A*) and coronal (*B*) oblique images through the right lung. In *A*, three subsegmental mucus plugs are visible in the right middle lobe medial segment (blue arrow), lateral segment (yellow arrow), and right lower lobe medial basal segment (red arrow), with complete opacification of the airways. In *B*, the blue arrow corresponds with the same plug as in *A*, which completely opacifies the airway lumen, with patent airway proximal and distal (white arrows). An additional plug in the same segment is also visible on this image (orange arrow). (*C* and *D*) Axial (*C*) and coronal (*D*) oblique images through the left lung. In *C*, three mucus plugs are visible in the left lower lobe posterobasal (red arrow), lateral basal (blue arrow), and anteromedial (yellow arrow) segments. In *D*, the blue arrow corresponds with the same plug in the lateral basal segment as in *C*. Note the complete occlusion of the airway, which is patent proximal and distal to the plug (white arrows). Also note that more proximally, there are nonocclusive filling defects in the airway lumen, which are not counted as plugs.

>13.0) (Figure 5A). This strong modifying effect of emphysema was true for other spirometric outcomes as well (Table E2 and Figure E1). In other analyses, we explored the independent effects of emphysema and mucus plugs on airflow obstruction by stratifying smokers by presence or absence of emphysema. In these analyses, we found that smokers with mucus plugs have a low post-bronchodilator FEV_1 even when emphysema is absent (Figure 5B). Notably, the median FEV_1 in smokers with a high mucus plug score and no emphysema is similar to the median FEV_1 in smokers with emphysema and a low mucus plug score (45% vs. 44%) (Figure 5B).

Mucus Plug Scores, Emphysema Percentage, and Sp_{O_2}

Among smokers, 28% were using supplemental oxygen, and oxygen use was higher in subgroups with a high mucus plug score and with emphysema (Table E1). Compared with smokers whose resting or postexercise Sp_{O_2} was >92%, those with resting or postexercise Sp_{O_2} <92% had higher mucus plug scores and higher emphysema percentage values (Table E3); this remained true when smokers using supplemental oxygen were excluded from the analyses (Table E3).

A DAG identified emphysema, airway wall thickness (Pi10), and smoking pack-years as the minimal sufficient adjustment set of covariates for estimating the total effect of mucus plug score on Sp_{O_2} (Figure 5F). Using regression models, we found that mucus plug score, emphysema percentage, and airway wall thickness were all independently related to resting and postexercise Sp_{O_2} in smokers, whereas smoking pack-years were not (Table 2). However, we found a strong modifying effect of emphysema percentage on the relationship between mucus plug scores and Sp_{O_2} (both resting and postexercise Sp_{O_2}) in smokers, as revealed by the interaction term constructed between mucus plug score and tertiles of emphysema and the Wald test for interaction ($P < 0.001$) (Figure 5C). The slope of the line describing the inverse relationship between mucus plug score and resting Sp_{O_2} was steeper in smokers in the lowest tertile of emphysema percentage values than in those in the highest tertile (Figure 5C). We also found strong modifying effects of emphysema on the relationship between mucus plug scores and postexercise Sp_{O_2} (P value test for interaction, $P < 0.001$) (Figure 5E). In other analyses, we explored the independent effects of emphysema and mucus plugs on Sp_{O_2} by stratifying smokers by presence or absence of emphysema. In these analyses, we found that smokers with mucus plugs have low resting and postexercise Sp_{O_2} values even when emphysema is absent (Figures 5D and 5F).

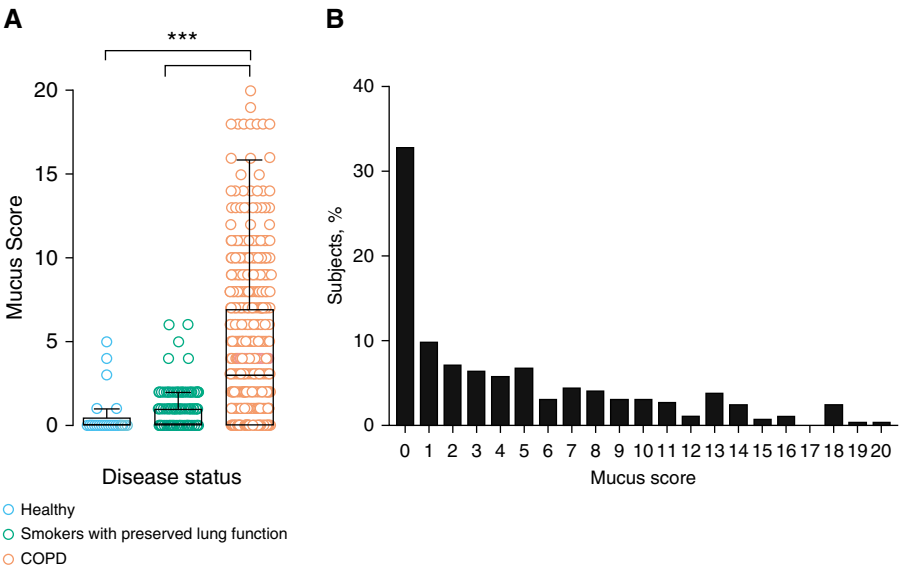


Figure 3. Mucus plug scores in smoker subgroups and control subjects. (*A*) The mucus plug score in healthy nonsmokers, smokers with preserved lung function, and smokers with chronic obstructive pulmonary disease (COPD). (*B*) The frequency distribution of mucus scores in smokers with COPD. ***Significant difference between the COPD group and the other two groups, $P < 0.001$.

Relationships between Mucus Plug Scores, Symptoms of Chronic Mucus Hypersecretion, COPD Control Outcomes, and Sputum Granulocytes

Among all smokers, 23% had symptoms of chronic mucus hypersecretion (CMH) using World Health Organization criteria (15), and the prevalence of CMH was higher in current smokers than in former smokers (35% vs. 16%; $P < 0.001$). In current smokers, the prevalence of CMH was similar in patients with a high mucus score and those with a low score (44% vs. 31%; $P = 0.18$), but the prevalence of CMH was higher in former smokers with a high mucus score than in those with a low score (27.9% vs. 11.2%; $P = 0.001$). Overall, the sensitivity of CMH symptoms for a high mucus plug score was low (33%) and the specificity was moderate (81%). Similarly, the sensitivity of sputum symptoms captured using the St. George's Respiratory Questionnaire data for a high mucus plug score in former smokers was also low (34%), as was the specificity (75%).

CAT scores were higher in patients with a high mucus plug score than in those with a low score (17.5 ± 7.6 vs. 14.3 ± 8.5; $P < 0.001$), and in a linear regression model that controlled for age, sex, race, and smoking status, the mucus plug score was significantly associated with CAT score ($\beta = 3.77$; 95% CI, 2.0–5.5; $P < 0.001$). In addition, the number of COPD exacerbations per person per year was higher in patients with a high mucus plug score than in those with a low score, and this was true when the exacerbation number was for the year before enrollment (0.75 ± 1.1 vs. 0.43 ± 0.85; Kruskal-Wallis test; $P = 0.004$) or for the year after enrollment (0.89 ± 1.3 vs. 0.43 ± 1.0; Kruskal-Wallis test; $P = 0.004$). In a negative binomial regression, the mucus plug score was associated with both the preenrollment COPD exacerbation number ($\beta = 0.63$; 95% CI, 0.27–1.0; $P = 0.001$) and the Year 1 postenrollment COPD exacerbation number ($\beta = 0.75$; 95% CI, 0.35–1.2; $P < 0.001$). The mucus plug score and the CAT score predicted exacerbations ($\geq$1 treatments with steroids in the 12 months from baseline to Year 1) and hospitalizations ($\geq$1 hospitalization for COPD in the 12 months from baseline to Year 1) similarly, as evidenced by similar areas under the curve in ROC curve analyses (exacerbations: 0.62 vs. 0.67; $P = 0.2$; hospitalizations: 0.59 vs. 0.66; $P = 0.18$). Finally, the 6MWT distance was lower in patients with a high mucus plug score than in those with a low score (329 ± 115 m vs. 392 ± 117 m; $P < 0.001$), and in a linear regression model controlled as above, the mucus plug score was significantly inversely associated with 6MWT distance ($\beta = -59.3$; 95% CI -85.4 to -33.3; $P < 0.001$).

Among the 400 smokers, 127 had data for neutrophil percentage and eosinophil percentage in induced sputum. The median [interquartile range] sputum neutrophil percentage was higher in patients with a high mucus plug score than in those with a low score (86% [71–94%] vs. 72% [52–83%]; $P < 0.001$). The median [interquartile range] sputum neutrophil number ($\times 10^6$/ml) was also higher in patients with a high mucus plug score than in those with a low score (1.12 [0.27–3.62] vs. 0.24 [0.09–0.59]; $P = 0.0002$). The sputum eosinophil percentage was similar in both subgroups (0.1 [0–1.1] vs. 0.3% [0–1.0]; $P = 0.55$), and the sputum eosinophil number was also similar (data not shown).

Stability of the Mucus Plug Phenotype in Smokers with COPD

To determine the stability of the mucus plug phenotype in smokers, we focused on analysis of mucus plugs in CT lung scans from a subset of the smokers with COPD (SPIROMICS stratum 3 and 4), and we took advantage of the fact that CT lung scans were repeated at 1 year of follow up in SPIROMICS participants. Specifically, we randomly selected 100 scan pairs from patients with COPD stratified by tertiles of segment score to ensure a broad representation of mucus plug scores. The scan reads were divided among the four radiologists. Scan pairs from each patient were read by the same radiologist to eliminate any between-reader error (within-reader variability was minimal, as previously described) (4). Radiologists did not score the same subject sequentially or have access to both scans at the same time to compare scores. In this way, we found that the mucus plug phenotype among patients with COPD was remarkably stable (Figure 6A). Patients with COPD with low mucus plug scores (0–1) at baseline tended to have low scores at Year 1, and patients with high mucus plug scores (7–20) at baseline tended to have high scores at Year 1 (Figure 6A). We also did analyses based on bronchopulmonary segments. Among segments in which mucus plugs were absent at baseline, the vast majority of segments remained free of mucus plugs 1 year later (Figure 6B); among segments in which mucus plugs were present at

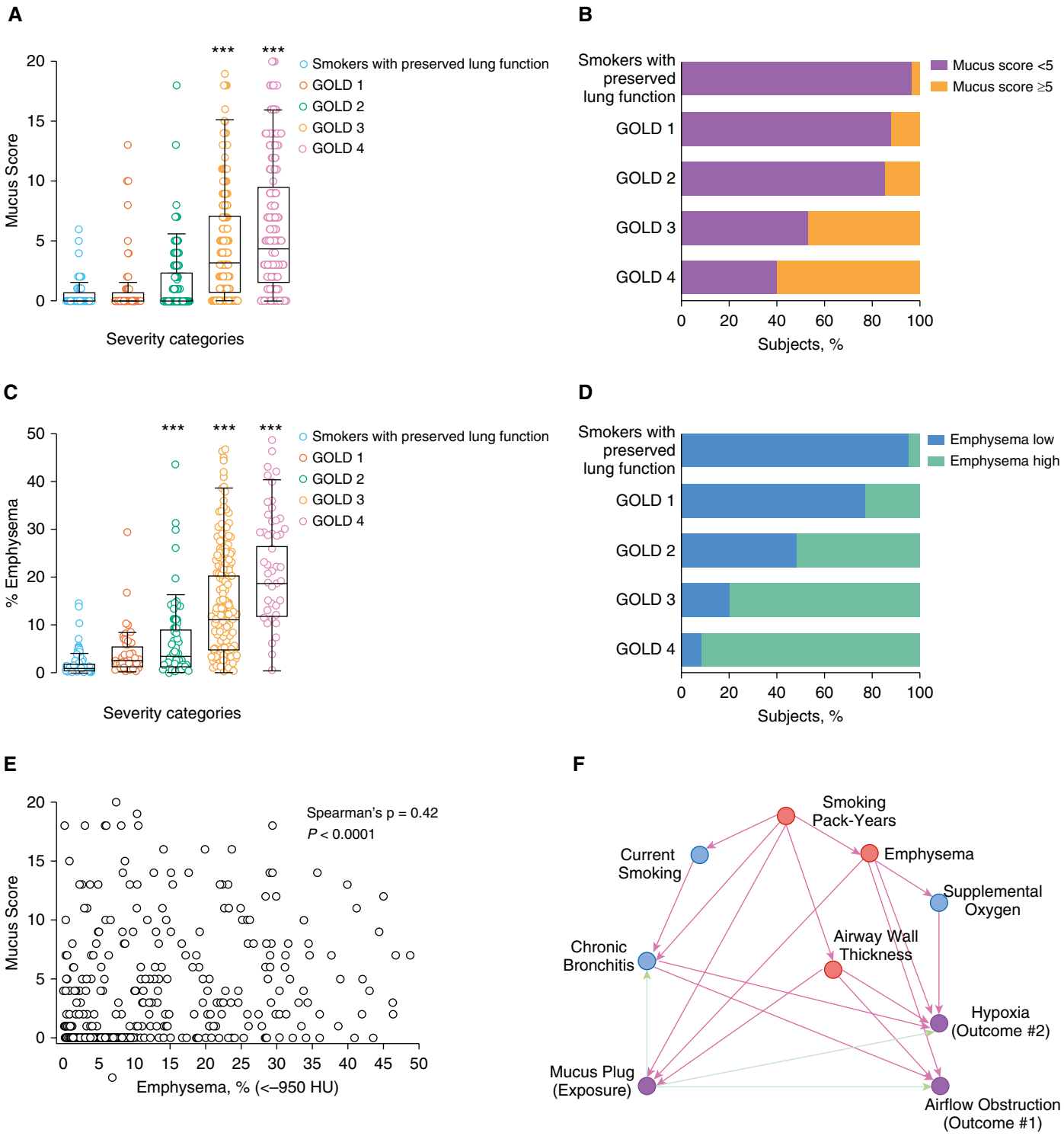
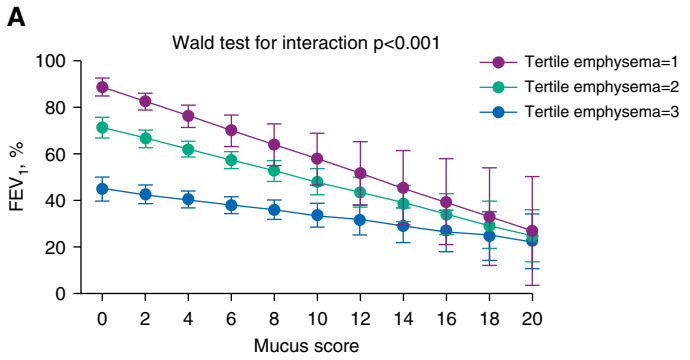


Figure 4. Mucus plugs, emphysema, and airflow obstruction in smokers. (*A*) The mucus plug score in smokers with preserved lung function and smokers with increasing severity of airflow obstruction classified by Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage. (*B*) The fraction of smokers with high mucus scores in the subgroups with and without airflow obstruction. (*C*) The emphysema percentage in smokers with preserved lung function and smokers with increasing severity of airflow obstruction classified by GOLD stage. (*D*) The fraction of smokers with emphysema in the subgroups with and without airflow obstruction. (*E*) The relationships between mucus plug scores and emphysema percentage in smokers. (*F*) The directed acyclic graph (DAG), or causal diagram, which formed the basis for the DAG-informed logistic regression models used to assess relationships among mucus plugs, emphysema, FEV_1, and peripheral oxygen saturation. Purple circles represent the predictor and outcome variables; blue circles represent ancestors of predictor or outcomes; and red circles represent ancestors of predictor and outcomes (confounders). Green arrows represent nonconfounded causal paths, and pink arrows represent biasing paths. ***Significantly different from smokers with preserved lung function, $P < 0.001$. HU = Hounsfield units.

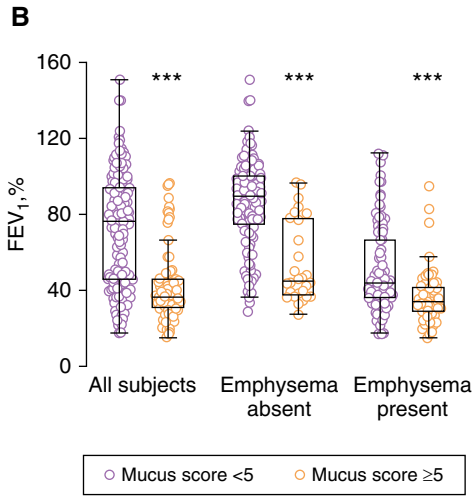
Table 2. Regression Models Predicting Lung Function Outcome Measures

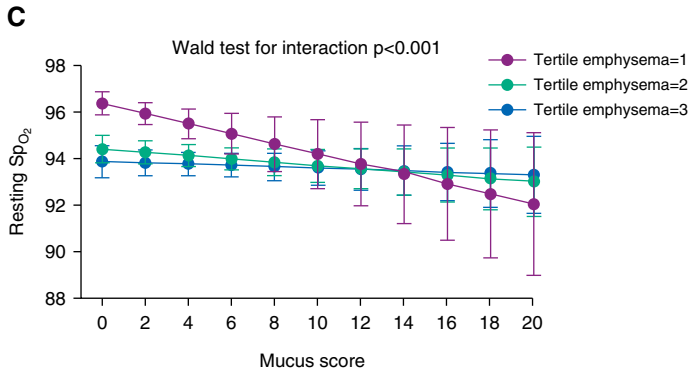
Predictor Variables*	Model 1			Model 2			Model 3			Model 4		
	β	95% CI	*P* Value	β	95% CI	*P* Value	β	95% CI	*P* Value	β	95% CI	*P* Value
Post-bronchodilator FEV_1% predicted												
Mucus score	−14.4	−16.9 to −11.9	<0.001	−10.0	−12.1 to −7.9	<0.001	−9.9	−12.1 to −7.6	<0.001	−9.6	−11.8 to −7.3	<0.001
Emphysema %				−15.1	−17.2 to −12.9	<0.001	−15.1	−17.2 to −12.9	<0.001	−14.9	−17.0 to −12.8	<0.001
Wall thickness Pi10, mm							−0.5	−2.7 to 1.6	0.63	−0.6	−2.7 to 1.6	0.60
Smoking pack-years										−3.1	−5.13 to −1.1	0.003
Post-bronchodilator FVC% predicted												
Mucus score	−6.5	−8.3 to −4.7	<0.001	−5.3	−7.2 to −3.5	<0.001	−4.5	−6.4 to −2.6	<0.001	−4.4	−6.3 to −2.5	<0.001
Emphysema %				−4.3	−6.1 to −2.4	<0.001	−4.4	−6.2 to −2.6	<0.001	−4.3	−6.2 to −2.5	<0.001
Wall thickness Pi10, mm							−2.4	−4.2 to −0.5	0.01	−2.4	−4.2 to −0.6	0.01
Smoking pack-years										−1.1	−2.8 to 0.7	0.23
Preexercise Sp_{O_2}, %												
Mucus score	−0.80	−1.1 to −0.51	<0.001	−0.62	−0.91 to −0.33	<0.001	−0.47	−0.77 to −0.17	0.003	−0.45	−0.75 to −0.15	0.004
Emphysema %				−0.65	−0.95 to −0.35	<0.001	−0.69	−0.99 to −0.39	<0.001	−0.67	−0.97 to −0.37	<0.001
Wall thickness Pi10, mm							−0.45	−0.75 to −0.16	0.003	−0.46	−0.75 to −0.16	0.003
Smoking pack-years										−0.22	−0.49 to 0.06	0.12
Postexercise Sp_{O_2}, %												
Mucus score	−1.9	−2.5 to −1.3	<0.001	−1.2	−1.8 to −0.67	<0.001	−0.85	−1.4 to −0.26	0.005	−0.79	−1.4 to −0.20	0.008
Emphysema %				−2.5	−3.1 to −1.9	<0.001	−2.6	−3.2 to −2.0	<0.001	−2.5	−3.1 to −1.9	<0.001
Wall thickness Pi10, mm							−1.2	−1.8 to −0.60	<0.001	−1.2	−1.7 to −0.6	<0.001
Smoking pack-years										−0.78	−1.3 to −0.2	0.004

Definition of abbreviation: CI = confidence interval; Sp_{O_2} = oxygen saturation as measured by pulse oximetry.
*Predictor variables are standardized for comparison.

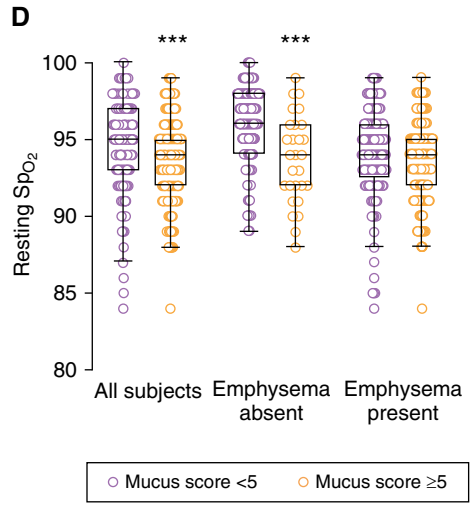


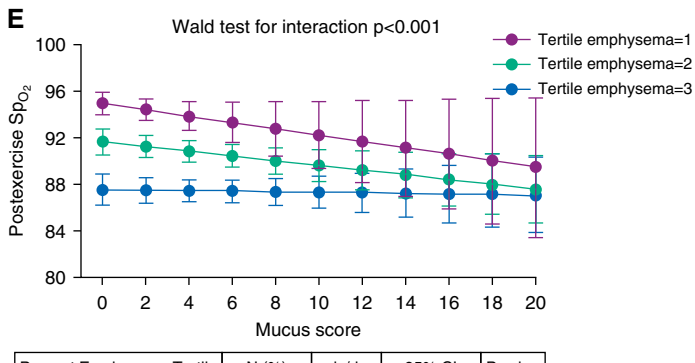
Percent Emphysema Tertile	N (%)	dy/dx	95% CI	P value
1 (0.04 – 2.4)	134 (33.6)	–3.1	–4.3, –1.9	<0.001
2 (2.5 – 13.0)	132 (33.1)	–2.3	–3.0, –1.7	<0.001
3 (13.1 – 48.8)	133 (33.3)	–1.1	–1.9, –0.4	0.004





Percent Emphysema Tertile	N (%)	dy/dx	95% CI	P value
1 (0.04 – 2.4)	134 (33.6)	–0.22	–0.34, –0.05	0.009
2 (2.5 – 13.0)	132 (33.1)	–0.07	–0.16, 0.02	0.12
3 (13.1 – 48.8)	133 (33.3)	–0.03	–0.13, 0.07	0.59





Percent Emphysema Tertile	N (%)	dy/dx	95% CI	P value
1 (0.04 – 2.4)	134 (33.6)	–0.27	–0.59, 0.04	0.09
2 (2.5 – 13.0)	132 (33.1)	–0.20	–0.37, –0.03	0.02
3 (13.1 – 48.8)	133 (33.3)	–0.02	–0.22, 0.18	0.83

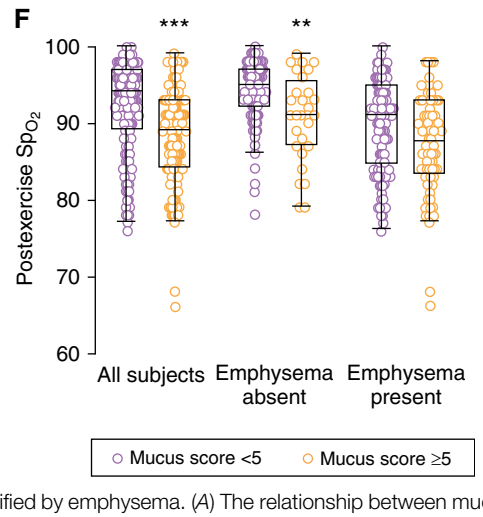


Figure 5. The relationship between mucus plug score and lung function outcomes are modified by emphysema. (*A*) The relationship between mucus plug score and FEV_1% predicted is modified by emphysema. Three linear regression lines demonstrate the relationships between mucus plug scores and FEV_1% predicted in smokers grouped by tertiles of emphysema percentage. The slope of the line describing the inverse relationship between mucus plug score and FEV_1 was steeper in smokers in the lowest tertile of emphysema percentage values than in those in the highest tertile. (*B*) The FEV_1 values in subgroups of smokers with and without a high mucus plug score and stratified by presence or absence of emphysema. (*C*) The relationship between mucus plug score and resting peripheral oxygen saturation (Sp_{O_2}-R) is modified by emphysema. The slope of the line describing the inverse relationship

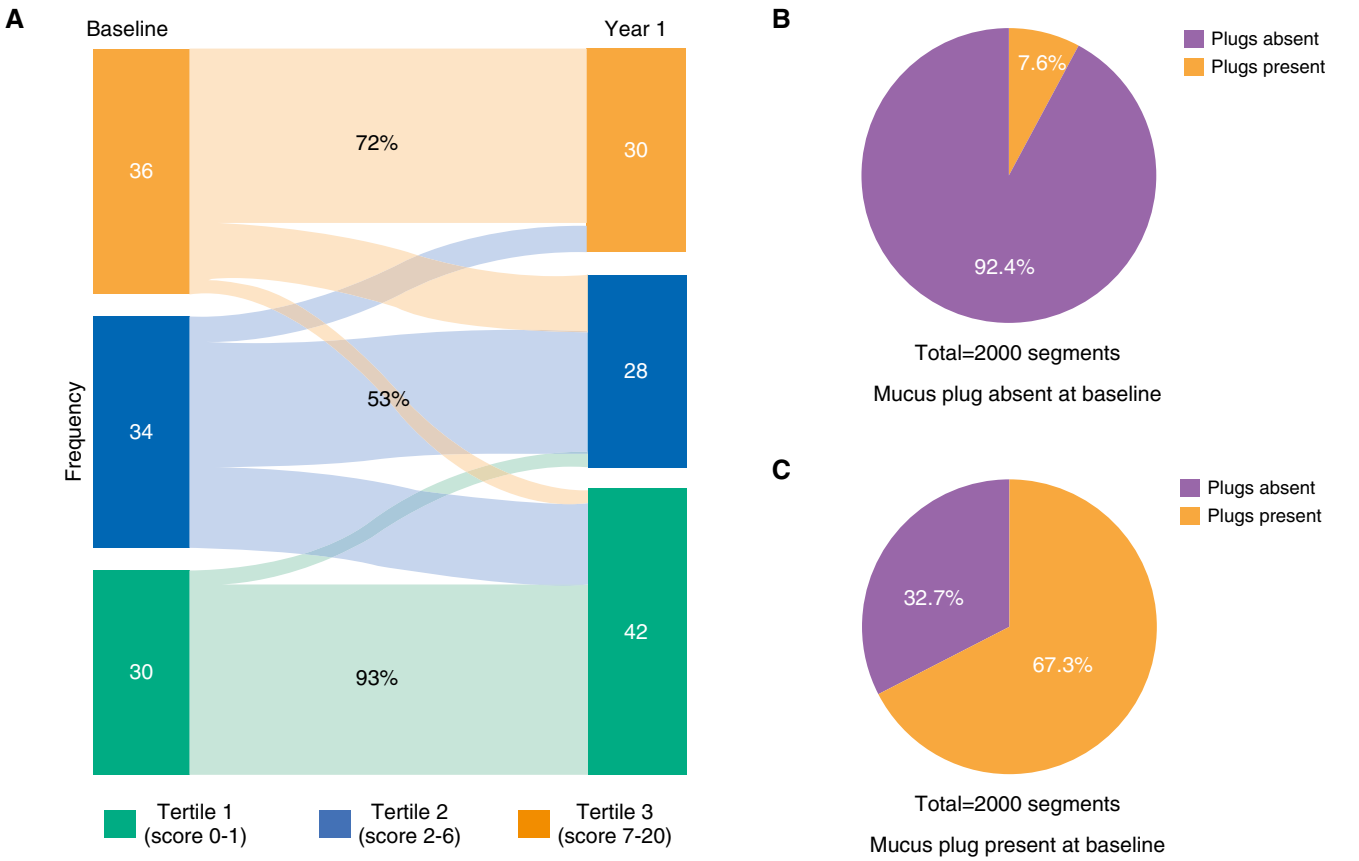


Figure 6. Mucus plugs persist for 1 year in smokers with chronic obstructive pulmonary disease (COPD). (*A*) A Sankey bar graph of mucus plug scores in 100 smokers with COPD at baseline and at 1 year of follow-up. Patients with COPD with low mucus plug scores (tertile 1) at baseline tended to remain in the lowest tertile for mucus plug scores at 1 year, whereas patients with COPD with high mucus plug scores (tertile 3) tended to remain in the highest tertile for mucus plug scores at 1 year. (*B*) Data on mucus plugs in bronchopulmonary segments with no airways plugged with mucus in baseline scans; 92% of these segments continued to have no airways plugged with mucus on the Year 1 scan. (*C*) Data on mucus plugs in bronchopulmonary segments with at least one airway plugged with mucus in baseline scans, 67% of these segments continued to have at least one airway plugged with mucus on the Year 1 scan.

baseline, the majority of segments still had mucus plugs 1 year later (Figure 6C).

Discussion

Smoking-related COPD, the fourth leading cause of death in the United States (16), is characterized by airflow obstruction, and hypoxemia occurs in a subset of patients with more severe disease. Here, we show that emphysema and mucus plugs are independently associated with lower FEV_1 and Sp_{O_2} in ever-smokers and that clinically significant abnormalities in airflow and oxygenation can occur in patient subgroups with prominent mucus plugging and little or no emphysema.

Our study leveraged advances in methods for detecting and quantifying mucus plugs and emphysema in MDCT lung images, and we report that both pathologies are very common in smokers with COPD. The mucus plug scores that we report in patients with COPD are higher than the scores recently reported in a COPD cohort of similar disease severity (17) for reasons that may relate to differences in methods or radiologist experience in identifying and scoring mucus plugs. The validity of the mucus plug scoring data we report here is supported by images and videos of the mucus plugs, by good agreement for mucus scores among readers, and by the extensive additional validation data provided in our prior publication (4).

Our major aim here was to determine the independent effects of mucus plugs and emphysema on measures of airflow obstruction and oxygenation in smokers with COPD. Because the correlation between mucus plug score and emphysema

Figure 5. (*Continued*). between mucus plug score and Sp_{O_2}-R was steeper in smokers in the lowest tertile of emphysema percentage values than in those in the highest tertile. (*D*) The Sp_{O_2}-R values in subgroups of smokers with and without a high mucus plug score and stratified by presence or absence of emphysema. (*E*) The relationship between mucus plug score and postexercise peripheral oxygen saturation (Sp_{O_2}-PE) is modified by emphysema. The slope of the line describing the inverse relationship between mucus plug score and Sp_{O_2}-PE was steeper in smokers in the lowest tertile of emphysema percentage values than in those in the highest tertile. (*F*) The Sp_{O_2}-PE values in subgroups of smokers with and without a high mucus plug score and stratified by presence or absence of emphysema. *dy/dx* is the marginal effect of mucus score on FEV_1 in *A*, resting Sp_{O_2} in *C*, and postexercise Sp_{O_2} in *E* stratified by quintiles of emphysema score. **Significantly different from smokers with mucus score of less than 5, $P < 0.01$. ***Significantly different from smokers with mucus score of less than 5, $P < 0.001$. CI = confidence interval.

percentage was relatively weak, we could use multivariate regression models to address this aim. We discovered that mucus plugs and emphysema are independently associated with FEV_1, a measure of airflow in larger airways, and that the effect sizes for the mucus plug score and emphysema percentage are similar. Thus, mucus plugs and emphysema are pathologies that both have important consequences for airflow limitation in smokers. Because mucus plugs were scored only if they completely occluded airways, often occurred in segmental and subsegmental bronchi, and remained significantly associated with airflow obstruction after accounting for airway wall thickness, our data support a causal relationship between mucus plugs and limits to airflow in these airways in smokers. Similarly, although the smokers with hypoxemia (resting or after exercise) had increased emphysema, they also had increased mucus plugging, and regression models revealed that emphysema and mucus plugs are independently associated with low Sp_{O_2}. A pathophysiologic effect of mucus plugs on ventilation–perfusion matching leading to low Sp_{O_2} is plausible. Mucus plugs that completely occlude airways will decrease the partial pressure of oxygen in the alveolar gas exchange units distal to the plugged airways and limit the oxygenation of capillary blood in these units. Poorly oxygenated blood returning to the left heart from these mucus-plugged lung units will be mixed with properly oxygenated blood from nonplugged lung units to create a venous admixture effect that will lower oxygen saturation in arterial blood (18, 19). Thus, mucus plug pathology is an underappreciated and potentially treatable mechanism of hypoxemia in COPD.

Although sputum cytology data were not available for many patients with more severe COPD, the available sputum data showed sputum neutrophilia, not eosinophilia, in the subgroup with high mucus plug scores. This finding stands in contrast to the eosinophilia that characterizes airway mucus plugs in severe asthma (4). This data and the fact that an asthma diagnosis was similarly prevalent in subgroups with and without mucus plugs argue against airway type 2 inflammation or asthma as a cause of mucus plugging in patients with COPD with high mucus plug scores.

Quantitative measures of mucus plug and emphysema pathology represent imaging biomarkers with the potential to advance precision medicine for patients with COPD. We clearly show that these imaging biomarkers can identify subgroups of smokers with emphysema-dominant and mucus-dominant disease. This becomes very relevant in the proper selection of patients for emphysema-based therapies (e.g., lung volume reduction surgery or bronchoscopic lung reduction therapies) or mucus-based therapies (mucoactive drugs or bronchoscopy-based treatments). In particular, our work provides the rationale for using MDCT imaging to select patients with COPD with prominent mucus plugs but limited emphysema for clinical trials of mucoactive treatments (20). Such trials could examine outcomes other than FEV_1 and Sp_{O_2}, including the CAT score, exacerbation frequency, and 6MWT distance, because all of these clinical outcomes are worse in the smoker subgroup with high mucus plug scores. Importantly, because many patients with COPD with high mucus plug scores did not have symptoms of CMH, these symptoms should not be used as surrogates for image-based disease classification.

In conclusion, this study reveals that mucus plugs are common in smokers with COPD and independently associate with airflow obstruction and hypoxemia. Our data offer mechanistic insights into the abnormal airway physiology of COPD and provide rationale for targeting patients with mucus-high/emphysema-low COPD for clinical trials of mucoactive treatments. ■

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

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