The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 SEPTEMBER 7, 2017 VOL. 377 NO. 10

Airway Mucin Concentration as a Marker of Chronic Bronchitis

Mehmet Kesimer, Ph.D., Amina A. Ford, M.S.P.H., Agathe Ceppe, M.S., Giorgia Radicioni, Ph.D., Rui Cao, Ph.D., C. William Davis, Ph.D., Claire M. Doerschuk, M.D., Neil E. Alexis, Ph.D., Wayne H. Anderson, Ph.D., Ashley G. Henderson, M.D., R. Graham Barr, M.D., Dr.P.H., Eugene R. Bleecker, M.D., Stephanie A. Christenson, M.D., Christopher B. Cooper, M.D., MeiLan K. Han, M.D., Nadia N. Hansel, M.D., Annette T. Hastie, Ph.D., Eric A. Hoffman, Ph.D., Richard E. Kanner, M.D., Fernando Martinez, M.D., Robert Paine III, M.D., Prescott G. Woodruff, M.D., Wanda K. O'Neal, Ph.D., and Richard C. Boucher, M.D.

ABSTRACT

BACKGROUND

Chronic obstructive pulmonary disease (COPD) is characterized by chronic bronchitic and emphysematous components. In one biophysical model, the concentration of mucin on the airway surfaces is hypothesized to be a key variable that controls mucus transport in healthy persons versus cessation of transport in persons with muco-obstructive lung diseases. Under this model, it is postulated that a high mucin concentration produces the sputum and disease progression that are characteristic of chronic bronchitis.

METHODS

We characterized the COPD status of 917 participants from the Subpopulations and Intermediate Outcome Measures in COPD Study (SPIROMICS) using questionnaires administered to participants, chest tomography, spirometry, and examination of induced sputum. Total mucin concentrations in sputum were measured with the use of size-exclusion chromatography and refractometry. In 148 of these participants, the respiratory secreted mucins MUC5AC and MUC5B were quantitated by means of mass spectrometry. Data from chronic-bronchitis questionnaires and data on total mucin concentrations in sputum were also analyzed in an independent 94-participant cohort.

RESULTS

Mean (±SE) total mucin concentrations were higher in current or former smokers with severe COPD than in controls who had never smoked (3166±402 vs. 1515±152 μg per milliliter) and were higher in participants with two or more respiratory exacerbations per year than in those with zero exacerbations (4194±878 vs. 2458±113 μg per milliliter). The absolute concentrations of MUC5B and MUC5AC in current or former smokers with severe COPD were approximately 3 times as high and 10 times as high, respectively, as in controls who had never smoked. Receiver-operating-characteristic curve analysis of the association between total mucin concentration and a diagnosis of chronic bronchitis yielded areas under the curve of 0.72 (95% confidence interval [CI], 0.65 to 0.79) for the SPIROMICS cohort and 0.82 (95% CI, 0.73 to 0.92) for the independent cohort.

CONCLUSIONS

Airway mucin concentrations may quantitate a key component of the chronic bronchitis pathophysiologic cascade that produces sputum and mediates disease severity. Studies designed to explore total mucin concentrations in sputum as a diagnostic biomarker and therapeutic target for chronic bronchitis appear to be warranted. (Funded by the National Heart, Lung, and Blood Institute and others.)

The authors' affiliations are listed in the Appendix. Address reprint requests to Dr. Kesimer at the Marsico Lung Institute, University of North Carolina Cystic Fibrosis Center, 7111 Marsico Hall, Chapel Hill, NC 27599-7248, or at kesimer@med.unc.edu.

Dr. Kesimer and Ms. Ford contributed equally to this article.

N Engl J Med 2017;377:911-22.
DOI: 10.1056/NEJMoa1701632

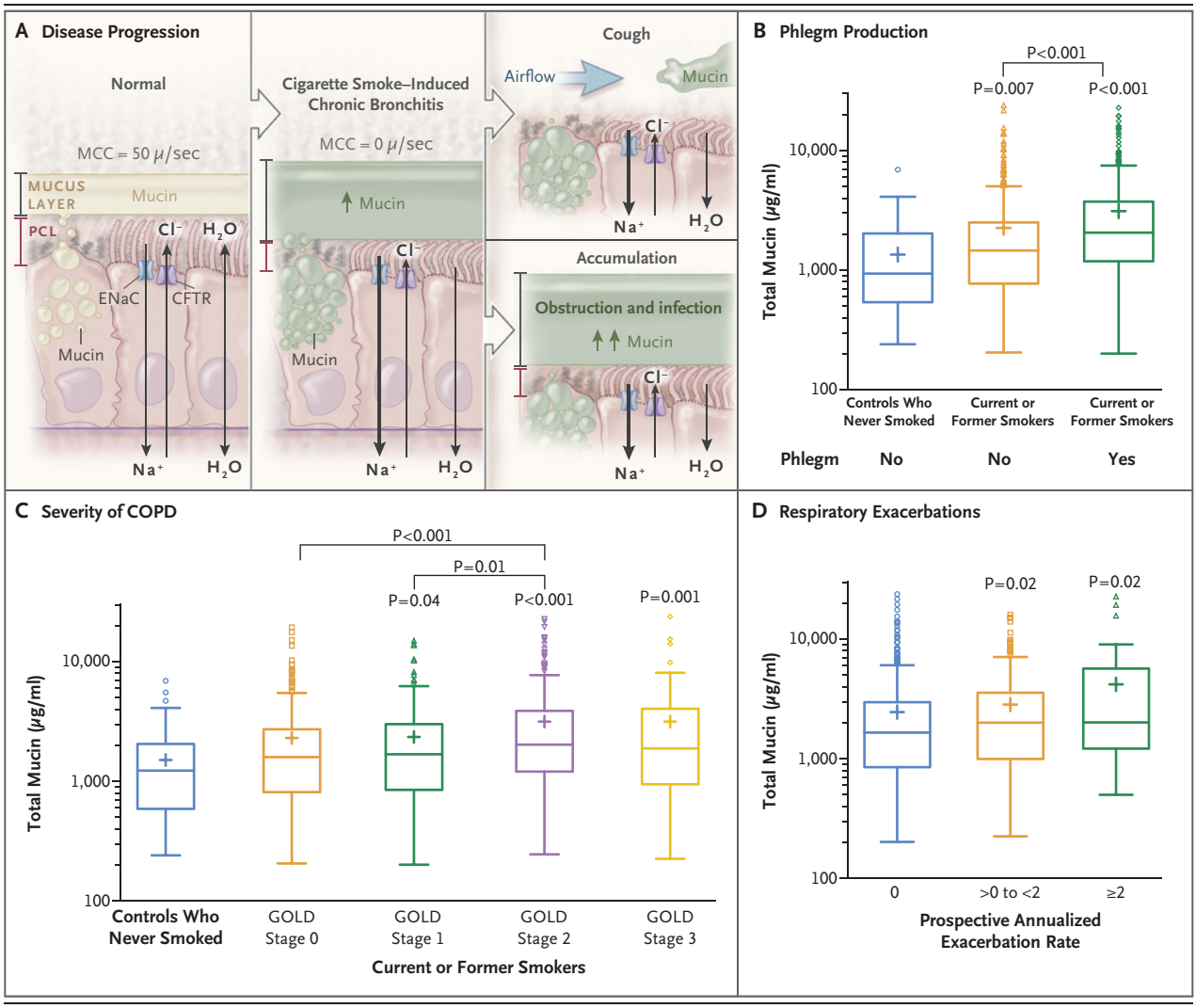
Human airway surfaces are protected from the noxious effects of inhaled substances by a mucus clearance system that traps deposited materials and clears them from the lung.[1] A failure of mucus transport, with pulmonary mucus accumulation, contributes to sputum production, airflow obstruction, and exacerbations in muco-obstructive pulmonary diseases.[2-6] One such muco-obstructive disease is chronic bronchitis, also known as chronic mucus hypersecretion. Chronic bronchitis is a syndrome that is associated with cigarette smoking; it is defined by patient-reported chronic cough and sputum production and, when associated with airflow obstruction, is a component of chronic obstructive pulmonary disease (COPD). Despite the importance of mucus accumulation in the pathogenesis of chronic bronchitis, a unifying hypothesis that describes the failure of mucus flow with consequent mucus accumulation in chronic bronchitis has been lacking, and there have been no laboratory methods to confirm a diagnosis of chronic bronchitis.

The mucus that forms the protective barrier on airway surfaces is composed of water (approximately 98%), ions, globular proteins, and polymeric mucin macromolecules.[1,7] The high-molecular-weight (>10^7 Da) mucin polymers, predominantly MUC5B and MUC5AC, provide the biophysical properties required for airway mucus transport and generate the perception of mucus as a "gel."[8] In one biophysical model (the "two-gel hypothesis"), it is posited that respiratory mucin concentrations govern the rates of mucus transport in the lung (Fig. 1A).[9] In this model, it is predicted that as mucin concentrations rise, a threshold concentration is exceeded, after which mucus transport ceases and adherent mucus plaques form on airway surfaces.[9] Ultimately, some accumulated mucus may be expectorated as sputum. Accumulated mucus that cannot be expectorated serves as the nidus for airflow obstruction, inflammation, and intermittent infection.[6,10,11] Thus, this model predicts that an increased mucin concentration will be associated with sputum production and disease severity in chronic bronchitis.

The hypothesis that total mucin concentration is a biochemical hallmark of the pathogenesis of chronic bronchitis was tested in an extensively

Figure 1 (facing page). Associations between Total Mucin Concentrations and Phlegm Production and Disease Severity in Chronic Obstructive Pulmonary Disease (COPD).

Panel A shows a model representing the progression from normal lung to cigarette smoke–induced chronic bronchitis. In healthy persons, the balance of active ion absorption (Na^+) versus secretion (Cl^-), passive osmotically entrained water transport, and mucin secretion generates a mucus layer with secreted mucin concentrations that are lower than the tethered mucin and other glycoconjugate concentrations in the periciliary layer (PCL). The result is a well-hydrated PCL and efficient mucociliary clearance (MCC). In persons with cigarette smoke–induced chronic bronchitis, an imbalance of ion transport coupled with mucin hypersecretion increases the mucin concentration in the mucus layer, producing osmotic compression of the PCL, adhesion of hyperconcentrated mucus to airway surfaces, and cessation of MCC. The adherent mucus may be expelled as phlegm or sputum by cough. Mucus that cannot be expelled by cough continues to accumulate, concentrates, and ultimately becomes the basis for airflow obstruction and the nidus for intermittent infection or exacerbation. CFTR denotes cystic fibrosis transmembrane regulator, and ENaC epithelial sodium channel. Panel B shows total mucin concentration in controls who had never smoked and who reported no phlegm (59 participants; 10 of 69 participants did not answer the questionnaire), current or former smokers who reported no phlegm (397 participants), and current or former smokers who reported bringing up phlegm (434 participants). Panel C shows total mucin concentrations and spirometrically defined disease severity in controls who had never smoked (69 participants) and in current or former smokers with a Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage of 0 (indicating an increased risk of disease; 303 participants), 1 (indicating mild COPD; 165 participants), 2 (indicating moderate COPD; 293 participants), or 3 (indicating severe COPD; 85 participants). Panel D shows total mucin concentration and the prospective annualized exacerbation rate from enrollment until the end of the study (defined as the number of days until follow-up or death). Rates were classified as zero exacerbations per year (596 participants), more than zero but fewer than two exacerbations per year (262 participants), and two or more exacerbations per year (36 participants). In Panels B through D, the P values shown but not connected by a bracket are for the comparison between the designated group and the first group shown. Other significant differences between groups are shown with a bracket. The horizontal line in the boxes represents the median, the cross represents the mean, and the bottom and top of the boxes represent the 25th and 75th percentiles, respectively. I bars represent the upper adjacent value (75th percentile plus 1.5 times the interquartile range) and the lower adjacent value (25th percentile minus 1.5 times the interquartile range), and the dots outliers. Bar plots of the data in Panels B through D are provided in the Supplementary Appendix, available with the full text of this article at NEJM.org. All P values were adjusted for multiple comparisons with the use of the Tukey–Kramer method.



phenotyped cohort of the Subpopulations and Intermediate Outcome Measures in COPD Study (SPIROMICS). Associations between total mucin concentrations[12] and sputum production, sputum characteristics, and disease severity, as indexed by means of spirometry and according to exacerbation frequency, were tested. Potential etiologic pathways for high mucin concentrations, including cigarette smoking and asthma, were also explored. Because previous data suggested that overproduction of MUC5B is associated with COPD[13] and that overproduction of MUC5AC is associated with asthma,[14] absolute concentrations of MUC5AC and MUC5B were measured in a SPIROMICS subgroup and the relationships of each mucin to chronic bronchitis and asthma[15] were tested. Finally, the usefulness of total mucin concentrations in sputum as an objective biomarker for the diagnosis of chronic bronchitis was explored in SPIROMICS participants and participants in an independent cohort.

METHODS

STUDY DESIGN

SPIROMICS was an observational study that examined COPD in 2981 participants who were recruited into four strata: controls who had never smoked cigarettes, current or former cigarette smokers without airflow obstruction, current or former cigarette smokers with mild-to-moderate airflow obstruction, and current or former ciga-

rette smokers with severe airflow obstruction. Current or former smokers had a smoking history of more than 20 pack-years. Chronic bronchitis was diagnosed by means of questionnaires that were administered to participants: one questionnaire reflected the classic definition of the disorder[16] (cough that is present during most days or nights, is present for 3 consecutive months of the year for 2 consecutive years, and results in phlegm production), whereas the St. George's Respiratory Questionnaire (SGRQ)[17] defined chronic bronchitis as cough and phlegm almost every day or several times a week. Emphysema was defined by the percentage of voxels in the lung field that showed attenuation of less than −950 Hounsfield units, as assessed by volumetric multidetector-row computed tomography (CT) of the lungs. Using this percentage, we classified a participant as having emphysema on the basis of normative values.[18] Detailed clinical definitions are available in the Supplementary Appendix.

Sputum that was induced by inhalation of hypertonic saline was collected from 917 participants who had a forced expiratory volume in 1 second of more than 35% of the predicted value, according to the protocol[19] and American Thoracic Society and European Respiratory Society standards,[16] for measurement of total mucin concentrations. Induced sputum samples were placed in a buffer of guanidine (6 mol per liter),[12] shipped to the SPIROMICS Biospecimen Processing Center, and stored at −4°C. The SPIROMICS protocol was approved by the participating universities, and participants provided written informed consent. (More information about the study and how to access SPIROMICS data is available at www.spiromics.org.)

Data from a questionnaire reflecting the classic definition of chronic bronchitis and data on total mucin concentrations in induced sputum were also collected from a single-site, 94-participant cohort of persons who had never smoked and current or former smokers with or without chronic bronchitis. Sputum collection and measurement technologies were identical to SPIROMICS procedures.

MUCIN QUANTITATION

Total mucin concentrations in sputum were measured with the use of size-exclusion chromatography and differential refractometry. Sputum samples were injected into size-exclusion columns to isolate mucins, and effluents were passed through a laser photometer (DAWN HELEOS II, Wyatt Technology) that was coupled to a refractometer (Optilab T-rEX, Wyatt Technology). Data were analyzed with the use of ASTRA software, version 6.1.1.7 (Wyatt Technology). Sputum samples for mass-spectrometric parallel-reaction monitoring–based quantitation of MUC5B and MUC5AC were reduced, alkylated, and digested with trypsin, and six MUC5B and MUC5AC heavy-labeled peptide internal standards were spiked into sputum digests (details are provided in the Supplementary Appendix). Samples were subjected to targeted selected-ion monitoring–data-independent acquisition analysis with the use of a hybrid quadrupole Orbitrap mass spectrometer with a nanospray source (Q Exactive, Thermo Scientific). To assess salivary contamination, 86 samples were randomly selected from all groups, and salivary protein amylase levels were measured with the use of a label-free proteomics approach.[20]

STATISTICAL ANALYSIS

Statistical analyses were performed with the use of SAS software, version 9.4 (SAS Institute). Total mucin, MUC5B, and MUC5AC concentrations were normalized through natural-log transformations. Analyses of variance were used to test the effects of categorical variables on total mucin, MUC5B, and MUC5AC concentrations. Pairwise comparisons between individual groups were adjusted with the Tukey–Kramer method. No adjustments for multiple testing were made. All tests were two-sided, and a P value of 0.05 or less was considered to indicate statistical significance. Data are presented as box plots, with bar graphs presented in the Supplementary Appendix. The analysis of the usefulness of sputum mucin for the diagnosis of chronic bronchitis used receiver-operating-characteristic (ROC) curve techniques. Additional details about study design, clinical definitions, methods, and statistical analysis are provided in the Supplementary Appendix.

RESULTS

PARTICIPANTS

The selection of participants from the entire SPIROMICS cohort for measurement of total mucin concentration is described in Figure S1 in

Table 1. Characteristics of Total Mucin Concentration Study as Compared with the Entire SPIROMICS Cohort at Baseline.*

Variable	Total Mucin Concentration Study						Entire SPIROMICS Cohort (N=2770)
	Controls Who Never Smoked (N=69)	Current or Former Smokers†				Total (N=917)	
		GOLD Stage 0 (N=303)	GOLD Stage 1 (N=165)	GOLD Stage 2 (N=293)	GOLD Stage 3 (N=85)		
Age — yr	56.7±10.5	60.2±9.9	66.4±8.4	64.8±7.8	66.8±8.4	63.1±9.4	63.0±9.3
Male sex — no. (%)	32 (46)	167 (55)	109 (66)	177 (60)	46 (54)	533 (58.1)	1449 (52)
Current smoking — no. (%)‡	0	161 (53)	58 (35)	130 (44)	24 (28)	374 (40.8)	1055 (38)
Chronic bronchitis — no. (%)§	2 (3)	57 (19)	30 (18)	89 (30)	22 (26)	201 (21.9)	549 (20)
Emphysema — no. (%)¶	3 (4)	24 (8)	45 (27)	123 (42)	62 (73)	259 (28.2)	875 (32)
Current asthma — no./total no. (%)	0/65	29/279 (10.4)	26/144 (18.1)	47/254 (18.5)	11/75 (14.7)	113/819 (13.8)	401/2638 (15.2)
FEV_1 — % of predicted value	101.5±12.1	96.7±14.3	91.7±10.3	65.9±8.3	43.2±4.1	81.2±21.5	78.4±23.9

* Plus–minus values are means ±SD. FEV_1 denotes forced expiratory volume in 1 second, and SPIROMICS the Subpopulations and Intermediate Outcome Measures in COPD Study.

† Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage 0 is defined by a ratio of the FEV_1 to the forced vital capacity of more than 0.70 and a cigarette smoking–related increased risk of disease. GOLD stage 1, indicating mild disease, is defined as an FEV_1 that is greater than or equal to 80% of the patient's predicted value; GOLD stage 2, indicating moderate disease, as an FEV_1 that is greater than or equal to 50% and less than 80% of the predicted value; and GOLD stage 3, indicating severe disease, as an FEV_1 that is greater than or equal to 30% and less than 50% of the predicted value. One participant had GOLD stage 4, indicating very severe disease, and data on GOLD stage were not available for another participant.

‡ Current smoking indicates that the participant reported smoking for the 3 months before the baseline visit.

§ Chronic bronchitis was diagnosed on the basis of a questionnaire administered to participants that reflected the classic definition of the disorder: cough that is present during most days or nights, is present for 3 consecutive months of the year for 2 consecutive years, and that results in phlegm production.

¶ Emphysema was defined by the percentage of voxels in the lung field that showed attenuation of less than –950 Hounsfield units, as assessed by volumetric multidetector-row computed tomography of the lungs. The presence of emphysema was determined with the use of normative equations.[18]

the Supplementary Appendix. Characteristics of the 917 participants in whom total mucin concentration was measured are shown in Table 1, and Table S1A through S1C in the Supplementary Appendix. Characteristics of the subgroup of 148 participants in whom absolute concentrations of MUC5B and MUCAC were measured are shown in Table S1B and S1D in the Supplementary Appendix. Characteristics of the 94-participant, single-site cohort are shown in Table S1E in the Supplementary Appendix.

TOTAL MUCIN CONCENTRATIONS AND PHLEGM PRODUCTION AND QUALITY

We first tested the relationships predicted by the two-gel hypothesis between total mucin concentrations in sputum and phlegm production and properties (Fig. 1A). Total mucin concentrations in sputum were higher in current or former smokers in SPIROMICS who reported phlegm production than in controls who had never smoked or current or former smokers with no phlegm production (Fig. 1B), findings consistent with the two-gel hypothesis. The relationship between mucin concentration and phlegm production remained significant after adjustment for smoking status, asthma status, and frequency of respiratory exacerbations (Table S2 in the Supplementary Appendix). Associations were also observed between total mucin concentrations and the mucoid, gel-like appearance of sputum and the numbers of gel-like plugs in sputum (Fig. S3 in the Supplementary Appendix).

Salivary contamination of sputum can bias measurements of mucin concentration. However, mass-spectroscopic analyses of salivary amylase revealed no significant between-group differences in amylase values and no correlations between amylase levels and mucin levels (Fig. S4 in the Supplementary Appendix).

TOTAL MUCIN CONCENTRATIONS AND DISEASE STATUS

We next tested relationships predicted by the two-gel hypothesis between total mucin concentration and disease progression (i.e., increase in severity of chronic bronchitis). Total mucin concentrations were associated with airflow obstruction as indexed by the spirometric criteria of the Global Initiative for Chronic Obstructive Lung Disease (GOLD; www.goldcopd.org) (Fig. 1C). For example, the mean (±SE) total mucin concentration was 3166±402 μg per milliliter in current or former smokers with a GOLD stage of 3 (indicating severe COPD) and 1515±152 μg per milliliter in controls who had never smoked. The association between mucin concentrations and GOLD status–defined airflow obstruction remained significant when the use of inhaled bronchodilator and glucocorticoid treatments was included in analysis-of-variance models ($P=0.001$). Relationships between total mucin concentrations and prospective exacerbation frequencies revealed that mucin concentrations were higher in SPIROMICS participants who had exacerbations than in those who had no exacerbations (≥2 exacerbations per year, 4194±878 μg per milliliter; >0 to <2 exacerbations per year, 2848±171 μg per milliliter; and 0 exacerbations, 2458±113 μg per milliliter) (Fig. 1D). Similar associations were observed for exacerbations requiring interactions with health care providers (Fig. S5 in the Supplementary Appendix).

EFFECT OF EXPOSURE TO CIGARETTE SMOKE AND ASTHMA ON TOTAL MUCIN CONCENTRATIONS

Laboratory data suggest that exposure to cigarette smoke decreases the hydration of airway surfaces and stimulates mucin production, both of which are predicted to increase mucin concentrations (Fig. 1A).[11,21-23] In the SPIROMICS cohort, a history of cigarette use, both past and current, was significantly associated with total mucin concentration (Fig. 2A).

On the basis of reports that asthma is associated with mucin hypersecretion,[1,24] relationships between a diagnosis of current asthma and total mucin concentration were tested. Approximately 15% of the current or former smokers in the SPIROMICS cohort reported current asthma (Table S1C in the Supplementary Appendix), and those participants had higher total mucin concentrations than controls who had never smoked (Fig. 2B). The influence of asthma on total mucin concentration was maintained after adjustment for a history of cigarette use ($P=0.002$). Similar relationships were observed for a diagnosis of asthma in childhood (Fig. S7 in the Supplementary Appendix). The total mucin concentrations of SPIROMICS participants were not significantly associated with asthma biomarkers, including sputum eosinophils, blood eosin-

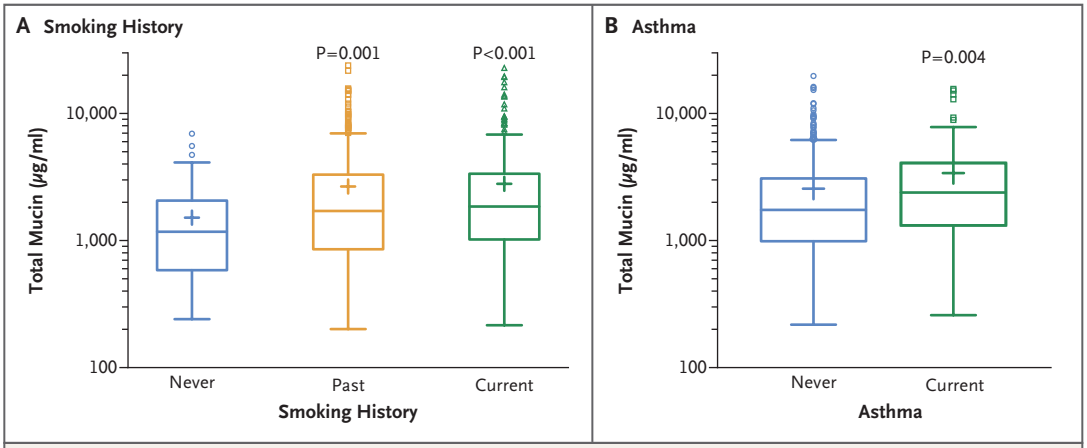


Figure 2. Association between Potential Etiologic Pathways and Total Mucin Concentrations in Sputum.

Panel A shows total mucin concentration and smoking history. Data are for 69 participants who had never smoked, 460 former smokers, and 374 current smokers. Panel B shows total mucin concentration and asthma status in participants with COPD (GOLD stages 1 through 3). Data are for 84 participants with current asthma and 389 participants who had never received a diagnosis of asthma. P values are for the comparison with participants who had never smoked or who had never received a diagnosis of asthma. The horizontal line in the boxes represents the median, the cross represents the mean, and the bottom and top of the boxes represent the 25th and 75th percentiles, respectively. I bars represent the upper adjacent value (75th percentile plus 1.5 times the interquartile range) and the lower adjacent value (25th percentile minus 1.5 times the interquartile range), and the dots outliers. All P values were adjusted for multiple comparisons with the use of the Tukey–Kramer method.

ophils, or blood IgE (Fig. S8 in the Supplementary Appendix).

SENSITIVITY ANALYSES BASED ON EXTREMES OF TOTAL MUCIN CONCENTRATIONS

We performed sensitivity analyses that examined current or former smokers with the lowest total mucin concentrations (lowest quartile), those with normal concentrations (interquartile range), and those with the highest concentrations (highest quartile). These analyses also showed that total mucin concentrations were related to phlegm production and disease severity (Table S3 in the Supplementary Appendix).

ABSOLUTE CONCENTRATIONS OF MUC5B AND MUC5AC

Two observations emerged from analyses of the contribution of the two secreted airway mucins to total mucin concentration. First, MUC5B was the dominant secreted mucin in control participants, with mean (±SE) concentrations approximately 10 times those of MUC5AC (108±20 vs. 10±4 pmol per milliliter) (Fig. 3A and 3B). Second, MUC5B concentrations increased proportionately with greater severity of COPD (by a factor of approximately 3, to 296±65 pmol per milliliter in current or former smokers with severe COPD), whereas MUC5AC concentrations increased disproportionately (by a factor of >10, to 108±31 pmol per milliliter in current or former smokers with severe COPD) (Fig. 3A and 3B). The mean (±SE) ratio of MUC5AC concentration to MUC5B concentration was significantly higher in current or former smokers with mild-to-moderate COPD than in controls who had never smoked (ratio, 0.5±0.1 vs. 0.1±0.04), but MUC5B remained the predominant mucin at all levels of disease severity (Fig. S10A in the Supplementary Appendix).

RELATIONSHIPS AMONG MUC5AC CONCENTRATIONS, CIGARETTE SMOKING, AND ASTHMA

To assess whether MUC5AC may be a biomarker for asthma in the population of cigarette smokers with COPD, relationships among cigarette smoking, asthma, and MUC5AC concentrations were investigated. The absolute concentration of MUC5AC and the ratio of MUC5AC concentration to MUC5B concentration were higher among current smokers than among controls who had never smoked (Fig. S10B and S10C in the Supplementary Appendix). This association persisted

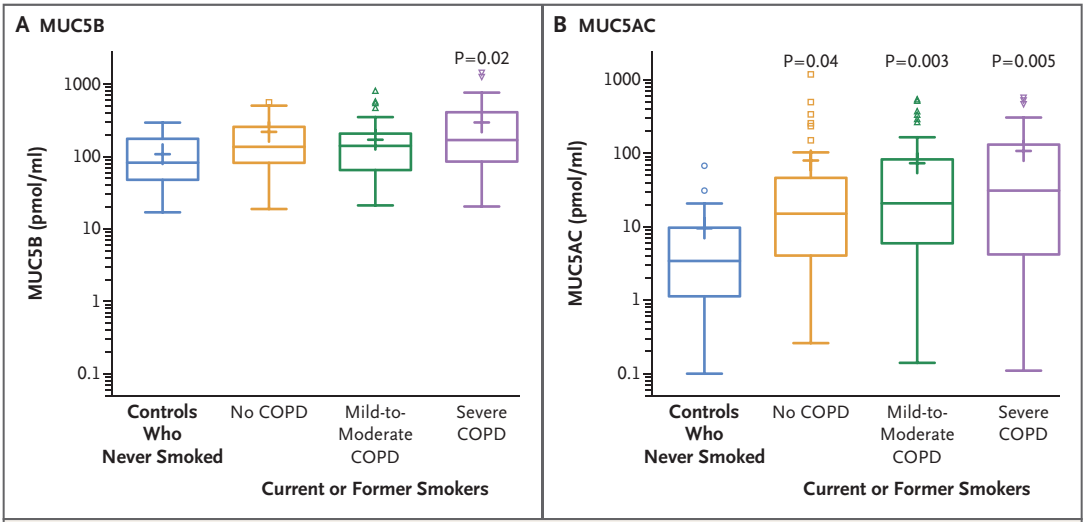


Figure 3. Absolute Concentrations of MUC5B and MUC5AC and Disease Severity.

Panels A and B show absolute concentrations of MUC5B and MUC5AC, respectively, in controls who had never smoked (19 participants), current or former smokers without spirometric evidence of COPD (42 participants), current or former smokers with mild-to-moderate COPD (59 participants), and current or former smokers with severe COPD (28 participants). P values are for the comparison with controls who had never smoked. The horizontal line in the boxes represents the median, the cross represents the mean, and the bottom and top of the boxes represent the 25th and 75th percentiles, respectively. I bars represent the upper adjacent value (75th percentile plus 1.5 times the interquartile range) and the lower adjacent value (25th percentile minus 1.5 times the interquartile range), and the dots outliers. All P values were adjusted for multiple comparisons with the use of the Tukey–Kramer method.

when participants with asthma were removed from the analysis (Fig. S10D in the Supplementary Appendix) and when asthma history was analyzed as a covariate ($P<0.001$ for MUC5AC and $P=0.001$ for the ratio of MUC5AC to MUC5B). In contrast, participants with current asthma or a childhood diagnosis of asthma did not have significantly higher MUC5AC concentrations than those who had never received a diagnosis of asthma (Fig. S10E in the Supplementary Appendix), and participants with high MUC5AC concentrations did not have significantly higher levels of sputum eosinophils or blood IgE than those with normal MUC5AC concentrations (Fig. S11 in the Supplementary Appendix).

RELATIONSHIPS AMONG MUCIN CONCENTRATIONS AND DIAGNOSIS OF CHRONIC BRONCHITIS

The observations suggesting that total mucin concentrations are linked to the pathophysiologic basis of chronic bronchitis raised the possibility that mucin concentrations may be an objective biomarker for chronic bronchitis. Total mucin concentrations in sputum were significantly higher in participants with either classically defined or SGRQ-defined chronic bronchitis than in those without chronic bronchitis (Fig. 4A), as were MUC5B and MUC5AC concentrations (Fig. S13 in the Supplementary Appendix).

The associations between mucin concentration and a diagnosis of chronic bronchitis were tested in additional analyses. First, on the basis of data indicating that mucins are produced by airways, not alveoli, the hypothesis that the chronic bronchitic component rather than the emphysematous component of COPD dominated total mucin concentrations in sputum was tested. Participants with classically defined or SGRQ-defined chronic bronchitis, with or without CT-defined emphysema, had higher mucin concentrations than participants without chronic bronchitis, with or without emphysema (Fig. S14 in the Supplementary Appendix). Second, a recent study showed that symptoms, including phlegm production, in smokers with normal findings on spirometry identified a population with a chronic bronchitis–like phenotype (e.g., respiratory exacerbations and activity limitation).[25] In our study, among

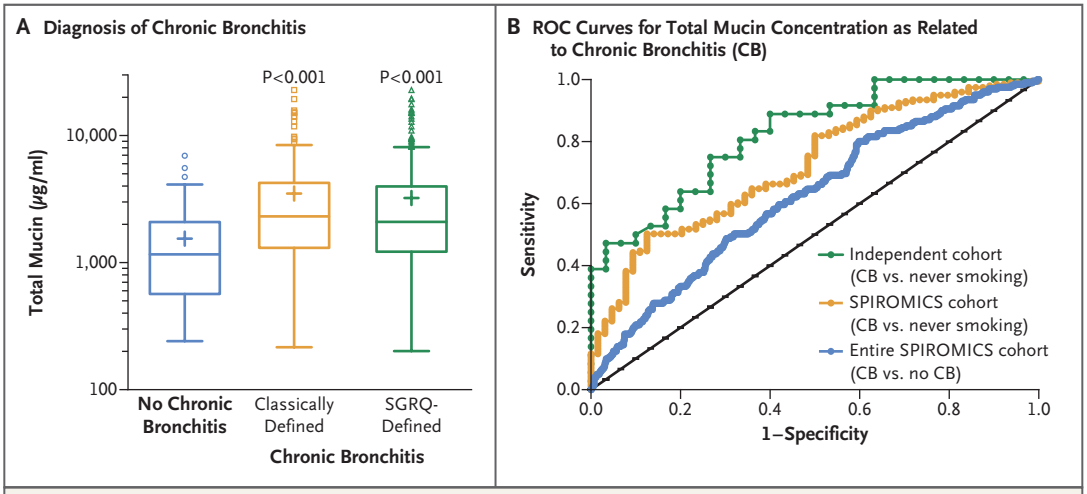


Figure 4. Total Mucin Concentration and Diagnosis of Chronic Bronchitis.

Panel A shows total mucin concentrations in current or former smokers who were identified as having chronic bronchitis by a questionnaire that reflected the classic definition of the disorder (199 participants), current or former smokers who were identified as having chronic bronchitis by the St. George's Respiratory Questionnaire (SGRQ) (382 participants), and controls who had never smoked and were not identified as having chronic bronchitis by either questionnaire (58 participants). P values are for the comparison with healthy controls who had never smoked. The horizontal line in the boxes represents the median, the cross represents the mean, and the bottom and top of the boxes represent the 25th and 75th percentiles, respectively. I bars represent the upper adjacent value (75th percentile plus 1.5 times the interquartile range) and the lower adjacent value (25th percentile minus 1.5 times the interquartile range), and the dots outliers. All P values were adjusted for multiple comparisons with the use of the Tukey–Kramer method. Panel B shows receiver-operating-characteristic (ROC) curves for total mucin concentration in participants with classically defined chronic bronchitis, as compared with controls who had never smoked, in the SPIROMICS cohort (orange curve; area under the curve [AUC], 0.72 [0.65 to 0.79]) and in the independent cohort (green curve; AUC, 0.82 [0.73 to 0.92]). The blue curve represents total mucin concentration in participants with classically defined chronic bronchitis, as compared with all participants without chronic bronchitis, in the entire SPIROMICS cohort (AUC, 0.62 [0.58 to 0.67]).

current or former smokers with normal spirometric values, those with symptoms had higher total mucin concentrations than those without symptoms (Fig. S15 in the Supplementary Appendix).

Finally, the sensitivity and specificity of total mucin concentration in sputum as a biomarker for chronic bronchitis were explored in both the SPIROMICS and independent cohorts. With the use of data on SPIROMICS participants with classically defined chronic bronchitis and healthy controls who had never smoked (Fig. 4A), the area under the ROC curve (AUC) for total mucin concentrations versus a diagnosis of classically defined chronic bronchitis was 0.72 (95% confidence interval [CI], 0.65 to 0.79) (Fig. 4B). Data on participants in the independent cohort (those with classically defined chronic bronchitis and healthy controls who had never smoked) yielded an AUC of 0.82 (95% CI, 0.73 to 0.92) (Fig. 4B). Similar relationships were observed when smokers without airflow obstruction were added to the control groups (Fig. S16 in the Supplementary Appendix). ROC analyses were also performed with respect to the entire SPIROMICS cohort (AUC, 0.62; 95% CI, 0.58 to 0.67) (Fig. 4B, and Fig. S17 in the Supplementary Appendix).

DISCUSSION

Chronic bronchitis is defined symptomatically by chronic mucus production and pathologically by airway inflammation, mucous-cell metaplasia and hyperplasia, and mucus plugging.[6,26] Clinically, the presence of the chronic-bronchitis phenotype (as compared with its absence) is associated with an accelerated loss of lung function and an increased frequency of COPD exacerbations.[3-5,27,28]

The two-gel hypothesis predicts that a high mucin concentration is the hallmark of the failed mucus transport and intrapulmonary mucus accumulation that are central to the pathogenesis of chronic bronchitis.[9] This hypothesis posits that there are two hydrogels on the airway surface (i.e., the mucus layer and the periciliary layer) that compete for hydration as a function of their relative osmotic pressures. Because concentrated mucins repel one another, the mucin contribution to osmotic pressure does not have a linear relationship with concentration but scales to more than the second power of concentration.[9] The periciliary layer is composed of membrane-tethered mucins and other glycoconjugates and, in healthy persons, is the more concentrated hydrogel and functions as a well-hydrated lubricant surface. In persons with chronic bronchitis, the concentration of secreted mucins in the mucus layer increases, reflecting increased mucin secretion, reduced hydration of airway surfaces, or both.[11,21,22] When mucus layer concentrations and osmotic pressures exceed those of the periciliary layer (i.e., the threshold value), the mucus layer compresses the periciliary layer and produces mucus stasis and adhesion.[10] Hence, we predicted that mucin concentration is the variable underlying the formation of the intraluminal mucus plaques that are expectorated with cough.

Data from the SPIROMICS cohort were consistent with this prediction. High total mucin concentrations were associated with both patient-reported phlegm production and the gel-like (mucoid) properties of the expectorated material. High total mucin concentrations were also associated with both the classic and the SGRQ definitions of chronic bronchitis, findings consistent with the inclusion of phlegm production in these definitions. High mucin concentrations were also associated with symptom-based evidence of airway disease (including phlegm) in the absence of airflow obstruction.[25]

The adherent intrapulmonary mucus that is not clearable by cough is predicted to contribute to airflow obstruction and exacerbation frequency in COPD (Fig. 1A). Increases in the degree of GOLD status–defined airflow obstruction were observed as a function of increased mucin concentrations in SPIROMICS participants. Increased mucin concentrations were also associated with increased prospective exacerbation frequencies. Because exacerbation frequency predicts the degree of airflow obstruction,[5] high total mucin concentrations may contribute to the loss of lung function in COPD through both intraluminal mucus obstruction and increased exacerbation frequencies.

The SPIROMICS cross-sectional sputum analyses allowed testing of associations, but not mechanistic links, between mucin concentration and the pathogenesis or severity of chronic bronchitis. It should be noted that previous studies involving participants with chronic bronchitis related an increase in mucus concentration to a decrease in mucociliary clearance, as predicted by the two-gel model.[29] The hypothesis that mucin concentration drives the pathogenesis of chronic bronchitis is also consistent with data from studies involving animal models, which have shown that dehydration of airway surfaces and mucin hyperconcentration produced the pathologic changes underlying COPD-like lung disease.[10] Consequently, we postulate that mucin concentration may quantitate the severity of a disease-causing pathway in chronic bronchitis and COPD.

The SPIROMICS data showed that having a history of cigarette use or having ever received a diagnosis of asthma was associated with high total mucin concentrations, findings consistent with previous in vitro, animal-model, and clinical studies.[14,21,30] Total mucin concentrations did not return to normal values in former smokers, a finding consistent with the persistence of airway inflammation after smoking cessation.[31,32] The association between asthma history and high total mucin concentrations suggests that mucin concentration may be a common pathway contributing to the worse outcomes in persons with the asthma–COPD overlap syndrome than in persons with either disease alone.[33]

With respect to the contributions of the two secreted mucins to disease pathogenesis, the increase in MUC5B concentration with disease severity is consistent with reports of MUC5B predominance in COPD.[13] However, the disproportionate increase in MUC5AC concentrations with disease severity was striking. The elevation in MUC5AC concentrations was dominated by

associations with cigarette-smoking status rather than asthma history or asthma biomarkers. This finding is consistent with a lower predictive power of asthma biomarkers in participants with asthma who smoke than in those who do not smoke[34] and the dominance in this population of non–type 2 helper T-cell pathways that regulate MUC5AC expression.[35] Practically, MUC5AC levels may not help define the asthma–COPD overlap syndrome in a SPIROMICS-like population with COPD.

The associations between total mucin concentration and the pathogenesis of chronic bronchitis suggest that this metric may be a quantitative biomarker for chronic bronchitis. Total mucin concentrations in sputum were associated with a diagnosis of chronic bronchitis based on each of the two types of self-report questionnaires: the questionnaire reflecting the classic definition of chronic bronchitis and the SGRQ. The ROC curves for total mucin concentration in SPIROMICS participants with a questionnaire-based diagnosis of chronic bronchitis versus control participants yielded a "fair" outcome (AUC, 0.72). A similar analysis performed in an independent, single-site cohort provided a "good" outcome (AUC, 0.82). The congruence of ROC outcomes argues for the usefulness of this measure, but the lower AUC for SPIROMICS participants suggests that variability among sites may be substantial. Future studies to test airway mucin concentrations as a diagnostic and prognostic biomarker for chronic bronchitis, as compared with questionnaire-based and airway biopsy–based metrics for the disease, appear to be warranted; such studies should include younger smokers at risk for COPD.[36]

Our results have therapeutic implications for chronic bronchitis. Higher total mucin concentrations (as compared with lower concentrations) were associated with asthma and exposure to cigarette smoke, suggesting that targeting upstream components of these pathways to inhibit MUC5B and MUC5AC production may be useful. Reports that exposure to hypertonic saline diluted mucin concentrations in sputum and accelerated mucus clearance in the short term among participants with chronic bronchitis suggest that therapies designed to hydrate airway surfaces and reduce mucin concentrations may also be effective.[21]

In conclusion, airway mucin concentrations describe a potential disease-causing, chronic-bronchitis pathway that is associated with sputum production and disease severity. If replicated, our results suggest that airway mucin concentrations may serve as a biomarker for the confirmation of the diagnosis of chronic bronchitis and the development of therapeutics for the disorder.

This SPIROMICS ancillary study is supported primarily by a grant (R01HL110906, to Dr. Kesimer) from the National Heart, Lung, and Blood Institute (NHLBI) of the National Institutes of Health under the NHLBI Ancillary Studies in Clinical Trials program. This study was also partially supported by grants R01HL103940, P50HL120100, and P01HL110808 from the NHLBI. This study used sputum samples and data collected through SPIROMICS, which was supported by contracts (HHSN268200900013C, HHSN268200900014C, HHSN268200900015C, HHSN268200900016C, HHSN268200900017C, HHSN268200900018C, HHSN268200900019C, and HHSN268200900020C) from the NHLBI; these contracts were supplemented by contributions made through the Foundation for the National Institutes of Health from AstraZeneca, Bellerophon Therapeutics, Boehringer Ingelheim Pharmaceuticals, Chiesi Farmaceutici, Forest Research Institute, GlaxoSmithKline, Grifols Therapeutics, Ikaria, Novartis Pharmaceuticals, Nycomed, Regeneron Pharmaceuticals, Sanofi, and Takeda Pharmaceutical.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank the SPIROMICS participants and participating physicians, investigators, and staff for making this research possible.

APPENDIX

The authors' affiliations are as follows: the Marsico Lung Institute, University of North Carolina at Chapel Hill, Chapel Hill (M.K., A.A.F., A.C., G.R., R.C., C.W.D., C.M.D., N.E.A., W.H.A., A.G.H., W.K.O., R.C.B.), and the Department of Internal Medicine, Wake Forest School of Medicine, Winston-Salem (E.R.B., A.T.H.) — both in North Carolina; the Department of Medicine, Columbia University Medical Center, and the Department of Epidemiology, Mailman School of Public Health at Columbia University (R.G.B.), and the Department of Medicine, Weill Cornell Medical College (F.M.), New York; the Division of Pulmonary, Critical Care, Allergy, and Sleep Medicine, Department of Medicine, University of California San Francisco Medical Center, San Francisco (S.A.C., P.G.W.); the Department of Medicine and Physiology, David Geffen School of Medicine, University of California, Los Angeles (C.B.C.); the Division of Pulmonary and Critical Care Medicine, University of Michigan Health System, Ann Arbor (M.K.H.); the Division of Pulmonary and Critical Care Medicine, Johns Hopkins University School of Medicine, Baltimore (N.N.H.); the Department of Radiology, Division of Physiologic Imaging, University of Iowa Hospitals and Clinics, Iowa City (E.A.H.); and the Department of Internal Medicine, Division of Pulmonary and Critical Care Medicine, University of Utah, Veterans Affairs Medical Center, Salt Lake City (R.E.K., R.P.).

REFERENCES

1. Fahy JV, Dickey BF. Airway mucus function and dysfunction. N Engl J Med 2010;363:2233-47.
2. Fletcher CM. The natural history of chronic bronchitis and emphysema: an eight-year study of early chronic obstructive lung disease in working men in London. Oxford, United Kingdom: Oxford University Press, 1976.
3. Kim V, Han MK, Vance GB, et al. The chronic bronchitic phenotype of COPD: an analysis of the COPDGene Study. Chest 2011;140:626-33.
4. Vestbo J, Prescott E, Lange P. Association of chronic mucus hypersecretion with FEV1 decline and chronic obstructive pulmonary disease morbidity. Am J Respir Crit Care Med 1996;153:1530-5.
5. Donaldson GC, Seemungal TA, Bhowmik A, Wedzicha JA. Relationship between exacerbation frequency and lung function decline in chronic obstructive pulmonary disease. Thorax 2002;57:847-52.
6. Hogg JC. Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. Lancet 2004;364:709-21.
7. Kesimer M, Kirkham S, Pickles RJ, et al. Tracheobronchial air-liquid interface cell culture: a model for innate mucosal defense of the upper airways? Am J Physiol Lung Cell Mol Physiol 2009;296:L92-L100.
8. Thornton DJ, Rousseau K, McGuckin MA. Structure and function of the polymeric mucins in airways mucus. Annu Rev Physiol 2008;70:459-86.
9. Button B, Cai LH, Ehre C, et al. A periciliary brush promotes the lung health by separating the mucus layer from airway epithelia. Science 2012;337:937-41.
10. Livraghi-Butrico A, Grubb BR, Wilkinson KJ, et al. Contribution of mucus concentration and secreted mucins Muc5ac and Muc5b to the pathogenesis of muco-obstructive lung disease. Mucosal Immunol 2017;10:395-407.
11. Rogers DF. Physiology of airway mucus secretion and pathophysiology of hypersecretion. Respir Care 2007;52:1134-46.
12. Henderson AG, Ehre C, Button B, et al. Cystic fibrosis airway secretions exhibit mucin hyperconcentration and increased osmotic pressure. J Clin Invest 2014;124:3047-60.
13. Kirkham S, Kolsum U, Rousseau K, Singh D, Vestbo J, Thornton DJ. MUC5B is the major mucin in the gel phase of sputum in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2008;178:1033-9.
14. Bonser LR, Zlock L, Finkbeiner W, Erle DJ. Epithelial tethering of MUC5AC-rich mucus impairs mucociliary transport in asthma. J Clin Invest 2016;126:2367-71.
15. Kumbhare S, Pleasants R, Ohar JA, Strange C. Characteristics and prevalence of asthma/chronic obstructive pulmonary disease overlap in the United States. Ann Am Thorac Soc 2016;13:803-10.
16. Ferris BG. Epidemiology Standardization Project (American Thoracic Society). Am Rev Respir Dis 1978;118:1-120.
17. Kim V, Crapo J, Zhao H, et al. Comparison between an alternative and the classic definition of chronic bronchitis in COPDGene. Ann Am Thorac Soc 2015;12:332-9.
18. Hoffman EA, Ahmed FS, Baumhauer H, et al. Variation in the percent of emphysema-like lung in a healthy, nonsmoking multiethnic sample: the MESA Lung Study. Ann Am Thorac Soc 2014;11:898-907.
19. Couper D, LaVange LM, Han M, et al. Design of the Subpopulations and Intermediate Outcomes in COPD Study (SPIROMICS). Thorax 2014;69:491-4.
20. Kesimer M, Cullen J, Cao R, et al. Excess secretion of gel-forming mucins and associated innate defense proteins with defective mucin un-packaging underpin gallbladder mucocele formation in dogs. PLoS One 2015;10(9):e0138988.
21. Clunes LA, Davies CM, Coakley RD, et al. Cigarette smoke exposure induces CFTR internalization and insolubility, leading to airway surface liquid dehydration. FASEB J 2012;26:533-45.
22. Dransfield MT, Wilhelm AM, Flanagan B, et al. Acquired cystic fibrosis transmembrane conductance regulator dysfunction in the lower airways in COPD. Chest 2013;144:498-506.
23. Borchers MT, Wert SE, Leikauf GD. Acrolein-induced MUC5ac expression in rat airways. Am J Physiol 1998;274:L573-L581.
24. Lachowicz-Scroggins ME, Yuan S, Kerr SC, et al. Abnormalities in MUC5AC and MUC5B protein in airway mucus in asthma. Am J Respir Crit Care Med 2016;194:1296-9.
25. Woodruff PG, Barr RG, Bleecker E, et al. Clinical significance of symptoms in smokers with preserved pulmonary function. N Engl J Med 2016;374:1811-21.
26. Hogg JC, Chu F, Utokaparch S, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. N Engl J Med 2004;350:2645-53.
27. Kim V, Zhao H, Boriek AM, et al. Persistent and newly developed chronic bronchitis are associated with worse outcomes in chronic obstructive pulmonary disease. Ann Am Thorac Soc 2016;13:1016-25.
28. Burge S, Wedzicha JA. COPD exacerbations: definitions and classifications. Eur Respir J Suppl 2003;41:46s-53s.
29. Anderson WH, Coakley RD, Button B, et al. The relationship of mucus concentration (hydration) to mucus osmotic pressure and transport in chronic bronchitis. Am J Respir Crit Care Med 2015;192:182-90.
30. Innes AL, Woodruff PG, Ferrando RE, et al. Epithelial mucin stores are increased in the large airways of smokers with airflow obstruction. Chest 2006;130:1102-8.
31. Lapperre TS, Postma DS, Gosman MM, et al. Relation between duration of smoking cessation and bronchial inflammation in COPD. Thorax 2006;61:115-21.
32. Willemse BW, ten Hacken NH, Rutgers B, Lesman-Leegte IG, Postma DS, Timens W. Effect of 1-year smoking cessation on airway inflammation in COPD and asymptomatic smokers. Eur Respir J 2005;26:835-45.
33. Postma DS, Rabe KF. The asthma–COPD overlap syndrome. N Engl J Med 2015;373:1241-9.
34. Gray T, Coakley R, Hirsh A, et al. Regulation of MUC5AC mucin secretion and airway surface liquid metabolism by IL-1beta in human bronchial epithelia. Am J Physiol Lung Cell Mol Physiol 2004;286:L320-L330.
35. O'Donnell RA, Richter A, Ward J, et al. Expression of ErbB receptors and mucins in the airways of long term current smokers. Thorax 2004;59:1032-40.
36. Allinson JP, Hardy R, Donaldson GC, Shaheen SO, Kuh D, Wedzicha JA. The presence of chronic mucus hypersecretion across adult life in relation to chronic obstructive pulmonary disease development. Am J Respir Crit Care Med 2016;193:662-72.