CHEST Online Supplement

Systemic Markers of Inflammation in Smokers With Symptoms Despite Preserved Spirometry in SPIROMICS

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e-Appendix 1.

Supplemental Methods:

Study Design and Participants

We obtained data from SPIROMICS, a prospective cohort study that enrolled 2,981 participants, ages 40-80 years old, across four strata (never smokers, smokers without COPD, mild/moderate COPD, and severe COPD)[1]. Ever smokers with a smoking history greater than 20 pack years, regardless of a prior diagnosis of obstructive lung disease, were recruited into the study. At baseline, all participants underwent spirometry before and following administration of albuterol and ipratropium and were then categorized using the GOLD staging system[2]. Smokers with preserved spirometry (n=830) were defined as current or former smokers with an FEV_1/FVC ratio greater than or equal to 0.70, and an FVC greater than or equal to the lower limit of normal. Participants with a concomitant asthma diagnosis were not excluded, however historical data regarding asthma diagnoses were collected, which we defined as "baseline asthma". Participants with unstable cardiac disease were excluded. We collected information on comorbid cardiovascular conditions including hypertension, coronary artery disease, congestive heart failure, valve disease, and vascular disease. We quantified symptom burden using the CAT, a validated eight-question health-status tool[3]. Consistent with our previous analyses, we categorized participants with a CAT score greater than or equal to ten as symptomatic, based on the GOLD system for symptom assessment[2,4]. We defined chronic bronchitis as self-reported cough and phlegm on most days for at least three months a year, over a period of two years or more[5]. All participants attempted to complete a six-minute walk test. We collected data on self-reported exacerbation history over the year prior to enrollment and prospective exacerbations over the study enrollment period. We defined exacerbations as the use of antibiotics, systemic glucocorticoids, and/or a health care utilization event (office visit, ER visit, or hospitalization) for a respiratory symptom "flare-up".

For this report we evaluated the subset of ever smokers who did not meet spirometric criteria for obstruction (n=830). We collected complete blood counts with differential and ELISA based measurements of serum IgE in these participants. Plasma protein biomarker levels of C-reactive protein (CRP), fibrinogen, and the two soluble TNF receptors (sTNFRSF1A [also known as sTNF-R55], sTNFRSF1B [also known as sTNF-R75]) were obtained in a subset of samples using luminex-based biomarker multiplex assays (n=429)[6,7]. Participants also underwent a sputum induction with nebulized saline, and sputum samples were processed locally and cell counts read centrally (University of North Carolina, Chapel Hill). We excluded sputum samples with a total leukocyte count of 100 or lower and those with greater than 80% squamous epithelial cells. The study protocol was approved by the institutional review boards (IRB) of all participating sites. All participants gave written informed consent.

e-Table 1: IRB documentation for SPIROMICS

Study center	IRB Committee name	Project approval #
Columbia University Medical Center	Columbia University Medical Center IRB	IRB-AAAE9315
Johns Hopkins University	Johns Hopkins Medicine Institutional Review Boards (JHM IRB)	NA_00035701 / CIR00004922
National Jewish Health	National Jewish IRB	HS-2678
Temple University	Temple University Office for Human Subjects Protections Institutional Review Board	21416
University of Alabama at Birmingham	The University of Alabama at Birmingham Institutional Review Board for Human Use	F120906004
University of California, Los Angeles	UCLA Office of the Human Research Protection Program	10-001740-CR-00004
University of California, San Francisco	UCSF Human Research Protection Program, Committee on Human Research	10-03169
University of Illinois at Chicago	UIC Office for the Protection of Research Subjects (OPRS)	2013-0939
University of Iowa	The University of Iowa Human Subjects Office/Institutional Review Board (IRB)	201308719
University of Michigan	Medical School Institutional Review Board (IRBMED)	HUM00036346
University of North Carolina at Chapel Hill	UNC-CH Office of Human Research Ethics (OHRE) Non-Biomedical IRB	10-0048
University of Utah	The University of Utah Institutional Review Board	00027298
Wake Forest University	Wake Forest University Health Sciences Office of Research Institutional Review Board	IRB00012805

Biomarker assays and normalization procedure

Included biomarkers had values above the lower limit of detection in at least 99% of the samples. We considered including interleukin (IL)-6, IL-8 and TNF-α in these analyses as additional markers of systemic inflammation classically associated with COPD[8], however greater than 40% of samples were below the lower limit of detection for these markers, failing our a-priori cutoff for acceptable biomarker quality. To limit the effect of outliers we "winsorized" the raw data (data points below the 0.3rd percentile were set at the 0.3rd percentile and data points above the 99.7th percentile were set at the 99.7th percentile)[9]. Protein analytes and cell differential counts were then log transformed and normalized by z-score. For sputum neutrophil, sputum eosinophil, and blood eosinophil count data, a value of one was added to all data points before applying log transformations.

Statistical Analyses

We compared demographic and clinical characteristics across groups using t-tests and chi-square tests as appropriate. We fit logistic regression models to assess the relationships of biomarker levels with symptom status and chronic bronchitis. We used Wilcoxon rank sum tests to evaluate raw marker values. We fit negative binomial regression models to evaluate the relationship between biomarker levels and exacerbation count. Prospective exacerbations over the entire study period were modeled with an offset term of the natural log of total follow-up time to accommodate for variable follow-up time across participants. We fit linear regression models to relate inflammatory marker levels to six-minute walk test distance (6MWD) and post-BD percent of predicted FEV1. Adjusted multivariate models were fit including age, gender, BMI, race, current smoking status, smoking pack years, baseline asthma history, study site, and experimental batch (for assays run in batches) as covariates. We used an ANOVA to evaluate differences in CRP between study strata, and Tukey-Kramer tests to evaluate pairwise comparisons. We compared baseline CRP measurements to one-year follow-up measurements using Pearson correlation and Bland-Altman analyses. We defined statistical significance as a p-value below 0.05. False discovery rate (FDR) adjusted p-values were calculated using the Benjamini-Hochberg procedure[10]. All analyses were performed using R 3.4.3.

e-Table 2: Clinical and demographic characteristics of study participants stratified by exacerbation history

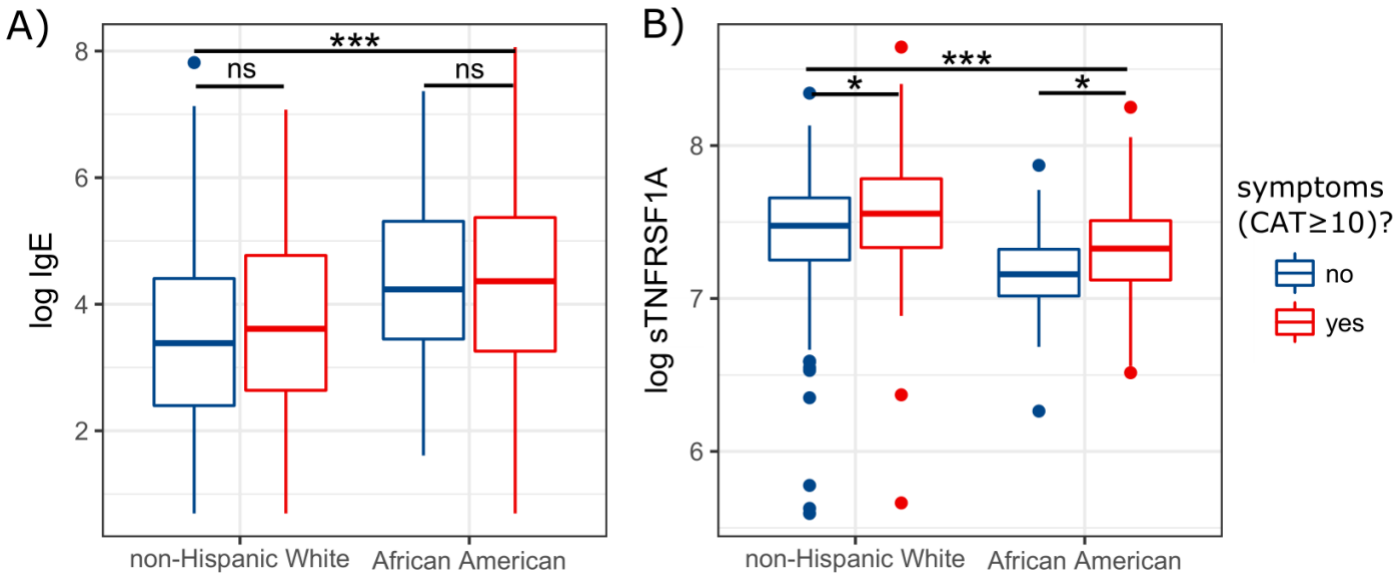
	Smokers with FEV1/FVC≥0.7 (GOLD 0)		
	No exacerbations in past 365 days n = 715	**≥1 exacerbation(s) in past 365 days n = 109**	**p-value**
Age	60 ± 9.7	58 ± 9.2	**0.007**
BMI	29 ± 5.1	30 ± 4.6	**0.003**
Female	358 (50%)	77 (71%)	**<0.001**
Race			
Caucasian	493 (68%)	60 (55%)	**0.003**
African American	185 (26%)	45 (41%)	
Other	43 (6%)	4 (4%)	
Current smokers	368 (52%)	59 (55%)	0.55
Smoking pack-years	43 ± 26	43 ± 19	0.93
History of asthma	83 (12%)	49 (63%)	**<0.001**
History of childhood asthma	32 (5%)	22 (22%)	**<0.001**
CAT Score ≥ 10	327 (46%)	88 (81%)	**<0.001**
Chronic bronchitis	111 (16%)	38 (37%)	**<0.001**
Post-BD FEV_1 % predicted	98 ± 13	97 ± 12	0.25
FEV_1/FVC	0.77 ± 0.05	0.78 ± 0.05	0.70
% BD reversibility	6.3 ± 6.6	8.1 ± 8.4	**0.03**

CAT = COPD Assessment Test, BMI = Body Mass Index, Post-BD FEV_1 = Post, bronchodilator Forced Expiratory Volume in 1 Second, FVC = Forced Vital Capacity, % BD Reversibility = Percentage improvement in FEV_1 following bronchodilator administration. Participants with and without symptoms were compared using t-tests for continuous variables and chi-square tests for categorical variables, as appropriate. Data are reported as mean ± standard deviation or n (%).

e-Table 3: Associations between inflammatory markers and functional measures of disease severity

		Unadjusted Model		Adjusted Model	
	Parameter	**Coefficient [95% CI]**	**p-value**	**Coefficient [95% CI]**	**p-value**
Six-minute walk test distance (meters)	**CRP**	**-16.1 [-24.2, -8.0]**	**<0.001****	**-11.4 [-19.7, -3.1]**	**0.007****
	sTNFRSF1A	**-16.2 [-24.8, -7.5]**	**<0.001****	**-12.2 [-21, -3.4]**	**0.007****
Post-bronchodilator FEV_1 % predicted	**CRP**	-1.99 [-3.14, -0.84]	**0.001****	-1.21 [-2.54, 0.12]	0.07
	sTNFRSF1A	-1.57 [-2.81, -0.34]	**0.01****	-1.15 [-2.56, 0.25]	0.11

CRP = C-reactive protein, sTNFRSF = soluble tumor necrosis factor super family, FEV_1 = forced expiratory volume in 1 second. * = FDR <0.1, **=FDR<0.05



e-Figure 1: IgE and sTNFRSF1A stratified by race | A) African American participants had higher levels of IgE, however IgE was not significantly different between symptom groups when stratified by race. B) African American participants had overall lower levels of sTNFRSF1a compared to non-Hispanic White participants, however in both groups, the symptomatic participants had significantly higher levels of sTNFRSF1A compared to participants without symptoms. ***=p<0.001, *=p<0.05, ns=non-significant, CAT = COPD assessment test.

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