SUPPLEMENTAL METHODS

Study Design and Setting

This is a cross-sectional analysis of the National Health and Nutrition Examination Survey (NHANES) data 2007-2008, 2009-2010, and 2011-2012. The NHANES is a national survey that collects biologic samples on a representative sample of the U.S. population, provided by the National Center for Health Statistics at the Centers for Disease Control and Prevention.[1]

Study Population

In this analysis, we identified all subjects aged ≥40 years with COPD, defined as a post-bronchodilator $FEV_1/FVC < 0.70$ with a history of smoking ≥100 cigarettes total in their life and at least one of following symptoms: cough, sputum production, wheezing, and shortness of breath on exertion.[2] According to the literature,[3] we defined asthma-COPD overlap syndrome (ACOS) as self-reported wheezing in past 12 months plus bronchodilator response (FVC increase of >200 ml and >12%) or self-reported physician diagnosis of asthma.

In the sensitivity analysis, as there is currently no standardized and validated definition of ACOS, we also analyzed the data by using an alternative definition of ACOS – defined by the presence of one major criterion or two minor criteria.[4] Major criteria were 1) history of asthma and 2) ≥15% and ≥400 ml of bronchodilator reversibility; minor criteria were 1) history of hay fever, 2) a bronchodilator response to salbutamol of ≥12% and 200 ml, and 3) blood eosinophils ≥5%.

Outcome and measurements

The primary outcome was fractional exhaled nitric oxide (FeNO) level (ppb). FeNO was

measured according to the NHANES protocol,[5] with the use of Aerocrine NIOX MINO ® (Aerocrine AB, Solna, Sweden). Subjects with a prohibitive medical condition (e.g., current chest pain, a physical problem with forceful expiration, current use of supplementary oxygen) were excluded from the FeNO measurement. The NHANES protocol required two reproducible FeNO measurements, in accordance with testing procedures recommended by the manufacturer and similar to those published by the American Thoracic Society (ATS) and the European Respiratory Society (ERS).[6] A reproducible measurement was defined as either <30 ppb and within 2ppb of each other, or >30 ppb and within 10% of each other. If the reproducibility criteria were not met within the first two exhalations, a participant had two additional exhalations to satisfy the criteria (up to a total of four trials).

Comorbidities were ascertained through the NHANES questions asking, "has a doctor or other health professional ever told you that you have [disease]?" Thus, comorbidities including coronary heart diseases (coronary heart disease, angina, heart attack, and heart failure), hypertension, diabetes, and renal failure were defined as a positive response to the each corresponding question.

Spirometry was conducted according to the ATS recommendations.[7] Participants who met the following criteria were excluded from spirometry for safety reasons: current chest pain or pain with forceful expiration; current use of daytime supplemental oxygen; had recent surgery of the eye, chest or abdomen; or had a recent heart attack, stroke, tuberculosis exposure, hemoptysis, a history of detached retina or pneumothorax. Participants who underwent a "baseline" (pre-bronchodilator) spirometry were selected for follow-up "post-bronchodilator" spirometry if their baseline spirometry values indicated possible airflow obstruction.[2,8,9] Possible airflow obstruction was defined as a pre-bronchodilator FEV_1/FVC ratio less than the lower limit

of normal representing the lower 5th percentile based on person's age, sex, height, and race/ethnicity.[2,8,9] In the post-bronchodilator assessment, participants who met following criteria were excluded due to safety reasons: active cardiovascular disease (uncontrolled blood pressure, irregular pulse on examination, taking medication for major arrhythmia, having an implanted defibrillator, or history of congenital heart disease) or taking certain prescription medications (a monoamine oxidase inhibitor, an anticonvulsant, a tricyclic antidepressant plus current treatment for cardiac disease, or potassium lowering drugs). Participants were also excluded from bronchodilator administration if they had recently taken a β_2-agnoist bronchodilator to avoid exceeding FDA recommended doses, or if they had had a previous adverse reaction to albuterol. GOLD stage was defined based on post-bronchodilator spirometry parameters (i.e., FEV_1/FVC and FEV_1 percent of predicted).[2] Predicted FEV_1 was calculated based on sex, age, and height according to a previous literature.[9]

REFERENCES

1. National Health and Nutrition Examination Survey. Centers for Disease Control and Prevention. http://www.cdc.gov/nchs/nhanes.htm. Accessed June 10, 2016.
2. Global Strategy for Diagnosis, Management, and Prevention of COPD. 2016; http://goldcopd.org/global-strategy-diagnosis-management-prevention-copd-2016/ Accessed June 10, 2016.
3. Menezes AM, Montes de Oca M, Perez-Padilla R, et al. Increased risk of exacerbation and hospitalization in subjects with an overlap phenotype: COPD-asthma. *Chest.* 2014;145(2):297-304.
4. Cosio BG, Soriano JB, Lopez-Campos JL, et al. Defining the Asthma-COPD overlap syndrome in a COPD cohort. *Chest.* 2015.
5. See KC, Christiani DC. Normal values and thresholds for the clinical interpretation of exhaled nitric oxide levels in the US general population: results from the National Health and Nutrition Examination Survey 2007-2010. *Chest.* 2013;143(1):107-116.
6. American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med.* 2005;171(8):912-930.

7. Standardization of Spirometry, 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med.* 1995;152(3):1107-1136.
8. Pellegrino R, Viegi G, Brusasco V, et al. Interpretative strategies for lung function tests. *Eur Respir J.* 2005;26(5):948-968.
9. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med.* 1999;159(1):179-187.

Supplemental Table 1. Characteristics in subjects with asthma-COPD overlap syndrome (alternative definition) and those with chronic obstructive pulmonary disease alone

Characteristics[a]	ACOS	COPD	P value
Unweighted sample, n	53	144	
Weighted estimate, n	777,935	2,049,856	
Participants' demographics			
Age (year), mean (SE)	56 (1.3)	58 (1.2)	0.26
Male sex	70 (49-85)	68 (60-75)	0.83
Race/ethnicity			0.39
Non-Hispanic white	87 (76-93)	91 (86-95)	
Non-Hispanic black	8 (4-16)	5 (3-9)	
Hispanics	5 (2-16)	2 (1-6)	
Others	0 (0-2)	1 (0-3)	
Primary health insurance			0.93
Medicare	9 (4-18)	13 (8-20)	
Medicaid	3 (1-10)	3 (1-5)	
Private	71 (52-85)	69 (59-77)	
No insurance	4 (1-14)	5 (2-10)	
Others	13 (5-31)	11 (6-20)	
Medical history			
Body mass index (kg/m^2), mean (SE)	29.8 (0.9)	27.1 (0.5)	0.03
Smoking status			

Current smoker	46 (32-62)	61 (48-71)	0.21
Pack-years, mean (SE)	30 (3.5)	37 (2.5)	0.11
≥10 pack-years	74 (57-86)	86 (76-92)	0.22
Symptoms			
Cough	30 (16-49)	38 (30-47)	0.47
Sputum	31 (17-49)	32 (23-43)	0.86
Wheezing	68 (46-84)	35 (27-45)	0.01
Shortness of breath on stairs/inclines	65 (48-79)	69 (59-77)	0.68
Comorbidities			
Coronary heart diseases[b]	18 (8-36)	13 (8-20)	0.47
Hypertension	40 (25-57)	40 (29-51)	0.99
Diabetes	9 (4-21)	12 (7-18)	0.54
Renal failure	8 (2-33)	2 (1-7)	0.36
Healthcare utilization			
Having a routine place for health care	94 (84-98)	93 (88-96)	0.83
Number of healthcare visits in the past year, mean (SE)	2 (0.2)	2 (0.2)	0.80
Number of healthcare visits for wheezing in the past year, mean (SE)	1 (0.6)	0.4 (0.1)	0.20
Overnight hospital stay in the past year	8 (3-20)	15 (9-24)	0.18
Oral steroid	1 (0-6)	3 (0-18)	0.52
Inhaled corticosteroids	0 (0-0)	4 (1-16)	0.19

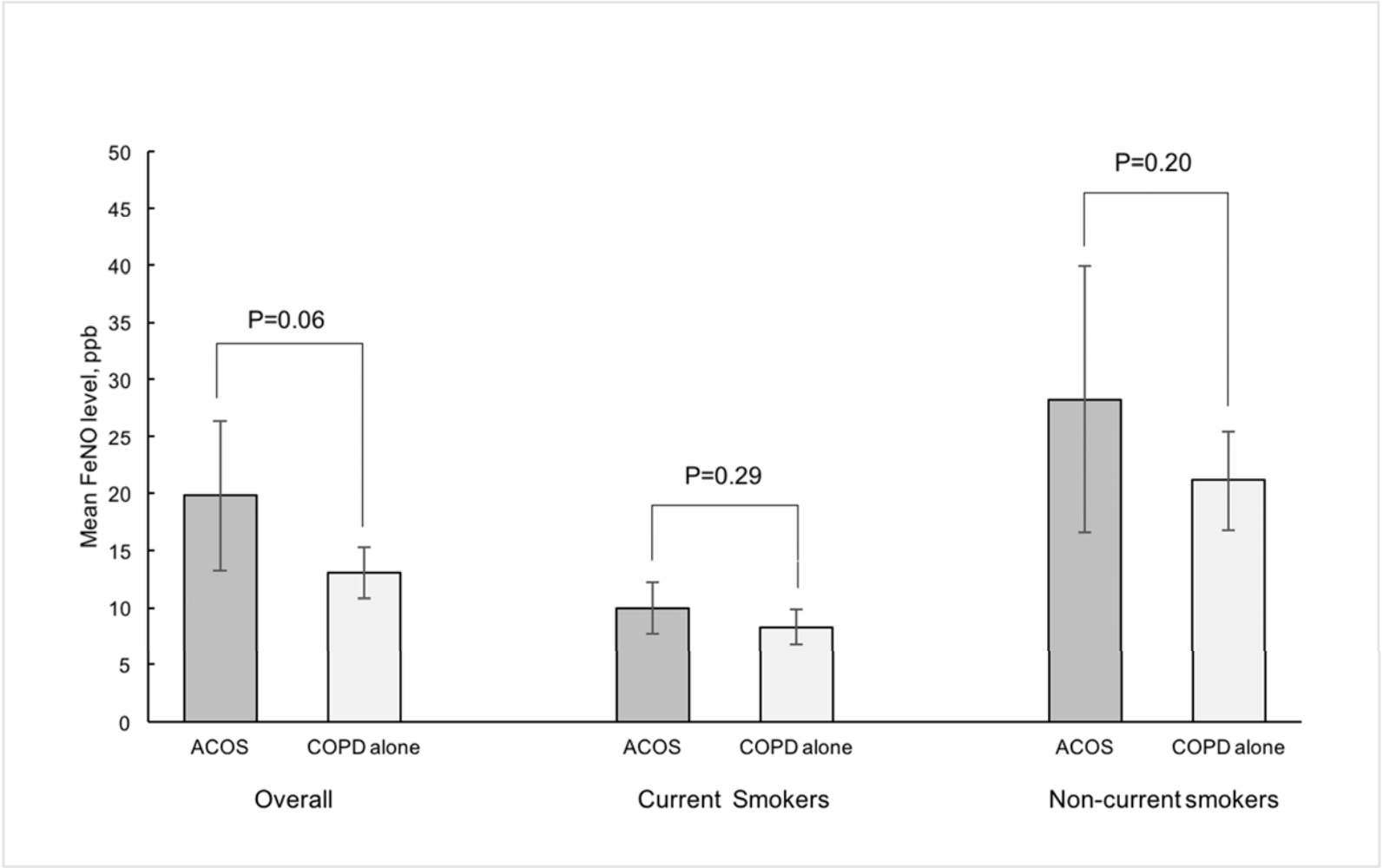
Laboratory tests			
White blood cell count (cells/μl), mean (SE)			
Total	7175 (261)	8074 (174)	0.01
Neutrophils	4334 (229)	5008 (136)	0.02
Lymphocytes	1958 (80)	2205 (83)	0.03
Eosinophils	293 (39)	226 (13)	0.13
Spirometry, mean (SE)			
Baseline FVC (L)	4.0 (0.2)	4.0 (0.1)	0.97
Baseline FEV_1 (L)	2.4 (0.1)	2.4 (0.1)	0.75
Baseline PEF (L/min)	388 (22)	375 (11)	0.58
Baseline FEV_1 % predicted	59 (56-63)	60 (56-64)	0.85
Baseline FEV_1/FVC %	60 (59-62)	59 (57-61)	0.30
Post-bronchodilator FVC (L)	4.2 (0.2)	4.0 (0.1)	0.52
Post-bronchodilator FEV_1 (L)	2.6 (0.1)	2.5 (0.1)	0.35
Post-bronchodilator PEF (L/min)	429 (26)	401 (12)	0.34
Post-bronchodilator FEV_1 % predicted	64 (60-68)	63 (59-66)	0.59
Post-bronchodilator FEV_1/FVC %	62 (60-65)	61 (59-63)	0.32
GOLD stage			0.03
I	2 (0-14)	17 (11-27)	
II	86 (76-92)	61 (48-73)	
III	12 (6-24)	20 (12-32)	
IV	0 (0-0)	1 (0-7)	

Abbreviations: FVC, forced expiratory volume; FEV_1, forced vital capacity per 1 second; PEF, peak flow; GOLD, the Global initiative of chronic Obstructive Lung Disease.

[a] Data are expressed as % (95% confidence interval) unless otherwise indicated

[b] Coronary heart diseases include ischemic heart disease, angina, myocardial infarction, and heart failure.

Supplemental Figure 1.



Supplemental Figure 2.