Original Article

Obstructive Sleep Apnea Risk, Asthma Burden, and Lower Airway Inflammation in Adults in the Severe Asthma Research Program (SARP) II

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What is already known about this topic? Obstructive sleep apnea (OSA) and asthma may be associated, but large studies in well-phenotyped subjects are lacking, and mechanisms are unknown. We tested this association and mechanisms in a subset of the Severe Asthma Research Program.

What does this article add to our knowledge? Our study shows that OSA risk was associated with worse indices of asthma control, increased health care utilization, and worse quality of life. Furthermore, OSA risk was associated with neutrophilic airway inflammation.

How does this study impact current management guidelines? Evaluating and treating OSA independent of obesity could be one new approach to include in clinical asthma programs.

BACKGROUND: Obstructive sleep apnea (OSA) may worsen asthma, but large studies are lacking and the underlying mechanisms are unknown.

OBJECTIVE: The objective of this study was to determine the prevalence of OSA risk among patients with asthma of different severity compared with normal controls (NC), and among asthmatics, to test the relationship of OSA risk with asthma burden and airway inflammation.

METHODS: Subjects with severe (SA, n = 94) and nonsevere asthma (NSA, n = 161), and NC (n = 146) were recruited in an add-on substudy, to the observational Severe Asthma Research Program (SARP) II; subjects completed sleep quality, sleepiness and OSA risk (Sleep Apnea scale of the Sleep Disorders Questionnaire [SA-SDQ]) questionnaires, and clinical assessments. Sputum was induced in a subset of asthmatics.

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Abbreviations used
AQLQ- Asthma Quality of Life Questionnaire
BMI- Body mass index (in kilograms per meter squared)
CI- Confidence interval (95% reported)
CPAP- Continuous positive airway pressure
ESS- Epworth Sleepiness Scale
FEV_1- Forced expiratory volume in first second of the forced vital capacity maneuver
FVC- Forced vital capacity
MDI- Metered dose inhaler
OSA- Obstructive sleep apnea
PC_{20}- Provocative concentration of methacholine, necessary to produce a 20% fall in FEV_1
PEF- Peak expiratory flow
PMNs- Polymorphonuclear neutrophils
PSG- Polysomnography (sleep study)
PSQI- Pittsburgh Sleep Quality Index
SAS- Statistical Analysis Software
SA-SDQ- Sleep Apnea scale of the Sleep Disorders Questionnaire
SD- Standard deviation

RESULTS: Relative to NC, despite similar sleep duration, the subjects with SA and NSA had worse sleep quality, were sleepier, and had higher SA-SDQ scores. Among asthmatics, higher SA-SDQ was associated with increased asthma symptoms, β-agonist use, health care utilization, and worse asthma quality of life. A significant association of SA-SDQ with sputum polymorphonuclear cells% was noted: each increase in SA-SDQ by its standard deviation (6.85 units) was associated with a rise in % sputum neutrophils of 7.78 (95% CI 2.33-13.22, $P = .0006$), independent of obesity and other confounders.
CONCLUSIONS: OSA symptoms are more prevalent among asthmatics, in whom they are associated with higher disease burden. OSA risk is associated with a neutrophilic airway inflammation in asthma, which suggests that OSA may be an important contributor to the neutrophilic asthma. Further studies are necessary to confirm these findings and better understand the mechanistic underpinnings of this relationship. Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2015;3:566-75)

Key words: *Asthma; Sleep apnea; Obstructive; Airway inflammation*

Asthma ranks among the most common chronic medical conditions and poses an increased health care burden. In the United States, the prevalence of asthma has risen from 7.3% (in 2001) to 8.2% (in 2011).[1] The health care costs associated with asthma are significant, and they correlate strongly with disease severity.[2] Despite significant progress in research and improved therapies, up to 75% of asthmatics do not achieve disease control and continue to be impaired in their quality of life.[1,3] For many, no apparent causes are found and optimized therapies remain inadequate.[4] These observations highlight the need for new interventions.

Obstructive sleep apnea (OSA) is another frequent breathing disorder[5] involving the upper airway, and is often associated with asthma. There is accumulating evidence for etiologic interactions between the 2 conditions, apart from mere coexistence due to their common occurrence.[6] On one hand, studies consistently report higher prevalence of OSA symptoms,[7-9] or polysomnography (PSG)-diagnosed OSA in asthmatic patients[10,11] in relationship with asthma severity.[10] Conversely, OSA appears to worsen asthma. OSA risk is associated with poor asthma control,[12] both during the day and at night.[13] Moreover, treatment for OSA improves asthma outcomes, such as symptoms,[14-16] bronchodilator use,[14] peak expiratory flow (PEF) rates,[14] and disease-specific quality of life.[17] Last, OSA is a risk factor for frequent exacerbations in difficult-to-control asthma.[18]

Among several plausible pathways put forth to explain the aggravation of asthma by OSA is that of inflammation.[6,13] In OSA, a neutrophil-predominant inflammation starts in the nose[19] and extends to the lower airways,[20,21] where it correlates with disease severity.[20] Underlying clinical asthma involves a complex inflammatory milieu that leads to bronchial reactivity, mucus secretion, and remodeling of the airway walls.[22] A variety of cells, including dendritic, eosinophils, mastocytes, and neutrophils, are involved. Traditionally, eosinophils have been thought to play the most prominent role because of their intense infiltration of asthmatic airways and because they release a variety of potent inflammatory mediators.[23] There is emerging evidence, however, that asthma is heterogeneous in its inflammatory cellular profiles.[24,25] Recent studies demonstrate that up to 60% of asthmatics who have persistent symptoms have a noneosinophilic, neutrophil-rich type of asthma[26] that responds poorly to standard therapies.[27,28] Whether neutrophils are attracted to the asthmatic airway as a result of smoking, obesity, or corticosteroid use remains unclear.[24,27,29] Nonetheless, the similarities discussed above give rise to the question: can OSA be an unrecognized contributor to the airway neutrophilia in asthma?

Although the aforementioned investigations represent important contributions to a nascent field, studies of OSA prevalence in large samples of subjects who have a wide range of baseline characteristics and different degree of asthma severity are lacking. In addition, the broad impact of OSA on the burden of asthma disease control and health care use, as well as underlying mechanisms, remains to be studied. The aims of the present study were to: (1) determine the prevalence of OSA symptoms among a large sample of patients with asthma who were well phenotyped for disease severity, relative to normal subjects; and (2) among asthmatics, to examine the relationships of OSA symptoms with asthma disease burden and the type of lower airway inflammation. Preliminary results have been published in abstract form.[30]

METHODS

This study began in October 2007, as an add-on substudy to the established and ongoing at the time Severe Asthma Research Program (SARP) II protocol,[31] which had been enrolling subjects since January 2007.

Subjects

Subjects with severe (SA) or nonsevere asthma (NSA) and normal controls (NC) were enrolled. Details on subject recruitment and group assignment are described in this article's Online Repository at www.jaci-inpractice.org. Of note, as part of the parent SARP study, at some of the sites, subjects with a clinical history of OSA or positive airway pressure (PAP) use were not enrolled. Each institutional review board approved the study and all subjects signed informed consent.

Of the 474 SARP subjects who completed the sleep questionnaires, 61 were excluded because they were younger than 18 years of age. Among the remaining 413 subjects, 28 had been diagnosed with

OSA. Because PAP treatment for OSA could influence OSA symptoms and asthma outcomes,[8,12-17] and confound the results of our analysis, the 12 subjects with OSA who were using PAP (4 in each group, ie, 3% of NC, 2% of NSA, and 4% of SA, $P = .75$) were excluded from further analyses.[8,12,13,32] Thus, our final sample included a total of 401 subjects (146 NC, 161 NSA, and 94 SA). Not all assessments were performed in all subjects; the numbers of subjects contributing to the analysis are presented with the respective variable in Table I. Of 132 NC subjects who underwent methacholine challenge, a provocative concentration of methacholine necessary to produce a 20% fall in FEV_1 (PC_{20}) could not be estimated in 125; the data from these subjects were not included when testing for group differences in baseline variables.

Clinical questionnaires

Clinical SARP-specific and validated questionnaires were administered. The SARP-specific instruments,[33] administered by study coordinators, included general information on demographics, smoking, asthma symptoms, medical history, including health care utilization (past year and lifelong) due to asthma, comorbidities, and medication history. The symptoms questions assessed cough, wheezing, chest tightness, shortness of breath, and nighttime asthma (waking from sleep, use of albuterol, early morning chest tightness) symptoms in the prior 3 months, on a scale 1-6 ranging from never to at least twice a day. Inhaled (via metered dose inhaler [MDI] or nebulized) rescue β-agonist use in the prior 3 months was ascertained on an identical scale.

The validated, self-administered questionnaires included: (1) the Sleep Apnea scale of the Sleep Disorders Questionnaire (SA-SDQ), which assesses OSA risk[34]; (2) a question on perceived excessive daytime sleepiness (EDS) ("Do you think you are overly (too) sleepy during the day?") and the Epworth Sleepiness Scale (ESS); (3) the Pittsburgh Sleep Quality Index (PSQI), which measures sleep quality (a PSQI score > 5 distinguishes poor from good sleepers, with good sensitivity and specificity); and (4) Asthma Quality of Life Questionnaire (AQLQ). These questionnaires are described in further detail in this article's Online Repository at www.jaci-inpractice.org.

Pulmonary function and inflammatory markers

Subjects completed pulmonary function testing, including spirometry, maximal bronchodilator reversibility (8 puffs of β-agonist), and methacholine challenge (if baseline forced expiratory volume in first second of the forced vital capacity maneuver [FEV_1] $\geq$ 50% predicted, adopted from the Asthma Clinical Research Network,[31,35] and approved by the SARP Data and Safety Monitoring Board[31]). In addition, subjects completed online exhaled nitric oxide (eNO) by chemiluminescence at a constant expiratory flow (50 mL/s) according to published guidelines,[36] and sputum induction in those without contraindication. The induction method (adopted from the Asthma Clinical Research Network[37]) and sample processing have been previously described in detail by the SARP group.[25] Percent WBC, bronchial and squamous epithelial cells were calculated for total cell counts. Samples with >80% squamous were considered inadequate. Percent neutrophils, eosinophils, macrophages, and lymphocytes were calculated for WBC count, with the former 2 populations being the focus of this analysis. Subjects had a venous blood sample drawn for measurement of total serum IgE, and a complete WBC count with differential for absolute count and % eosinophils.

Data analysis

Obesity was defined by body mass index (BMI) $\geq$ 30 kg/m^2, following the Centers for Disease Control's guidelines. African Americans—a group with particular susceptibility for worse asthma[1]—were compared with Caucasians and all other races (American Indian or Alaskan Native, Asian, Native Hawaiian, and others) combined. From the SA-SDQ, habitual snoring was identified by scores of 4 or 5 ("usually" or "always"). The validated SA-SDQ scores $\geq$36 for men and $\geq$32 for women defined high OSA risk.[34] Because of the small numbers of participants with asthma meeting these cutoffs, the SDQ was normalized (ie, each individual score divided by the sample standard deviation). This approach was also taken to facilitate the interpretation of results in a clinical context. Asthma symptoms and bronchodilator use were categorized as occurring at least daily (scores $\geq$ 5). Global variables of "any asthma symptoms" (wheezing, cough, chest tightness, or shortness of breath) or "any (via MDI or nebulized) bronchodilator use" were constructed, and similarly categorized by their daily occurrence (scores $\geq$ 5). PC_{20} methacholine and total IgE data were $\log_{(10)}$-transformed before analysis, to assure a normal distribution. Asthma sputum inflammatory phenotypes were defined using the cutoffs previously published by the SARP group: eosinophilic (eosinophils $\geq$ 2% and neutrophils $<$ 40%), neutrophilic (eosinophils $<$ 2% and neutrophils $\geq$ 40%), mixed granulocytic neutrophilic (eosinophils $\geq$ 2% and neutrophils $\geq$ 40%), and paucigranulocytic phenotypes (eosinophils $<$ 2% and neutrophils $<$ 40%).[25]

SAS statistical software Version 9.3 (SAS Institute, Cary, NC) was used for analyses. Categorical variables are summarized as counts and percentages, and continuous variables as means and standard deviations (SD) unless otherwise specified. Chi-squared or Fisher's exact tests were used, as appropriate, to analyze categorical variables.

To test for differences between the 3 groups in baseline and sleep measures (Tables I and II—both univariate and multivariate models with adjustment for age, gender, race, obesity, and smoking history), we used general linear models with *post hoc* contrasts. Because this was an exploratory ancillary study, we did not control for multiple comparisons.

Among subjects with asthma, we used Student *t*-tests to compare continuous baseline variables between the 2 groups (Table I). General linear models were used to test for associations of normalized SDQ with continuous asthma outcomes (Tables III and IV) and inflammatory markers (Table V) in both univariate and multivariate analyses. For dichotomous-dependent variables (Tables III and IV), logistic regression was employed.

When we assessed the associations of the normalized SA-SDQ score with asthma outcomes, we included age, gender, BMI, and race as covariates.[38] For associations of the normalized SA-SDQ score with inflammatory markers, we included covariates that have been proposed to impact noneosinophilic pathways in the lower airway (smoking history, obesity, and current inhaled or systemic corticosteroid use).[24,27,29]

All tests were 2-tailed, with significance set at $P < .05$ and trends noted for $P = .05$-$.10$.

RESULTS

Baseline characteristics of study subjects

Table I presents the baseline characteristics of subjects. Both asthma groups were significantly older than NC, and had higher prevalence of obesity and increased African American representation. In 7 control subjects, the PC_{20} could be estimated, but after corroborating with their lack of symptoms and with other clinical data available, these subjects were found appropriate for enrollment by their center PIs. Only 1 control subject reported nightly symptoms, but had an FEV_1% reversibility = 4.95 from

TABLE I. Baseline characteristics of normal controls (NC) and subjects with nonsevere (NSA) and severe asthma (SA)

	NC (n = 146)*	NSA (n = 161)*	SA (n = 94)*	*P* value†
Age (y)	31 (11)	34 (13)	44 (13)	<.0001‡
Female, n (%)	91 (62%)	94 (58%)	57 (61%)	.78
BMI (kg/m^2)	26 (7)	30 (7)	32 (10)	<.0001‡
Obese, n (%)	30 (21%)	59 (37%)	51 (54%)	<.0001‡
Race, n (%)				.22
Caucasians	101 (69%)	98 (61%)	61 (65%)	
African Americans	31 (21%)	45 (28%)	31 (33%)	
Others	14 (10%)	18 (11%)	2 (2%)	
Hx/o smoking, n (%)	21 (14%)	35 (22%)	27 (29%)	.03¥
Asthma duration (y)	-	21 (12)	26 (17)	.008
Pre-bronchodilator FEV_1 % predicted	97 (10)	84 (18)	63 (22)	<.0001‡
Pre-bronchodilator FVC% predicted	98 (11)	92 (16)	78 (19)	<.0001‡
Pre-bronchodilator FEV_1/FVC% predicted	99 (7)	90 (11)	79 (16)	<.0001‡
Methacholine PC_{20} (log) (7/124/39)	0.62 (1.23)	0.14 (0.70)	−0.23 (0.90)	<.0001¥,€
FEV_1 (%) reversibility to 8 puffs of albuterol (n = 143/160/91)	5 (3)	16 (18)	29 (35)	<.007‡
Daily symptoms, n (%)				
Wheezing	0	29 (18%)	38 (40%)	<.0001‡
Cough	0	27 (17%)	38 (40%)	<.0001‡
Chest tightness	0	18 (11%)	34 (36%)	<.0001‡
Shortness of breath	0	29 (18%)	47 (50%)	<.0001‡
Any	0	52 (32%)	63 (67%)	<.0001‡
Nightly symptoms, n (%)	1	24 (15%)	39 (41%)	<.0001‡
Daily rescue β-agonist use, n (%)				
Inhaled	0	43 (27%)	44 (47%)	<.0001‡
Nebulized	0	1 (1%)	19 (20%)	<.0001¥,€
Any	0	43 (27%)	48 (53%)	<.0001‡
Health care utilization due to asthma in the past year, n (%): (n = 143/160/93)				
Hx/o MD visits	0	95 (60%)	82 (87%)	<.0001‡
Hx/o ER visits	0	23 (14%)	47 (50%)	<.0001‡
Hx/o hospitalizations	0	4 (3%)	29 (31%)	<.0001¥,€
Hx/o ER visits or hospitalizations	0	25 (16%)	49 (52%)	<.0001‡
Hx/o ICU admission	0	2 (1%)	11 (12%)	<.0001¥,€
Health care utilization ever due to asthma, n (%): (n = 143/159/92)				
Hx/o ER visits	0	96 (60%)	80 (85%)	<.0001‡
Hx/o hospitalizations	0	56 (35%)	63 (67%)	<.0001‡
Hx/o ICU admission	0	11 (7%)	32 (34%)	<.0001¥,€
Hx/o assisted ventilation	0	5 (3%)	18 (20%)	<.0001¥,€
AQLQ (n = 51/155/89)				
Total score	6.95 (0.07)	4.91 (1.19)	3.97 (1.22)	<.0001‡
Symptoms	6.92 (0.12)	4.97 (1.22)	3.87 (1.39)	<.0001‡
Activities	6.95 (0.10)	4.91 (1.27)	4.13 (1.18)	<.0001‡
Emotions	7.00 (0)	4.93 (1.50)	3.73 (1.63)	<.0001‡
Environment	6.98 (0.08)	4.66 (1.43)	4.12 (1.52)	<.0001‡
Inflammatory markers				
IgE (log) (n = 103/64)	-	2.20 (0.64)	2.07 (2.94)	.27
Blood Eos% (n = 103/64)	-	3.60 (2.63)	3.57 (2.97)	.97
eNO (n = 111/64)	-	32 (25)	63 (64)	.0004
Sputum eosinophils % (n = 94/45)	-	4 (10)	8 (14)	.07
Sputum PMNs% (n = 94/45)	-	44 (25)	49 (24)	.26

AQLQ, Asthma Quality of Life Questionnaire; *BMI*, body mass index; *eNO*, fraction of exhaled nitric oxide; *FEV_1*, forced expiratory volume in first second of the forced vital capacity maneuver; *FVC*, forced vital capacity; *PC_{20}*, the provocative concentration of methacholine, necessary to produce a 20% fall in FEV_1; *PMNs*, polymorphonuclear neutrophils.

Values are means (standard deviations) or counts (%).

*Numbers of subjects except for PC_{20}, FEV_1 reversibility, health care utilization, AQLQ, and inflammatory markers (where numbers are shown for NC/NSA/SA).

†Denotes overall *P* values from general linear models (for 3 groups) or from *t*-tests (for 2 groups' analyses). Significant pairwise comparisons between the 3 groups from the general linear analysis with *post hoc* contrasts are shown as follows: ‡all groups differ, ¥SA vs NC; €SA vs NSA.

TABLE II. Sleep measures, obstructive sleep apnea (OSA) symptoms and OSA risk in normal controls (NC) and subjects with nonsevere (NSA) and severe asthma (SA), in univariate and multivariate (adjusted for age, gender, obesity, race, and smoking history) analyses

					Adjusted *P* values*		
	NC (n = 146)	NSA (n = 161)	SA (n = 94)	Unadjusted *P* value (3 groups)†	SA vs NC	NSA vs NC	SA vs NSA
Sleep duration (h)	7 (1)	7 (2)	7 (3)	.75	.97	.97	.99
Daytime sleepiness, n (%)	18 (12%)	64 (40%)	38 (40%)	<.0001‡,§	<.0001	<.0001	.44
ESS	5 (4)	7 (4)	8 (5)	<.0001‡,§	<.0001	<.0001	.33
Abnormal ESS (score > 10), n (%)	11 (8%)	31 (19%)	29 (31%)	<.0001‖	<.0001	.004	.01
PSQI	7 (3)	9 (3)	11 (3)	<.0001‖	<.0001	<.0001	.0003
Abnormal PSQI (score > 5), n (%)	126 (86%)	158 (98%)	94 (100%)	<.0001‡,§	.0003	<.0001	.74
Any snoring, n (%)	68 (47%)	114 (71%)	68 (72%)	<.0001	.03	.0004	.46
Habitual snoring, n (%)	10 (7%)	29 (18%)	19 (20%)	.004‡,§	.15	.06	.93
Witnessed apnea, n (%)	10 (7%)	31 (19%)	24 (26%)	.0003‡,§	.04	.03	.74
SA-SDQ	19 (6)	26 (7)	29 (6)	<.0001‖	<.0001	<.0001	.25
High OSA risk¶, n (%)	4 (3%)	26 (16%)	24 (26%)	<.0001‖	.04	.02	.85

ESS, Epworth Sleepiness Scale; *PSQI*, Pittsburgh Sleep Quality Index; *SA-SDQ*, Sleep Apnea scale of the Sleep Disorders Questionnaire.
Values are means (standard deviations) or numbers (%).
**P* values adjusted for age, gender, obesity, race, and hx/o smoking.
†Denotes overall *P* values from general linear models. Significant group pairwise comparisons from the general linear analysis *post hoc* contrasts are shown as follows: ‖all groups differ ($P < .0001$ for each comparison), ‡SA vs NC $P < .0001$, §NSA vs NC $P < .0001$.
¶defined as SA-SDQ scores ≥36 for men and ≥32 for women.

TABLE III. Associations of obstructive sleep apnea risk* with asthma control indices

	Univariate analyses†		Multivariate analyses†,‡	
	Parameter estimate or OR (95% CI)	*P* value	Parameter estimate or OR (95% CI)	*P* value
Daily symptoms				
Wheezing	2.22 (1.60, 3.07)	<.0001	1.75 (1.15, 2.66)	.009
Cough	1.86 (1.36, 2.53)	<.0001	1.25 (0.82, 1.91)	.29
Chest tightness	1.68 (1.21, 2.34)	.002	1.38 (0.88, 2.14)	.16
Shortness of breath	1.92 (1.41, 2.59)	<.0001	1.37 (0.92, 2.05)	.13
Any	2.44 (1.79, 3.33)	<.0001	1.56 (1.05, 2.31)	.03
Nightly symptoms	1.89 (1.38, 2.58)	<.0001	1.76 (1.16, 2.67)	.008
Daily β-agonist rescue use				
Inhaled	1.66 (1.25, 2.19)	.0004	1.46 (1.00, 2.14)	.05
Nebulized	1.59 (0.99, 2.53)	.05	1.61 (0.85, 3.07)	.14
Any	1.70 (1.28, 2.24)	.0002	1.52 (1.04, 2.22)	.03
Lung function				
FEV_1% predicted	−6.46 (−9.19, −3.72)	<.0001	−0.01 (−3.62, 3.59)	.99
FVC% predicted	−5.91 (−8.11, −3.70)	<.0001	0.82 (−1.94, 3.58)	.56
FEV_1/FVC% predicted	−1.83 (−3.67, 0.01)	.05	−1.08 (−3.65, 1.48)	.41
Log PC_{20}	−0.14 (−0.26, −0.02)	.03	−0.06 (0.23, 0.12)	.54
FEV_1 reversibility to 8 puffs of inhaled albuterol	2.44 (−0.83, 5.71)	.14	−0.41 (−5.00, 4.18)	.86

CI, Confidence interval; *FEV_1*, forced expiratory volume in first second of the forced vital capacity maneuver; *FVC*, forced vital capacity; *OR*, odds ratios; *PC_{20}*, provocative concentration of methacholine, necessary to produce a 20% fall in FEV_1.
*Sleep Apnea scale of the Sleep Disorders Questionnaire score normalized by its standard deviation.
†Analyses used general linear models (for continuous dependent variables) or logistic regression (for dichotomous dependent variables).
‡Adjusted for age, gender, race, and obesity.

baseline, a negative methacholine challenge (PC_{20} could not be estimated), and interestingly endorsed frequent snoring.

Among asthmatics, SA had a longer duration of disease, worse lung physiology and methacholine PC_{20}, and increased bronchial reversibility compared with NSA. As expected, both asthma groups showed worse lung physiology measures, health care use, and quality of life than NC; among individuals with asthma, SA uniformly had worse such outcomes than NSA. With the exception of higher eNO levels and a trend for higher sputum eosinophil % among subjects with SA, the other markers of airway inflammation were similar between the 2 groups.

Sleep measures and sleep apnea symptoms among all study subjects

As shown in Table II, despite similar duration of sleep, subjects with NSA and SA reported EDS, scored higher on ESS, and had abnormal ESS scores more often than NC. In addition, compared with normal subjects, subjects with NSA and SA had

TABLE IV. Associations of obstructive sleep apnea risk* with asthma-related health care utilization and quality of life

	Univariate analyses†		Multivariate analyses†,‡	
	Parameter estimate or OR (95% CI)	*P* value	Parameter estimate or OR (95% CI)	*P* value
Health care utilization in the past year				
Hx/o MD visits	1.50 (1.13, 2.01)	.006	1.15 (0.76, 1.73)	.52
Hx/o ER visits	1.48 (1.11, 1.97)	.008	1.35 (0.91, 2.00)	.14
Hx/o hospitalizations	1.96 (1.33, 2.89)	.0007	1.50 (0.90, 2.52)	.12
Hx/o ER visits or hospitalizations	1.54 (1.16, 2.05)	.003	1.30 (0.88, 1.93)	.18
Hx/o ICU admission	1.61 (0.92, 2.81)	.09	1.41 (0.68, 2.95)	.36
Health care utilization ever				
Hx/o ER visits	1.62 (1.21, 2.18)	.001	1.45 (0.96, 2.20)	.08
Hx/o hospitalizations	1.46 (1.13, 1.90)	.005	1.10 (0.76, 1.59)	.60
Hx/o ICU admission	1.59 (1.11, 2.29)	.01	1.35 (0.83, 2.20)	.23
Hx/o assisted ventilation	2.06 (1.32, 3.22)	.002	1.87 (1.05, 3.35)	.03
AQLQ				
Total score	−0.53 (−0.67, −0.38)	<.0001	−0.40 (−0.61, −0.20)	.0002
Symptoms	−0.53 (−0.70, −0.37)	<.0001	−0.42 (−0.65, −0.19)	.0004
Activities	−0.49 (−0.65, −0.34)	<.0001	−0.34 (−0.55, −0.13)	.002
Emotions	−0.59 (−0.79, −0.39)	<.0001	−0.48 (−0.76, −0.21)	.0007
Environment	−0.51 (−0.69, −0.33)	<.0001	−0.41 (−0.66, −0.10)	.001

OR, Odds ratios; *CI*, confidence interval; *AQLQ*, Asthma Quality of Life Questionnaire.
*Sleep Apnea scale of the Sleep Disorders Questionnaire score normalized by its standard deviation.
†Analyses used general linear models (for continuous dependent variables) or logistic regression (for dichotomous dependent variables).
‡Adjusted for age, gender, race, and obesity.

TABLE V. Associations of obstructive sleep apnea risk* with blood and airway inflammatory markers in subjects with asthma

	Univariate analyses†		Multivariate analyses†,‡	
	Parameter estimate (95% CI)	*P* value	Parameter estimate (95% CI)	*P* value
Log IgE	−0.04 (−0.16, 0.07)	.42	−0.12 (−0.28, 0.03)	.11
Blood eosinophil count	−0.001 (−0.04, 0.03)	.94	−0.004 (−0.05, 0.04)	.87
Blood eosinophils%	−0.12 (−0.55, 0.32)	.60	0.01 (−0.57, 0.59)	.97
eNO	−3.43 (−10.21, 3.35)	.32	−0.30 (−9.14, 8.54)	.94
Sputum eosinophils%	0.61 (−1.24, 2.45)	.52	0.28 (−2.27, 2.82)	.83
Sputum PMNs%	5.68 (1.76, 9.60)	.005	7.78 (2.33, 13.22)	.006

CI, Confidence interval; *eNO*, fraction of exhaled nitric oxide; *PMNs*, polymorphonuclear neutrophils.
*Sleep Apnea scale of the Sleep Disorders Questionnaire score normalized by its standard deviation.
†Analyses used general linear models.
‡Adjusted for obesity, smoking history (yes/no), and any (inhaled or systemic) current corticosteroid use.

higher PSQI global scores; these scores were almost uniformly abnormal.

Other OSA symptoms, such as snoring and witnessed apneas, were also more prevalent among asthmatics. SA-SDQ scores and prevalence of high OSA risk (Table II) were higher among asthmatics. The average SA-SDQ score among all asthmatics was 26.9 (SD 6.9).

Associations of SA-SDQ with disease control measures among participants with asthma

In univariate analyses (Table III), higher SA-SDQ scores were significantly associated with daily (wheezing, cough, chest tightness, shortness of breath, or any) and nightly asthma symptoms, daily (via MDI or nebulization) β-agonist rescue use, measures of airway obstruction, and methacholine PC_{20}. After adjusting for covariates such as age, gender, obesity, and race (Table III), SA-SDQ scores remained significantly associated with daily wheezing or any asthma symptom, nightly asthma symptoms, and use of any β-agonist rescue; no relationships with pulmonary physiologic measures (FEV_1% and FVC% predicted, and log PC_{20}) were found.

Associations of SA-SDQ with disease-related health care use and quality of life among subjects with asthma

In univariate analyses, higher SA-SDQ scores were associated with more frequent visits to physicians and emergency department, and hospitalizations in the prior year; additionally, higher SA-SDQ scores were associated with increased odds for lifetime emergency department visits, hospitalizations, ICU admissions, and need for assisted ventilation (Table IV). In multivariate analyses, a significant association of SA-SDQ was maintained with a lifetime history of assisted ventilation.

Higher SA-SDQ was related to worse AQLQ global and individual domain scores (Table IV), with estimates of the relationships almost always surpassing the established minimal clinically important difference of 0.5.[39] With adjustment for covariates, the significance of all these relationships was

maintained (Table IV); although the magnitude of the estimates was reduced slightly, they generally remained within the reported range (0.42-0.58) for a minimal clinically important difference.[39]

Associations of SA-SDQ with asthma-related inflammatory markers

Analyses of sputum cells showed higher % polymorphonuclear neutrophils (PMNs) in asthma subjects with high OSA risk compared with those without high OSA risk ($P = .001$), whereas % sputum eosinophils were similar ($P = .66$) (Figure 1). Overall, subjects with high OSA risk tended to have higher representation of any inflammatory (neutrophilic, eosinophilic, or mixed) versus paucigranulocytic phenotypes than individuals without high OSA risk (80% vs 63%, $P = .07$). In analyses of specific inflammatory phenotypes (Figure 2), individuals with high OSA risk trended toward higher proportions of neutrophilic and mixed granulocytic, and lower proportions of eosinophilic and paucigranulocytic phenotypes (43% vs 30%, 31% vs 18%, 6% vs 15%, and 20% vs 37%, respectively, Fisher's exact $P = .06$ for overall relationship).

In univariate analyses, SA-SDQ was not associated with markers of eosinophilic inflammation from blood and airway samples (Table V). In contrast, there was a strong positive association of SA-SDQ with sputum neutrophils; the strength of this association was further magnified when it was adjusted for the covariates (obesity, prior smoking history, and current inhaled or systemic corticosteroid use): each increase in SD-SDQ by its standard deviation (6.85 units) was associated with a rise in % sputum neutrophils of 7.78 (95% confidence interval = [2.33, 13.22], $P = .006$).

DISCUSSION

In this large sample of well-characterized subjects, OSA symptoms were more prevalent among subjects with asthma, apart from traditional risks, and were associated with worse asthma control indices, more frequent disease-related health care use, and lower quality of life. In subjects with asthma, a greater risk for OSA was significantly associated with airway neutrophilia, independent of obesity and other confounders. This suggests that OSA may be an important, previously unrecognized contributor to neutrophilic asthma. Further studies with objective measures of OSA are necessary to confirm these findings and better understand the underlying mechanisms.

By studying a large asthma population that has been well phenotyped for disease severity, our study adds key strengths to existing evidence that suggests a bidirectional relationship of OSA with asthma. On one hand, we find that—despite similar sleep duration—asthmatics, compared with normal controls, reported increased sleepiness and worse sleep quality (Table II). At the same time, they more often reported snoring and witnessed apneas, scored higher on SA-SDQ, and more often met the definition of high OSA risk (Table II). Several clinical studies have shown a high prevalence of OSA symptoms or PSG-diagnosed OSA in patients with asthma[7-11]; this prevalence is higher than that in normal controls[7,10] or in other types of patients.[9] Underlying this relationship may be factors more unique to this population (including the disease itself and inhaled corticosteroid usage).[38,40] Conversely, our study finds that the risk of OSA is related to an increased asthma burden. Higher OSA risk was significantly associated with several indices of poor disease control, such as increased asthma symptoms and rescue inhaler use

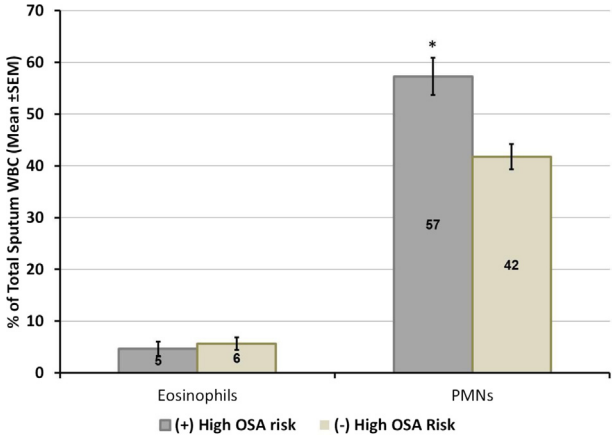

FIGURE 1. Sputum eosinophils and PMNs (expressed as percentage of sputum total white blood cell counts) in asthmatic subjects with and without high OSA risk (defined as scores on Sleep Apnea scale of the Sleep Disorders Questionnaire [SA-SDQ] $\geq$ 36 for men and $\geq$32 for women). In subjects with high OSA risk compared with those without high OSA, there were higher % PMNs, whereas % sputum eosinophils were similar between the 2 groups. *P value = .001 (2-sample Student t-test). *OSA*, Obstructive sleep apnea; *PMNs*, polymorphonuclear neutrophils.

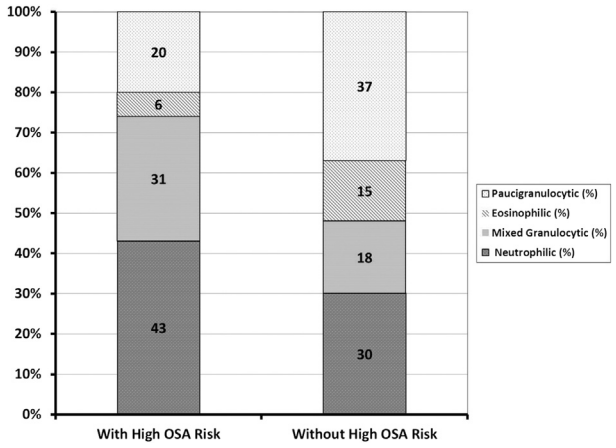

FIGURE 2. Proportion of sputum inflammatory phenotypes in asthmatic subjects with and without high OSA risk (defined as scores on Sleep Apnea scale of the Sleep Disorders Questionnaire [SA-SDQ] $\geq$ 36 for men and $\geq$32 for women). Asthmatic subjects with high OSA risk, compared with those without high OSA risk, tended to have higher proportions of neutrophilic and mixed granulocytic, and lower proportions of eosinophilic and paucigranulocytic phenotypes ($P = .06$, Fisher's exact test). *OSA*, obstructive sleep apnea.

(Table III). We also show that high OSA risk was associated with increased health care utilization and lower quality of life (Table IV). Thus, our study strengthens previous findings from patients with asthma where higher risk for OSA was associated with worse overall asthma control,[12] daytime and nighttime symptoms,[13] and with increased exacerbations.[18] Likewise, these data align well with studies of continuous positive airway pressure

(CPAP) therapy for OSA in small sets of patients with asthma, in which this treatment improved asthma outcomes, including daytime[14] and nighttime[14-16] symptoms, rescue bronchodilator use,[14] PEF rates,[14] and disease-specific quality of life.[17]

Our study provides novel evidence for a potential mechanism whereby OSA aggravates asthma through noneosinophilic inflammatory pathways. We found that higher OSA risk was associated with the enhanced presence of PMNs in the sputum (Figure 1). In subjects with high OSA risk relative to those without high OSA risk, we also observed a 2.5-fold decreased prevalence of eosinophilic asthma and a 1.4-fold increased risk of neutrophilic asthma (Figure 2). Furthermore, after adjusting for covariates, higher SA-SDQ was significantly associated with sputum PMNs%, whereas no association was found with sputum % or peripheral blood markers of eosinophilic inflammation (Table V). This suggests that comorbid OSA may influence the pathogenesis of a neutrophil-rich rather than an eosinophilic type of asthma. There is increased recognition of the heterogeneity of asthma, encompassing phenotypes with potentially different underlying inflammatory pathologies. For example, a neutrophilic phenotype of asthma has been observed more often in patients with chronic persistent, severe, and even fatal asthma.[26,27] PMNs can contribute to tissue damage and inflammation, mucus hypersecretion, and airway remodeling, all of which are characteristic of asthma. PMNs secrete a salvo of powerful cytotoxic and extracellular matrix—disrupting enzymes such as elastase, myeloperoxidase, and matrix metalloproteases. The neutrophil elastase, for example, is a potent secretagogue and enhancer of vascular permeability, which leads to excess mucus production. PMNs produce mediators, such as leukotrienes, with pleiotropic activity on eosinophils and T cells, which release Th2 cytokines and promote bronchospasm. PMNs are an important source of TGF-β, implicated in angiogenesis, smooth muscle proliferation, and trafficking of cells involved ultimately in airway remodeling.[41] At the same time, there is an increasing body of evidence that OSA induces neutrophilic inflammation of the airways. Patients with OSA, compared with normal subjects, have increased neutrophil numbers in the induced sputum,[20,21] and these correlate with the apnea-hypopnea index.[20] In OSA, neutrophil activation was documented via increased reactive oxygen species formation and NF-κB upregulation.[42] Additionally, neutrophil apoptosis, unlike that of other cells, is inhibited, rather than induced, by hypoxia.[43] In this context, our novel results in patients with asthma suggest that OSA—a common occurrence in these patients—may contribute to the noneosinophilic, neutrophilic inflammation, and more severe asthma. These alterations in the lower airway cellular profile could relate to OSA's key features—intermittent hypoxia, mechanical stress from increased work of breathing, and sleep fragmentation. Indeed, a recent experimental study found that exposure to chronic intermittent hypoxia during allergen-induced inflammation in rats shifted the pattern of airway inflammation from its traditional Th-2 eosinophilic to more Th-1-phenotype, with airway infiltration by monocytes.[44] More importantly, these immune alterations were associated with airflow limitation. This physiologic deficit resulted from heterogeneous structural changes that consisted of proximal airway collagen deposition, and co-occurred with distal matrix degradation in the small airways and lung parenchyma.[44] Whether the other OSA features have different effects, and how all these often coexistent features interact in an individual patient with asthma to modulate the lower airway inflammatory milieu, needs to be studied.

There are several limitations to our study. First, at some of the centers, to be enrolled in the parent SARP study, subjects had to lack a clinical history of OSA or PAP use. As a consequence, we likely studied a narrower range, that is, milder sleep-disordered breathing, and lacked more severe cases. Furthermore, in difficult-to-control asthma, OSA is highly prevalent (88%-95.5%),[10,11] but because of exclusions in the parent study, we likely missed some OSA cases. Thus, our data likely represent an underestimation of the true relationships between OSA and asthma. Future studies will need to be conducted in unselected patients, in order to incorporate a wide range of severity of OSA. Another study limitation is its cross-sectional design that precludes causal inferences. For example, sleepiness in asthma could be due to asthma-related sleep disturbance,[45] OSA,[8,46] or a combination thereof. Furthermore, although the interaction of OSA with asthma is likely to be bidirectional,[6,46] interventional studies suggest an influential role of coexistent OSA in asthma. Altogether, these studies call for further investigations of the bidirectional links between OSA and asthma. Last, a questionnaire-based assessment, rather than an objective diagnosis of OSA, was employed. This approach has been common in the OSA and OSA/asthma literature,[9] where the expense of technology and personnel time would have made carrying out such a large study cost-prohibitive. We note that the SA-SDQ is a well-established assessment instrument for OSA, with good performance in sleep-based and other clinic populations, when compared with PSG.[34,47]

We want to acknowledge that obesity is a frequent confounder in studies of OSA and/or asthma. In our study, subjects with SA were more obese than subjects with NSA and normal subjects, similar to previous reports.[48] Some of our findings were attenuated by adjustment for covariates, including obesity (Tables III-V). It is possible that obese subjects in our study were at high risk for both OSA and asthma simply because of their obesity,[49] or it is possible that OSA mediates the relationship between obesity and asthma. Obesity has been suggested to have an important role in promoting the development and/or severity of asthma.[50] However, obesity is also a well-established risk factor for OSA.[5] Our analyses on associations of OSA symptoms with asthma control measures are adjusted for obesity and suggest a role of OSA in asthma control that is independent of obesity. Furthermore, when accounting for SA-SDQ (Table V), obesity was not associated with sputum PMNs% or eosinophils% (−7.13 [−18.39, 4.13], $P = .21$; and −0.46 [−5.72, 4.79], $P = .86$, respectively), or with other markers of eosinophilic inflammation shown in Table V (data not shown). This agrees with recent literature that suggests that adipose tissue can directly affect the airway, rather than through enhanced airway inflammation.[50] This phenomenon may explain why studies that addressed the relationship of obesity with lower airway inflammation in asthma have provided negative results,[49,51] prompting calls to consider OSA in this type of investigations.[29,49] In addition, interventional studies of CPAP treatment for OSA report improvement in asthma outcomes.[14,15,17] Although weight changes are not reported, it is unlikely that substantial loss could have occurred this quickly (2 weeks to 2 months of follow-up) to yield these improvements in asthma; in contrast, a meta-analysis of several experimental CPAP studies showed trivial, nonsignificant rather

weight gain, following OSA treatment.[52] Last, in SARP, neutrophils were highest in cluster 5 that had also the highest proportion of obesity and possibly OSA.[53] Whether the neutrophilia in this cluster is driven by OSA, or something intrinsic to this subphenotype, remains to be further studied.

In summary, our study of a large and well-characterized sample of individuals with asthma shows associations of OSA symptoms with worse asthma control indices, health care utilization, and disease-specific quality of life; these associations were independent of other factors, such as obesity, that can affect asthma, and suggest that the underlying mechanisms may act through noneosinophilic, neutrophilic inflammatory pathways. This study represents an important step forward in our understanding of the interaction of asthma and OSA, beyond common risk factors such as obesity. Further studies that incorporate objective assessments of OSA which allow to differentiate its pathogenic features may deepen our understanding of these mechanisms, and may offer new interventions for a phenotype that responds poorly to current standard therapies.

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METHODS

Subjects

Subjects were recruited as part of an add-on substudy to the observational multicenter Severe Asthma Research Program (SARP) II. The criteria for enrollment of subjects with severe (SA) or nonsevere asthma (NSA) in stable condition, as well as normal controls, all of whom were current nonsmokers, were detailed previously.[1] In brief, asthma was defined according to the National Asthma Education and Prevention Program guidelines: episodic respiratory symptoms, reversible airflow obstruction (documentation of variability of forced expiratory volume in first second of the forced vital capacity maneuver and/or of forced vital capacity by 12% and 200 cm^3 either spontaneously or after 2 puffs of inhaled albuterol), and/or a positive methacholine bronchoprovocation test.[2] SARP Clinical Center PIs had the discretion to exclude from recruitment subjects with a clinical history of obstructive sleep apnea (OSA) or positive airway pressure. Participants with asthma were classified according to the American Thoracic Society's definition of refractory asthma.[3] To be classified as SA, 1 of the 2 major criteria (continuous oral corticosteroid or high-dose inhaled corticosteroid use) and at least 2 of the 7 minor criteria were required. Those not fulfilling the criteria for SA were categorized as NSA. Normal controls were healthy individuals with no history of asthma who had normal pulmonary function, including spirometry and $\leq$5% reversibility after albuterol. Subjects were enrolled at 5 sites (via recruitment databases, physician-to-physician contact, email, and paper advertisements): the University of Wisconsin, the Cleveland Clinic, University of Pittsburgh, University of Virginia, and Wake Forest University.

Clinical questionnaires

The validated, self-administered questionnaires included: (1) the Sleep Apnea scale of the Sleep Disorders Questionnaire[4] (SA-SDQ), which has been validated in a large sample of patients to assesses OSA risk.[4] This scale includes 8 symptom items that ask about loud snoring disruptive to the bed partner, stopping breathing during sleep, sudden gasping arousals, worsening of snoring while supine or after alcohol, nocturnal sweating and nasal congestion, and a history of hypertension. Responses are recorded on a 5-point Likert scale (from "never" to "always"). Data on weight, age, smoking, and body mass index are rated on a 1-5 scale. Scores across all items are then summed to yield a total score that can range from 12 to 60. (2) A second questionnaire asked about perceived excessive daytime sleepiness (EDS) ("Do you think you are overly (too) sleepy during the day?") and was followed by the Epworth Sleepiness Scale, which is a validated and widely used tool that measures subjective daytime sleepiness.[5] It rates from 0 (no chance) to 3 (high chance) the likelihood of dozing in 8 specific situations commonly encountered in daily life. Total score is the sum of all items and can range from 0 to 24. A score of >10 indicates EDS.[5] This scale demonstrated a high level of internal consistency among its 8 items, high test-retest reliability, and ability to distinguish between patients with EDS from normal subjects.[6,7] (3) The Pittsburgh Sleep Quality Index (PSQI) measures sleep quality.[8] Nineteen individual items generate 7 "component" scores: sleep quality, latency, duration, efficiency and disturbances, use of sleeping medication, and daytime dysfunction. Each component is scored from 0 to 3 and yields an overall score that can range from 0 to 21, with a higher score indicating worse quality of sleep. A PSQI score > 5 distinguishes poor from good sleepers with good sensitivity and specificity[8]; (4) Asthma Quality of Life Questionnaire is a validated 32-item disease-specific, widely used quality of life instrument.[9,10] It includes questions on activities, symptoms, emotions, and exposures over the past 2 weeks rated on a 7-point Likert scale (1 = maximal impairment to 7 = no impairment). The total score is the average of all 32 responses. The minimal clinically important difference is roughly 0.5 (range: 0.42-0.58).[11]

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