Title

Inflammation biomarkers in OSA, chronic obstructive pulmonary disease, and chronic obstructive pulmonary disease/OSA overlap syndrome.

Journal

Journal of Clinical Sleep Medicine, 19(8)

ISSN

1550-9389

Authors

Sanchez-Azofra, Ana

Gu, Wanjun

Masso-Silva, Jorge A

et al.

Publication Date

2023-08-01

DOI

10.5664/jcsm.10600

Peer reviewed

https://doi.org/10.5664/jcsm.10600

JCSM | Journal of Clinical Sleep Medicine

SCIENTIFIC INVESTIGATIONS

Inflammation biomarkers in OSA, chronic obstructive pulmonary disease, and chronic obstructive pulmonary disease/OSA overlap syndrome

Ana Sanchez-Azofra, MD[1,2]; Wanjun Gu, BA, BS[1,3]; Jorge A. Masso-Silva, PhD[1,4]; David Sanz-Rubio, PhD[5]; Marta Marin-Oto, MD, PhD[5]; Pablo Cubero, BS[5]; Ana V. Gil, BS[5]; Esteban A. Moya, PhD[1]; Laura A. Barnes, MD[1]; Omar A. Mesarwi, MD[1]; Traci Marin, PhD[1,6]; Tatum S. Simonson, PhD[1,7]; Laura E. Crotty Alexander, MD[1,4]; Jose M. Marin MD, PhD[5,8,*]; Atul Malhotra, MD[1,7,*]

*[1]Division of Pulmonary, Critical Care, and Sleep Medicine and Physiology, Department of Medicine, University of California, La Jolla, California; [2]Division of Pulmonary and Sleep Medicine. Hospital Universitario de la Princesa, Universidad Autónoma de Madrid, Madrid, España; [3]Herbert Wertheim School of Public Health and Longevity Sciences, University of California, San Diego, La Jolla, California; [4]Section of Pulmonary and Critical Care, VA San Diego, La Jolla, California; [5]Translational Research Unit, IIS Aragón, Hospital Universitario Miguel Servet, Zaragoza, Spain; [6]Health Sciences, Department of Respiratory Therapy, Victor Valley College, Victorville, California; [7]Center for Physiological Genomics of Low Oxygen, University of California, La Jolla, California; [8]CIBERES Instituto Salud Carlos III, and Department of Medicine, University of Zaragoza School of Medicine, Zaragoza, Spain; *Contributed equally*

Study Objectives: The coexistence of obstructive sleep apnea (OSA) and chronic obstructive pulmonary disease (COPD) in a single individual, also known as overlap syndrome (OVS), is associated with higher cardiovascular risk and mortality than either OSA or COPD alone. However, the underlying mechanisms remain unclear. We hypothesized that patients with OVS have elevated systemic inflammatory biomarkers relative to patients with either disease alone, which could explain greater cardiovascular risk observed in OVS.

Methods: We included 255 participants in the study, 55 with COPD alone, 100 with OSA alone, 50 with OVS, and 50 healthy controls. All participants underwent a home sleep study, spirometry, and a blood draw for high-sensitivity C-reactive protein and total blood count analysis. In a randomly selected subset of 186 participants, inflammatory protein profiling was performed using Bio-Rad Bio-Plex Pro Human Cytokine 27-Plex Assays. Biomarker level differences across groups were identified using a mixed linear model.

Results: Levels of interleukin 6 (IL-6), high-sensitivity C-reactive protein (hs-CRP), and granulocyte colony stimulating factor (G-CSF) were higher in participants with OVS and COPD compared with healthy controls and participants with OSA. Furthermore, participants with OVS had higher circulating levels of leukocytes and neutrophils than those with COPD, OSA, and controls.

Conclusions: COPD and OVS are associated with higher systemic inflammation relative to OSA and healthy controls. This work proposes the potential utilization of interleukin 6, granulocyte colony stimulating factor, and high-sensitivity C-reactive protein as screening biomarkers for COPD in patients with OSA. Inflammatory pathways may not fully explain the higher cardiovascular risk observed in OVS, indicating the need for further investigation.

Keywords: cardiovascular risk, sleep-disordered breathing, obstructive sleep apnea, chronic obstructive pulmonary disease, overlap syndrome, c-reactive protein, granulocyte colony stimulating factor, interleukin 6, interleukin 8

Citation: Sanchez-Azofra A, Gu W, Masso-Silva JA, et al. Inflammation biomarkers in OSA, chronic obstructive pulmonary disease, and chronic obstructive pulmonary disease/OSA overlap syndrome. *J Clin Sleep Med*. 2023;19(8):1447–1456.

BRIEF SUMMARY

Current Knowledge/Study Rationale: The coexistence of obstructive sleep apnea (OSA) and chronic obstructive pulmonary disease (COPD) in a single individual, also known as the overlap syndrome, is associated with higher cardiovascular risk and mortality than either disease alone. However, to our knowledge there are minimal data regarding the underlying mechanisms.

Study Impact: In this study, overlap syndrome and COPD were associated with higher systemic inflammation levels relative to OSA and healthy controls. This work proposes the potential utilization of interleukin 6, granulocyte colony stimulating factor, and high-sensitivity C-reactive protein as screening biomarkers for COPD in patients with OSA. Our new findings suggest that inflammatory pathways are unlikely to explain fully the increased cardiovascular risk in overlap syndrome compared to OSA or COPD alone, suggesting other causal pathways should perhaps be sought in subsequent studies.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) and obstructive sleep apnea (OSA) represent 2 of the most prevalent chronic respiratory disorders in clinical practice, and cardiovascular diseases represent a major comorbidity in each disorder.[1,2] The "overlap syndrome" (OVS) is a term used to describe the concurrent presence of COPD and OSA in a single individual.[3] This term was first introduced by D.C. Flenley in 1985.[3] The prevalence of OVS in the general population is estimated at 1–3.6%, with a higher prevalence among those patients with either OSA or COPD.[4] OSA is characterized by intermittent nocturnal desaturations associated with periodic collapse of the upper airway during sleep. Upper airway flow limitation in patients with COPD without apnea/hypopnea per se also contributes to nocturnal desaturation. Individuals with OVS present

even greater degrees of oxygen desaturation, as a consequence of lower baseline oxygen saturation during sleep, predisposing to pulmonary hypertension.[4]

COPD and OSA are linked to increased cardiovascular morbidity and mortality,[5,6] which is most likely due to a combination of intermittent hypoxia, systemic inflammation, increased oxidative stress, metabolic abnormalities, and activation of the sympathetic nervous system.[7–9] COPD, once considered a respiratory disease, is now recognized as a systemic inflammatory disease.[10] According to the ECLIPSE Investigator Group, high serum levels of high-sensitivity C-reactive protein (hs-CRP) and interleukin-6 (IL-6) in patients with COPD were associated with 3-year all-cause mortality.[11] The link between OSA and systemic inflammation has been extensively studied for more than 20 years.[12–14] Intermittent hypoxia and chronic sleep fragmentation can both lead to adipose tissue inflammation, which in turn causes increased release of IL-6 and hs-CRP.[15,16] However, obesity is an important confounding factor in this relationship. In fact, in some series of patients with OSA, once adjusted for body mass index (BMI), no differences in circulating inflammatory cytokines have been found between patients with OSA and those without OSA.[17–19]

There is currently minimal data on the inflammatory response in OVS. The alveolar lavage fluid of patients with OVS showed a significantly higher proportion of neutrophils, as well as higher concentrations of tumor necrosis factor α (TNFα) and interleukin-8 (IL-8), than that of patients with COPD.[20] In a small study of COPD patients with severe comorbidities (n = 38), those with OVS (n = 17) had a higher serum percentage of peripheral neutrophils than those with COPD alone.[21]

A recent meta-analysis suggests that OVS patients have higher risk of hypertension, peripheral vascular disease, ischemic heart disease, heart failure, and cerebrovascular disease compared to either disease alone.[22] Patients with OVS have poor cardiovascular outcomes compared with either disease alone, and greater time on positive airway pressure (PAP) was associated with reduced mortality.[23,24] Sharma et al[25] observed an increased mass of the right ventricle in patients with OVS compared to COPD alone, suggesting higher pulmonary arterial pressures in OVS. In a prospective study, the prevalence of hypertension and coronary heart disease was higher in patients with OVS than in patients with either disease alone.[26] In this study, patients with OVS had higher levels of endothelial dysfunction markers, such as, soluble vascular cell adhesion molecule-1b and TNF-α.[26] However, regardless of these poor outcomes, OVS has not been adequately studied, and the underlying mechanisms contributing to OVS-increased cardiovascular risk are unknown.

Plasma levels of IL-6 and hs-CRP have been shown to predict the risk of cardiovascular diseases.[27,28] This increased IL-6 and hs-CRP release can interact with endothelial cells and promote nuclear factor-κB-dependent endothelial dysfunction and atherosclerosis.[29] The presence of systemic inflammation in COPD and OSA, as evidenced by elevated levels of hs-CRP and IL-6, may contribute to an increased cardiovascular risk in OVS patients.

Based on this conceptual framework, we tested the hypothesis that patients with OVS would have elevated levels of systemic inflammatory cytokines and chemokines compared with patients with either OSA alone, COPD alone, or healthy controls. We focused our primary outcome on IL-6 and hs-CRP based on existing literature and the known role of these biomarkers in mediating cardiovascular risk. However, as an exploratory analysis we measured a broad panel of inflammatory modulating proteins to search for new biomarkers.

METHODS

This retrospective study was approved by the Aragon Research Institute, Human Research Protection Program (C.I.PI 13/0008). The project respected the fundamental principles established in the Declaration of Helsinki. Written informed consent was obtained from each participant upon receiving a detailed description of the study protocol. A total of 255 participants was consecutively recruited from the outpatient clinic in Hospital Universitario Miguel Servet, a large teaching hospital in Zaragoza, Spain, during scheduled appointments from 2011 to 2015, as shown in the flowchart in **Figure 1**. The participants with COPD and those with OVS are part of the ongoing CHAIN cohort,[30] and the controls and participants with OSA are part of the ongoing EPIOSA cohort.[31]

All participants were clinically stable and those with COPD were free of exacerbations for at least 8 weeks prior to the study. In the EPIOSA cohort, participants were excluded if they were younger than 20 or older than 60 years old, if they had any history of cigarette or tobacco use, alcohol abuse, or any comorbidities.[31] In the CHAIN cohort, participants were excluded if they were younger than 35 years old, and if they had neoplasia or serious comorbidities that prevented the performance of the tests.[30] In the present study, blood samples were collected from all groups (controls/OSA/COPD/OVS) at baseline, the morning after their sleep study, under fasting conditions. For the OSA group, a second blood sample was collected during the follow-up visit after 1 year. Forty participants in the OSA group declined treatment, and the other 60 participants started treatment with PAP with good adherence as defined by the use of the PAP device for more than 4 hours per night throughout the year.[32] Two participants in the OSA-treated group discontinued the study. Given the retrospective design of the current study, we did not have follow-up serum samples for the COPD and OVS groups.

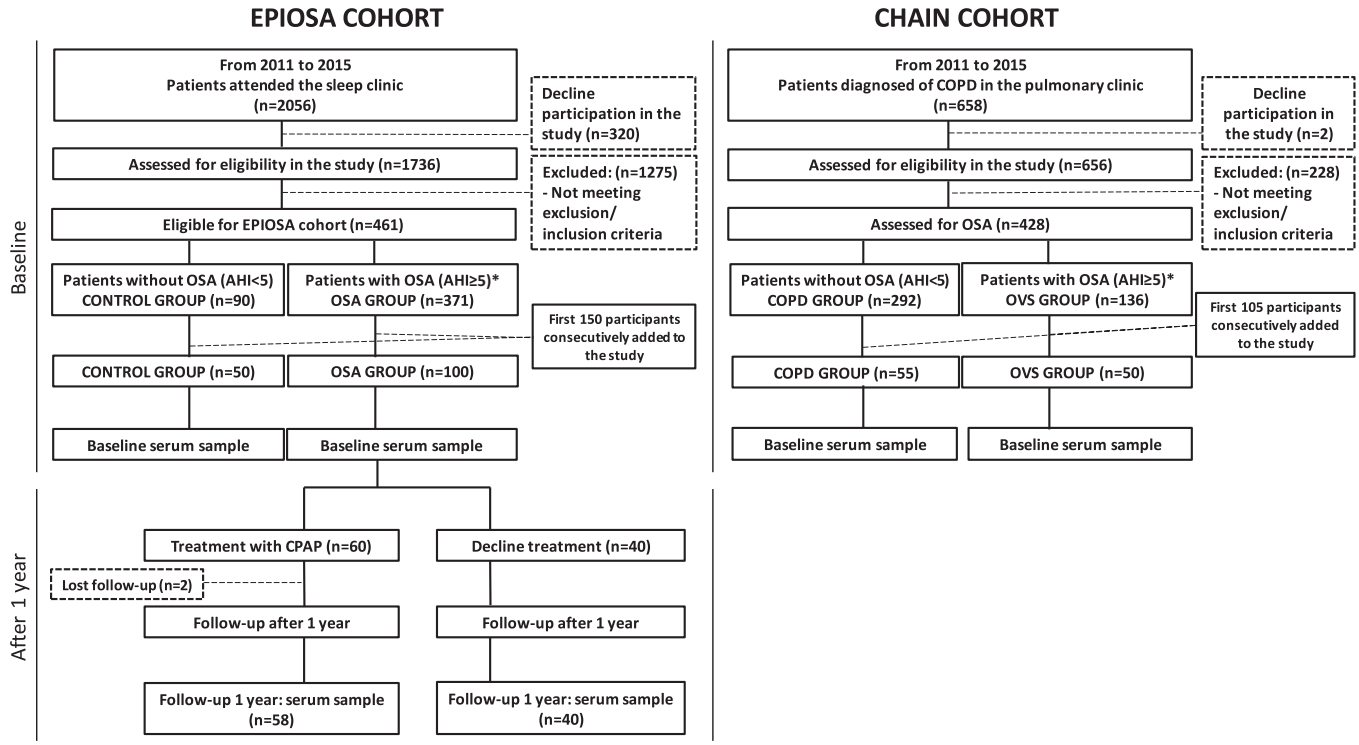
Clinical data

The following clinical and physiological metrics were measured: sociodemographic data, weight, height, BMI and neck circumference, smoking history, past medical history, presence of comorbidities, and medication use. Daytime somnolence was assessed with the Epworth Sleepiness Scale.

Sleep apnea test and spirometry

A respiratory polygraph (ApneaLink Air, ResMed, San Diego, CA), which is a portable monitoring device, classified as a type III home sleep test, was used to perform the sleep studies. Sleep studies were scored by a blinded, experienced sleep technician

Figure 1—Flowchart of the study.



For EPIOSA cohort, a total of 2,056 individuals attended the sleep clinic from 2011 to 2015. From those, 320 individuals declined participation in the study and 1,275 were excluded for not meeting the inclusion/exclusion criteria. A total of 461 participants was eligible for the EPIOSA study, 90 in the control group and 371 in the OSA group. The first 150 participants consecutively added to the EPIOSA cohort were included in our study, 100 with OSA and 50 healthy controls. The participants with OSA were divided into 2 groups depending on whether they accepted or declined treatment with PAP. These 2 groups of participants with OSA were followed up for 1 year. For the CHAIN cohort, a total of 658 participants were screened and consented, from those 2 declined participation in the study, 228 were excluded for not meeting the inclusion/exclusion criteria. A total of 428 participants was assessed for OSA, from those 292 were in the COPD group and 136 were in the OVS group. The first 105 participants consecutively added to the CHAIN cohort were included in our study, 55 with COPD and 50 OVS. *Patients with OSA candidates for treatment following the Spanish Guidelines obstructive sleep apnea.[57] AHI = apnea-hypopnea index, COPD = chronic obstructive sleep apnea, OSA = obstructive sleep apnea, OVS = overlap syndrome, PAP = positive airway pressure.

and staged according to standard criteria.[33] OSA was defined as an apnea-hypopnea index (AHI) ≥ 5 events/h of recording. Optimal titration of continuous PAP was performed by using auto-continuous PAP (Autoset-T; ResMed, Sydney, Australia), according to previous validation procedures by the Spanish Sleep and Breathing Group.[34] Adherence to continuous PAP was measured at each visit using the device's internal timers.

All participants underwent at baseline pre- and postbronchodilator spirometry according to current recommendations.[35] COPD was defined by persistent respiratory symptoms and a postbronchodilator forced expiratory volume in 1 second (FEV_1)/forced vital capacity < 0.70 according to the Global initiative for Chronic Obstructive Lung Disease (GOLD) guidelines, and disease severity was defined by GOLD stage.[10]

Laboratory measurements

Whole blood samples were obtained at baseline for all participants, and for the OSA group, a second blood sample was obtained after one year for each participant. In all participants within 2 hours after collection, hs-CRP was measured based on a particle-enhanced turbidimetric immunoassay technique. About 5 mL of blood without anticoagulant was obtained for serum preparation and stored at −80 °C. These samples were shipped to the University of California San Diego on dry ice under a material transfer agreement. Serum cytokine levels were analyzed in a randomly selected subset of 186 participants using a Bio-Rad Bio-Plex Pro Human Cytokine 27-Plex Assay (cat. No. M500KCAF0Y) and the Bio-Plex 200 System, MAGPIX Multiplex Reader (Bio-Rad Laboratories, Life Science Group 2000, Hercules, CA). Briefly, the Luminex xMAP technology based on immunoassay methods is capable of simultaneously quantifying 27 targets: IL-1β, IL-1, IL-1 receptor antagonist, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, eotaxin, fibroblast growth factor basic, granulocyte colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), interferon gamma, interferon gamma-induced protein 10, monocyte chemoattractant protein 1, macrophage inflammatory protein 1α, macrophage inflammatory protein 1β, platelet-derived growth factor BB, vascular endothelial growth factor, and TNFα. The concentration of each cytokine was extrapolated from the calibration curve (individualized for each cytokine), determined independently for each experiment (each plate). All samples were analyzed in duplicate.

Statistical analysis

Cytokine measurements of each participant underwent rank-based inverse normal transformation to improve the normality of the biomarker level distribution. A mixed linear model was used to reveal a COPD, OSA, and overlap effect on cytokine measurements while controlling for the fixed effects of participant sex, age, BMI, AHI, and FEV_1 (%), as well as the random batch effect of each experiment (each plate). The model can be written as:

$$\text{Cytokine} = \beta_0 + \beta_{COPD}\text{COPD} + \beta_{OSA}\text{OSA} + \beta_{overlap}(\text{COPD} * \text{OSA}) + \beta_{sex}\text{Sex} + \beta_{age}\text{Age} + \beta_{BMI}\text{BMI} + \beta_{AHI}\text{AHI} + \beta_{FEV_1}\text{FEV}_1 + \sum_{i=1}^{7}\beta_{plate_i}\text{Plate}_i$$

where COPD and OSA are indicator variables whose values are 1 if the participant has COPD and/or OSA, else 0. The interaction between the COPD and OSA term is denoted as COPD*OSA, whose value will be 1 if the participant has OVS. For biomarkers significantly associated with COPD, OSA, and/or OVS outcomes, stepwise regression analyses were also conducted (**Table 1**). In addition to the fully adjusted model (M3) used for the pre-hoc test, the stepwise regression predicts the levels of biomarkers considering, respectively, a simplistic model M1, where COPD, OSA, and OVS are the only predictors of biomarker levels as well as an intermediate model M2, where sex and age are added as covariates but neither AHI nor FEV_1 were controlled. Post hoc 1-way ANOVA tests were performed to compare transformed and adjusted cytokine levels between the groups (control, COPD, OSA, and OVS) if a significant COPD, OSA, and/or OVS effect was observed. To reveal the effect of comorbidities on cytokine levels, post hoc significant cytokine levels were further tested for association with common comorbidities including smoking status, arterial hypertension, dyslipidemia, diabetes, and cardiovascular complications while controlling for covariates. Corrections for multiple comparisons based on the total number of cytokines measured were conducted using the Bonferroni method to account for increased false discovery rate. A corrected *P* value less than or equal to .05 was used to establish statistical significance. For visualization purposes, transformed and adjusted biomarker levels were plotted across the four groups of participants to mitigate the effect of extreme values and account for the differences in sex, age, BMI, AHI, and FEV_1 between groups. To demonstrate similarities and differences of cytokine profiles of participants, principal component analysis was performed considering all 27 cytokines measured. The principal components were scaled and recentered to control for batch effects. All data curation, transformation, and statistical analyses were conducted using R v4.2.0.

RESULTS

Baseline characteristics

Baseline characteristics of our population are described in **Table 2**. There were fewer women in the COPD and OVS

Table 1—Stepwise regression summary statistics.

	M1	M2	M3	M1	M2	M3
	IL8			IL6		
OSA	−0.19 (0.190)	−0.20 (0.212)	−0.287 (0.150)	−0.07 (0.686)	0.09 (0.629)	−0.11 (0.651)
COPD	0.78 (< 0.001)*	0.50 (0.048)*	0.337 (0.237)	0.84 (< 0.001)*	1.12 (< 0.001)*	1.11 (0.002)*
OVS	−0.49 (0.023)*	−0.45 (0.046)*	−0.457 (0.042)*	0.02 (0.938)	−0.14 (0.605)	−0.11 (0.702)
Sex		−0.24 (0.109)	−0.199 (0.181)		−0.40 (0.029)*	−0.38 (0.040)*
Age		0.01 (0.020)*	0.013 (0.023)		−0.00 (0.769)	−0.00 (0.792)
BMI		0.01 (0.658)	0.004 (0.753)		−0.01 (0.738)	−0.01 (0.464)
AHI			0.002 (0.547)			0.01 (0.171)
FEV_1			−0.004 (0.216)			−0.00 (0.846)
	G-CSF			hs-CRP		
OSA	0.07 (0.646)	−0.03 (0.869)	−0.27 (0.232)	0.232 (0.215)	0.13 (0.509)	−0.07 (0.761)
COPD	1.20 (< 0.001)*	0.75 (0.008)*	0.79 (0.013)*	0.851 (< 0.001)*	0.56 (0.087)	0.18 (0.601)
OVS	−0.61 (0.011)*	−0.45 (0.070)*	−0.40 (0.111)	−0.002 (0.995)	0.07 (0.816)	−0.01 (0.966)
Sex		0.12 (0.474)	0.13 (0.441)		−0.15 (0.400)	−0.13 (0.453)
Age		0.01 (0.040)*	0.01 (0.034)*		0.01 (0.201)	0.01 (0.231)
BMI		−0.01 (0.728)	−0.01 (0.365)		0.03 (0.035)*	0.03 (0.079)
AHI			0.01 (0.072)			0.01 (0.139)
FEV_1			0.00 (0.872)			−0.01 (0.005)*

Values expressed as effect size (*P* value). *$P < .05$. AHI = apnea-hypopnea index, BMI = body mass index, COPD = chronic obstructive pulmonary disease, FEV_1 = forced expiratory volume in 1 second, G-CSF = granulocyte colony stimulating factor, hs-CRP = high-sensitivity C-reactive protein, IL-6 = interleukin-6, IL-8 = interleukin 8, OSA = obstructive sleep apnea, OVS = COPD-OSA overlap syndrome.

Table 2—Baseline characteristics of study participants.

	Control	COPD	OSA	OVS
n	50	55	100	50
Age	41 ± 10	71 ± 8*‡	47 ± 10*†	67 ± 7*‡†
Sex	42.9	94.7*‡	76.1*†	95.2*‡
BMI	26.6 ± 3.6	29.1 ± 4.4	29.6 ± 3.7*	31.1 ± 6.0*
Cigarette use (packs/y)	2.3 ± 4.0	58.1 ± 25.0*‡	2.6 ± 3.7	60.2 ± 26.8*‡
FEV_1 post (%)	98.3 ± 10.4	63.2 ± 21.4*‡	97.4 ± 10.4	53.7 ± 19.0*‡†
FEV_1/FVC	81.4 ± 4.8	56.8 ± 12.3*‡	81.0 ± 4.6	51.0 ± 13.0*‡†
AHI (events/h)	3.6 ± 1.8	0.6 ± 1.8‡	36.7 ± 19.9*	28.2 ± 22.0*‡†
CT90	1.29 ± 2.5	9.3 ± 19.7	15.9 ± 18.1*	47.3 ± 31.2*‡†
Nadir saturation	88.3 ± 3.9	86.8 ± 4.0‡	79.2 ± 7.6*	76.1 ± 8.1*†
Epworth Sleepiness Scale	9.6 ± 5.5	6.8 ± 4.2*‡	9.5 ± 4.8	8.5 ± 3.3

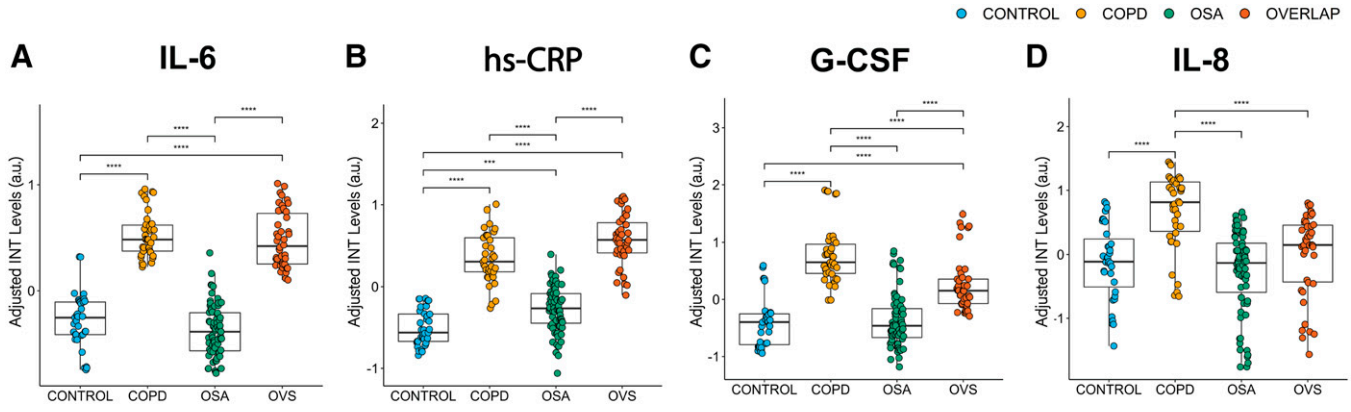
Values expressed as mean ± SD or percentages. *P < .05, controls vs OSA, COPD and OVS. ‡P < .05, OSA vs COPD and OVS. †P < .05, COPD vs OSA and OVS. AHI = apnea-hypopnea index, BMI = body mass index, COPD = chronic obstructive pulmonary disease, CT 90 = cumulative sleep time percentage with oxyhemoglobin saturation (SpO_2) < 90%, FEV_1 = forced expiratory volume in 1 second, FVC = forced vital capacity, OSA = obstructive sleep apnea, OVS = COPD-OSA overlap syndrome.

groups compared with OSA and control groups. The average age of the participants with COPD and OVS was also higher than in the OSA and control groups. BMI was similar between COPD, OSA, and OVS groups and lower in the control group. The average airway obstruction severity measured by FEV_1 was lower in the OVS group compared with the COPD group (53.7% ± 19.0% and 63.2% ± 21.4%, respectively). AHI and cumulative sleep time percentage with oxyhemoglobin saturation (SpO_2) < 90% (CT90) were higher in the OSA and OVS group, and nadir saturation was lower in these 2 groups. All these factors were corrected in the models used for our analysis.

Cytokines and chemokines levels

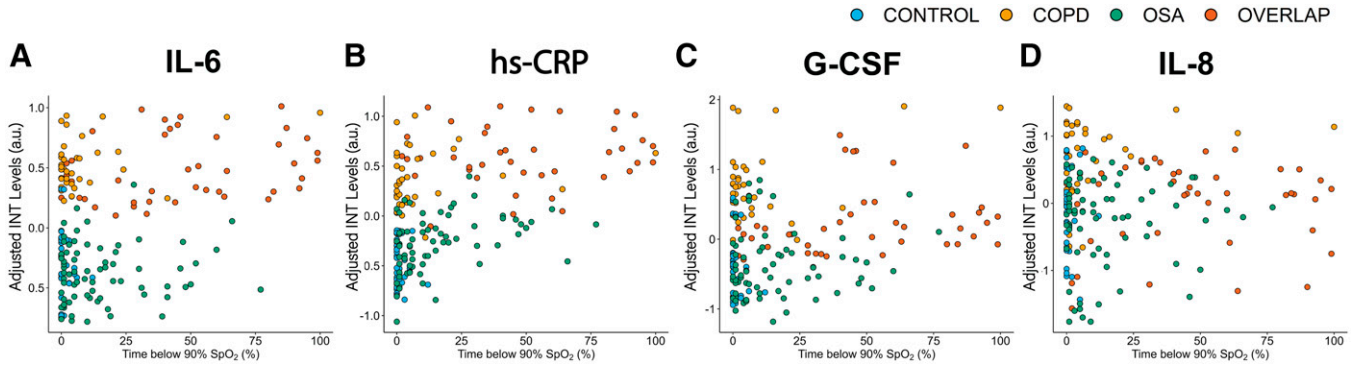
Levels of IL-6 were higher in the COPD and OVS groups (P < .001), but not the OSA group (P = .204), (**Figure 2A**) compared with controls. The OVS group had higher levels of IL-6 compared with the OSA group (P < .001), but similar to those seen in COPD. hs-CRP levels were higher in COPD, OSA, and OVS groups compared with controls (P < .001). Both OVS and COPD had similar levels of hs-CRP, but higher compared with the OSA group (P < .001) (**Figure 2B**). Furthermore, G-CSF levels were higher in the COPD and OVS groups (P < .001), but not in the OSA group (P = .992), compared with controls (**Figure 2C**). The OVS group had higher G-CSF levels compared

Figure 2—Rank-base inverse normal transformed levels of IL-6, hs-CRP, G-CSF, and IL-8 in patients with OSA, with COPD, with OVS and healthy controls adjusted for sex, age, BMI, AHI, and FEV_1.



(A) Levels of IL-6 were higher in the COPD and OVS groups compared to the control group. OSA levels of IL-6 were similar to the control group. **(B)** hs-CRP levels were higher in COPD, OSA, and OVS compared to controls. OVS had higher levels of hs-CRP than OSA, but similar to COPD. **(C)** G-CSF levels in OVS were higher than in OSA but lower than in COPD. **(D)** IL-8 levels were higher in COPD compared to OVS and OSA. COPD = chronic obstructive pulmonary disease, G-CSF = granulocyte colony stimulating factor, hs-CRP = high-sensitivity C reactive protein, IL-6 = interleukin-6, IL-8 = interleukin 8, INT = inverse normal transformation, OSA = obstructive sleep apnea, OVS = overlap syndrome. ***P < .001, ****P < .0001.

Figure 3—Regression analysis of the adjusted levels of IL-6, hs-CRP, G-CSF, and IL-8 against CT90.



The levels of IL-6, hs-CRP, G-CSF, and IL-8 were not correlated with the severity of hypoxia measured by the CT90. COPD = chronic obstructive pulmonary disease, CT90 = cumulative sleep time percentage with oxyhemoglobin saturation (SpO_2) < 90%, G-CSF = granulocyte colony stimulating factor, hs-CRP = high-sensitivity C-reactive protein, IL-6 = interleukin-6, IL-8 = interleukin 8, INT = inverse normal transformation, OSA = obstructive sleep apnea, OVS = overlap syndrome.

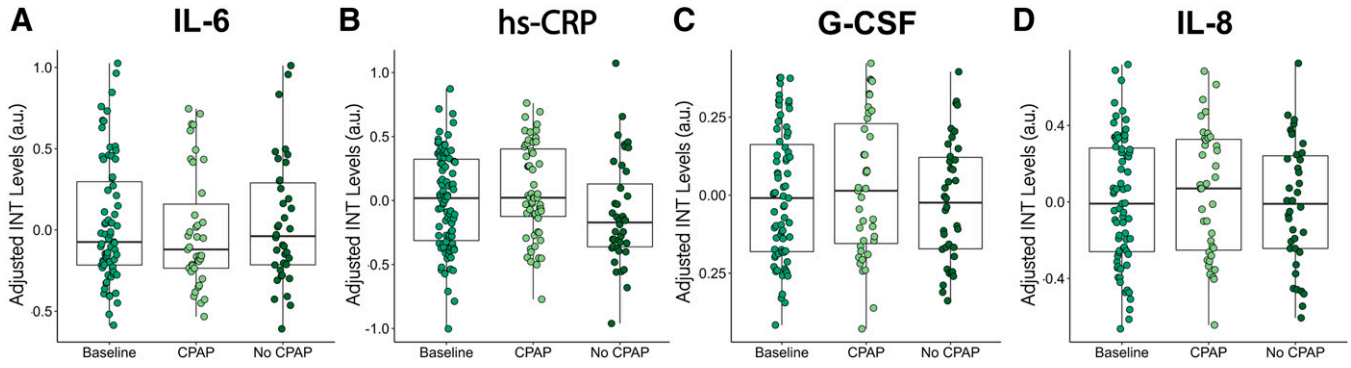
with the OSA group ($P < .001$), but lower compared with the COPD group ($P < .001$). The COPD group had higher IL-8 levels compared with the control group ($P < .001$), but the OVS ($P = .815$) and OSA ($P = .628$) groups did not (**Figure 2D**). We found no differences between groups for the remaining cytokines in the assay. Furthermore, systemic levels of IL-6, hs-CRP, G-CSF, and IL-8 were not correlated with the severity of hypoxemia measured by the cumulative time spent with $SpO_2 < 90\%$ (CT90) (**Figure 3**). Finally, in the OSA group, no differences were detected in any inflammatory protein level after a year of treatment with PAP (**Figure 4**).

Principal component analysis was conducted on all 27 cytokines and chemokines while controlling for sex differences and potential batch effects of the individual plates used to run the cytokine assays. Inflammatory protein profiles of participants were consistently clustered and projected onto the first 2 principal components. As shown by the projection, control, COPD, OSA, and OVS participants all appeared in distinctive clusters, with participants with OVS and COPD mainly in the top left quadrant and participants with OSA and controls localized in the lower right quadrant (**Figure 5**), suggesting these 3 diseases might have different inflammation profiles. Furthermore, participants with OVS were mainly clustered between participants with COPD and OSA, demonstrating a unique cytokine profile of OVS that shares similarities with both COPD and OSA, but was distinguishable from them.

Inflammatory cells

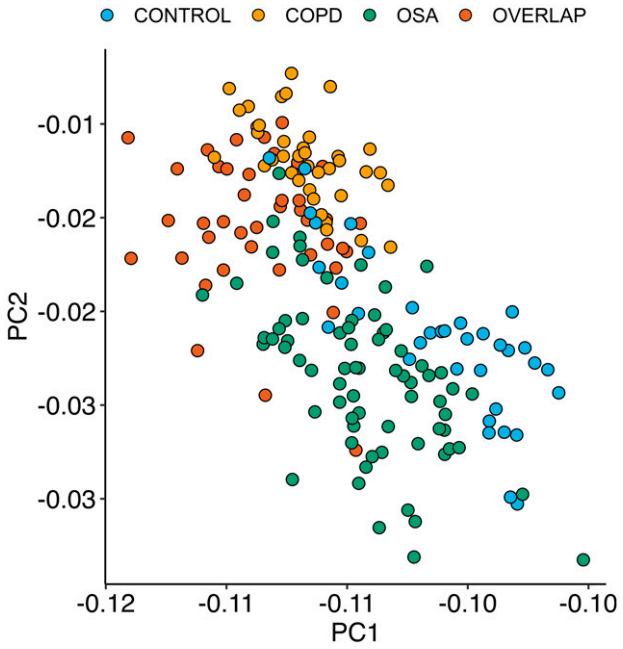
Leukocyte and neutrophils levels were also elevated in all 3 groups—COPD, OSA, and OVS—compared with controls ($P < .001$), with participants with OVS having higher levels than OSA ($P < .001$) and COPD ($P < .001$; **Figure 6A** and **Figure 6B**). Lymphocyte levels were significantly lower in COPD compared with

Figure 4—Rank-base inverse normal transformed levels and adjusted for sex, age, BMI, AHI, and FEV_1 of IL-6, hs-CRP, G-CSF, IL-8 in patients with OSA at baseline and 1 year after treatment with CPAP or 1 year with no treatment.



In the OSA group, no differences were detected in any inflammatory protein levels after a year of treatment with PAP. AHI = apnea-hypopnea index, BMI = body mass index, CPAP = continuous positive airway pressure, FEV1 = forced expiratory volume in 1 second, G-CSF = granulocyte colony stimulating factor, hs-CRP = high-sensitivity C reactive protein, IL-6 = interleukin-6, IL-8 = interleukin 8, INT = inverse normal transformation, OSA = obstructive sleep apnea.

Figure 5—Dimension reduction plot of the adjusted levels of all 27 cytokines in healthy control participants (blue), individuals with COPD (yellow), individuals with OSA (green), and individuals with OVS (red) participants.



A principal component analysis of the adjusted levels of all 27 cytokines was performed and the adjusted cytokine levels were centered and projected onto the first 2 principal components. COPD = chronic obstructive pulmonary disease, OSA = obstructive sleep apnea, OVS = overlap syndrome, PC1 = principal component 1, PC2 = principal component 2.

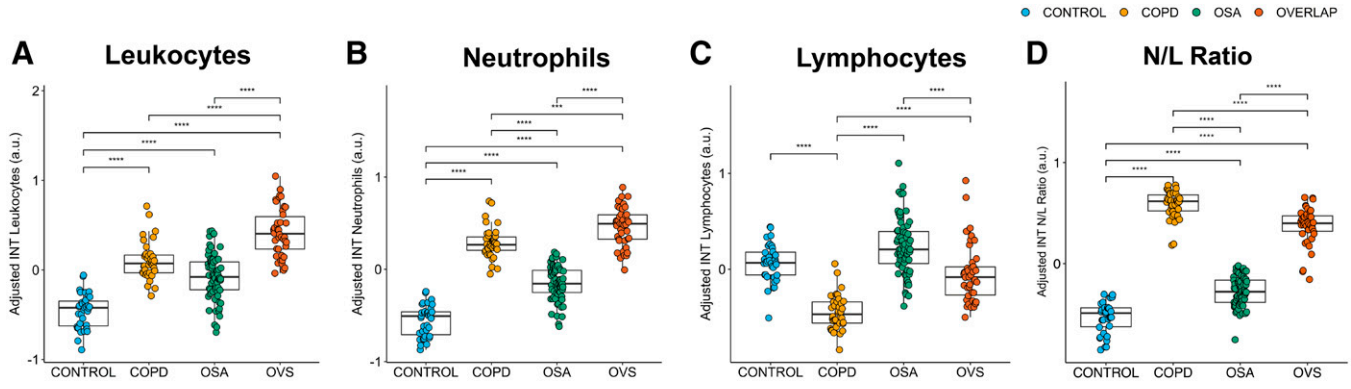
controls and OSA (**Figure 6C**). The neutrophil-to-lymphocyte ratio was higher in COPD, OSA, and COPD when compared with healthy controls (**Figure 6D**).

DISCUSSION

The present study shows that serum levels of the inflammatory markers IL-6, hs-CRP, and G-CSF are increased in patients with COPD and OVS compared with healthy controls. Furthermore, levels of IL-6, hs-CRP, and G-CSF were higher in participants with COPD and OVS compared with OSA alone. Second, we showed that participants with OVS have higher systemic inflammation levels as measured by leukocytes and neutrophils levels than COPD and OSA alone. Third, we observed no major impact of PAP therapy on cytokine levels in OSA.

Chronic low-grade inflammation is associated with the initiation and progression of atherosclerotic disease, heart failure, and obesity-related metabolic disorders.[36,37] In the past 20 years, there has been an increased understanding of the role the innate immune response pathway plays in the development of vascular diseases through biomarkers such as IL-1, IL-6, and CRP.[38] CRP is an important serum inflammation marker. It is synthesized by the liver, and its production is mostly controlled by the proinflammatory cytokine IL-6. IL-6 is a circulating cytokine known to be secreted from a number of different cells, including activated macrophages, lymphocytes, and adipocytes.[39] Inflammation is the main stimulus for IL-6 production, but other stimuli also exist, such as cigarette smoke and adiposity.[39] Given that an elevated neutrophil-to-lymphocyte ratio is considered indicative of a state of subclinical systemic inflammation in chronic diseases, our data confirm that OSA, COPD, and OVS exhibit a profile of subclinical inflammatory states (with the latter being primarily driven by the COPD component).[40,41] The higher neutrophil-to-lymphocyte ratio in COPD compared with OVS could be explained by the lower levels of lymphocytes observed in COPD. The present findings corroborate existing literature that suggests a link between the occurrence of COPD and a decrease in lymphocyte levels.[42] Our linear regression model controlled for the effect of FEV_1, so we do not believe that the

Figure 6—Rank-base inverse normal transformed levels of leukocytes, total count of neutrophils, total count of lymphocytes, and total count neutrophils/lymphocytes ratio in participants with OSA, participants with COPD, participants with OVS and healthy controls participants adjusted for sex, age, BMI, AHI and FEV_1.



(A) Leukocytes levels were higher in COPD, OSA, and OVS compared to healthy controls. OVS had higher levels of leukocytes than OSA and COPD. **(B)** Neutrophils levels were higher in COPD, OSA, and OVS compared to healthy control participants. OVS had higher levels of neutrophils than OSA and COPD. **(C)** Lymphocytes levels were lower in COPD, compared to healthy control participants. **(D)** The neutrophil/lymphocyte ratio was higher in COPD, OSA, and OVS compared with healthy controls. ***P < .001, ****P < .0001. COPD = chronic obstructive pulmonary disease, INT = inverse normal transformation, N/L = neutrophil/lymphocyte, OSA = obstructive sleep apnea, OVS = overlap syndrome.

more severe pulmonary impairment in OVS, as indicated by a lower FEV_1 value of 53% compared to 63% in COPD, is necessarily driving the differences in cytokine levels observed between these 2 groups.

OVS has been associated with an increased cardiovascular risk compared to OSA and COPD alone, and inflammation may play a role in this relationship.[22–24,26] However, scarce information on the inflammatory response in OVS is available in the scientific literature. In this work, we addressed the presence of inflammation in patients with OVS. To the best of our knowledge, this is the first study to investigate a broad range of inflammatory biomarkers (over 30 markers) in a large sample of individuals (n = 186). In our study, levels of leukocytes and neutrophils were higher in the OVS group compared with either disease alone. This finding could be of great importance since several studies have demonstrated the role of neutrophils in the initiation of atherosclerosis and in the instability of the atherosclerotic lesions.[43] Neutrophil secretion of reactive oxygen species[44] and neutrophil extension traps, which can induce endothelial dysfunction and trigger coagulation, have been detected in atherosclerotic and thrombotic lesions in humans and mice.[45,46] Similar to our results, in a small study of patients with COPD (n = 38), Macrea et al[21] showed that those with OVS (n = 17) had a higher serum percentage of peripheral neutrophils than those with COPD alone. In our study we found no differences between the COPD and OVS groups for levels of IL-6, hs-CRP, and G-CSFs. Likewise, in a recent study, Marin-Oto et al[47] found that the levels of the soluble isoform of the receptor for advanced glycationn end products, a molecule that inhibits the activation of proinflammatory signaling cascades, were lower in OVS compared with healthy controls, but similar to patients with COPD. On the contrary, Wang et al[20] found that the alveolar lavage of patients with OVS showed higher levels of neutronphils, TNFα, and IL-8 than that of patients with COPD.

This work proposes the potential utilization of G-CSF as a novel biomarker of COPD in OSA patients. G-CSF is a hematopoietic growth factor named for its role in the proliferation and differentiation of cells of the myeloid lineage. The deletion of G-CSF in a COPD mouse model led to reduced airway inflammation, reduced lung tissue destruction, and attenuated systemic inflammation.[48] Armstrong et al[49] identified some glucocorticoid-insensitive cytokines, including GM-CSF, G-CSF and IL-8, that may be involved in the progression of airway inflammation in patients with COPD. In our study, we did see increased levels of IL-8 in the COPD group compared with controls, but there was no difference in the OVS or OSA groups. Furthermore, the observation that COPD had higher G-CSF and IL-8 levels compared to those with OVS was unexpected. The reason for this observation is unclear but could reflect inadequate matching of disease severity between groups, or perhaps some protective effect of OSA, which has been postulated related to ischemic preconditioning.[50] The interactions of sustained plus intermittent hypoxia require further study in both basic and clinical studies.[51] We did not find any correlation between COPD severity, measured by FEV_1%, and G-CSF levels.

Our findings imply that nocturnal hypoxemia may not be a major contributor (or may be a minor variable) in systemic inflammation. This finding could underlie the lack of association between inflammatory cytokines and the degree of nocturnal hypoxemia, which is observed in intermittent hypoxemia (as seen in OSA and OVS) and continuous hypoxia (as seen in COPD).

Finally, although randomized trials have shown benefit to PAP therapy in OSA,[52] the bulk of the literature has not shown a major impact of PAP on cardiovascular risk.[53,54] The reason for this finding is unclear but may reflect issues with study design in which high-risk patients are not a priori identified prior to enrollment. In theory, patients with OVS may represent a high-risk group, although, to our knowledge, this patient group has not been assessed in the context of a randomized controlled trial. Thus, therapeutic uncertainty remains, but we believe rigorous studies may well be guided by studies such as the present one to facilitate robust future research.

In the case of COPD, several studies have suggested potential therapeutic benefit to PAP interventions.[55,56] However, to our knowledge, the majority of these studies have either excluded or not assessed OSA in the context of COPD. Thus, the optimal treatment of this large group of patients remains unclear.

Despite our study's strengths, we acknowledge a number of limitations. First, our patient groups were not perfectly matched, given the different comorbidities that are commonly observed in these patient populations. We regard our findings as informative for a large group of patients but acknowledge they do not generalize to other patient groups not studied. Thus, our conclusions are limited to the groups we included based on our study design. Second, we only had PAP therapy data from patients with OSA and thus we cannot draw any conclusions regarding the impact of therapeutic interventions on patients with COPD or OVS. Third, leukocyte and neutrophil counts were higher in OVS compared to COPD alone, but no significant differences were detected between these 2 groups in the panel of cytokines analyzed. This finding may potentially be explained by the fact that the cytokines we investigated in this study may not be sufficiently sensitive for the inflammatory pathways that contribute to systemic inflammation seen in OVS. It would be of interest for future studies to explore other -omic biomarkers (proteomic, epigenomic, transcriptomic, etc.) and analyze urine samples in this population to understand better the pathways that drive cardiovascular risk in OVS. We acknowledge that although IL-6, G-CSF, and hs-CRP are not specific biomarkers of COPD and OVS, they can still be useful as a screening of COPD in patients with OSA. Furthermore, we recognize that our study design for our primary analysis was cross sectional and thus the findings represent correlation rather than causation. Despite these limitations, we believe that our findings are robust and hope that they encourage further research in this area.

CONCLUSIONS

COPD and OVS were associated with higher levels of systemic inflammation biomarkers relative to OSA and healthy controls. This work proposes the potential utilization of IL-6, G-CSF, and hs-CRP as screening biomarkers for COPD in patients with

OSA. This fact is of great importance given the high prevalence of COPD among the population with OSA and the importance of an early diagnosis of the OVS, giving the higher association with cardiovascular risk. Our new findings suggest that inflammatory pathways are unlikely to fully explain the increased cardiovascular risk in OVS compared to OSA or COPD alone, suggesting other causal pathways should be investigated in subsequent studies.

ABBREVIATIONS

AHI, apnea-hypopnea index
BMI, body mass index
COPD, chronic obstructive pulmonary disease
CT90, cumulative sleep time percentage with oxyhemoglobin saturation < 90%
FEV_1, forced expiratory volume in 1 second
GOLD, Global Initiative for Chronic Obstructive Lung Disease
G-CSF, granulocyte colony stimulating factor
GM-CSF, granulocyte-macrophage colony stimulating factor
hs-CRP, high-sensitivity C-reactive protein
IL-6, interleukin 6
IL-8, interleukin 8
OSA, obstructive sleep apnea
OVS, overlap syndrome
PAP, positive airway pressure
TNFα, tumor necrosis factor α

REFERENCES

1. Benjafield AV, Ayas NT, Eastwood PR, et al. Estimation of the global prevalence and burden of obstructive sleep apnoea: a literature-based analysis. *Lancet Respir Med.* 2019;7(8):687–698.
2. Halpin DMG, Celli BR, Criner GJ, et al. It is time for the world to take COPD seriously: a statement from the GOLD board of directors. *Eur Respir J.* 2019; 54(1):1900914.
3. Flenley DC. Sleep in chronic obstructive lung disease. *Clin Chest Med.* 1985;6(4): 651–661.
4. Shawon MSR, Perret JL, Senaratna CV, Lodge C, Hamilton GS, Dharmage SC. Current evidence on prevalence and clinical outcomes of co-morbid obstructive sleep apnea and chronic obstructive pulmonary disease: a systematic review. *Sleep Med Rev.* 2017;32:58–68.
5. Tietjens JR, Claman D, Kezirian EJ, et al. Obstructive sleep apnea in cardiovascular disease: a review of the literature and proposed multidisciplinary clinical management strategy. *J Am Heart Assoc.* 2019;8(1):e010440.
6. André S, Conde B, Fragoso E, Boléo-Tomé JP, Areias V, Cardoso J; GI DPOC-Grupo de Interesse na Doença Pulmonar Obstrutiva Crónica. COPD and cardiovascular disease. *Pulmonology.* 2019;25(3):168–176.
7. Balbirsingh V, Mohammed AS, Turner AM, Newnham M. Cardiovascular disease in chronic obstructive pulmonary disease: a narrative review. *Thorax.* 2022;77(9): 939–945.
8. Tobaldini E, Costantino G, Solbiati M, et al. Sleep, sleep deprivation, autonomic nervous system and cardiovascular diseases. *Neurosci Biobehav Rev.* 2017; 74(Pt B):321–329.
9. Orrù G, Storari M, Scano A, Piras V, Taibi R, Viscuso D. Obstructive sleep apnea, oxidative stress, inflammation and endothelial dysfunction-an overview of predictive laboratory biomarkers. *Eur Rev Med Pharmacol Sci.* 2020;24(12): 6939–6948.
10. Gupta N, Malhotra N, Ish P. GOLD 2021 guidelines for COPD - what's new and why. *Adv Respir Med.* 2021;89(3):344–346.
11. Celli BR, Locantore N, Yates J, et al; ECLIPSE Investigators. Inflammatory biomarkers improve clinical prediction of mortality in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 2012;185(10):1065–1072.
12. Horvath P, Tarnoki DL, Tarnoki AD, et al. Complement system activation in obstructive sleep apnea. *J Sleep Res.* 2018;27(6):e12674.
13. Perrini S, Cignarelli A, Quaranta VN, et al. Correction of intermittent hypoxia reduces inflammation in obese subjects with obstructive sleep apnea. *JCI Insight.* 2017;2(17):e94379.
14. Zychowski KE, Sanchez B, Pedrosa RP, et al. Serum from obstructive sleep apnea patients induces inflammatory responses in coronary artery endothelial cells. *Atherosclerosis.* 2016;254:59–66.
15. Poroyko VA, Carreras A, Khalyfa A, et al. Chronic sleep disruption alters gut microbiota, induces systemic and adipose tissue inflammation and insulin resistance in mice. *Sci Rep.* 2016;6(1):35405.
16. Gileles-Hillel A, Kheirandish-Gozal L, Gozal D. Biological plausibility linking sleep apnoea and metabolic dysfunction. *Nat Rev Endocrinol.* 2016;12(5):290–298.
17. Ryan S, Nolan GM, Hannigan E, Cunningham S, Taylor C, McNicholas WT. Cardiovascular risk markers in obstructive sleep apnoea syndrome and correlation with obesity. *Thorax.* 2007;62(6):509–514.
18. Wali SO, Manzar MD, Abdelaziz MM, et al. Putative associations between inflammatory biomarkers, obesity, and obstructive sleep apnea. *Ann Thorac Med.* 2021;16(4):329–336.
19. Taheri S, Austin D, Lin L, Nieto FJ, Young T, Mignot E. Correlates of serum C-reactive protein (CRP)–no association with sleep duration or sleep disordered breathing. *Sleep.* 2007;30(8):991–996.
20. Wang Y, Hu K, Liu K, et al. Obstructive sleep apnea exacerbates airway inflammation in patients with chronic obstructive pulmonary disease. *Sleep Med.* 2015;16(9):1123–1130.
21. Macrea M, Campbell S, Martin T, Oursler KA. The peripheral neutrophils in subjects with COPD-OSA overlap syndrome and severe comorbidities: a feasible inflammatory biomarker? *Adv Clin Exp Med.* 2018;27(12):1677–1682.
22. Shah AJ, Quek E, Alqahtani JS, Hurst JR, Mandal S. Cardiovascular outcomes in patients with COPD-OSA overlap syndrome: a systematic review and meta-analysis. *Sleep Med Rev.* 2022;63:101627.
23. Marin JM, Soriano JB, Carrizo SJ, Boldova A, Celli BR. Outcomes in patients with chronic obstructive pulmonary disease and obstructive sleep apnea: the overlap syndrome. *Am J Respir Crit Care Med.* 2010;182(3):325–331.
24. Stanchina ML, Welicky LM, Donat W, Lee D, Corrao W, Malhotra A. Impact of CPAP use and age on mortality in patients with combined COPD and obstructive sleep apnea: the overlap syndrome. *J Clin Sleep Med.* 2013;9(8):767–772.
25. Sharma B, Neilan TG, Kwong RY, et al. Evaluation of right ventricular remodeling using cardiac magnetic resonance imaging in co-existent chronic obstructive pulmonary disease and obstructive sleep apnea. *COPD.* 2013; 10(1):4–10.
26. Wang J, Li X, Hou WJ, Dong LX, Cao J. Endothelial function and T-lymphocyte subsets in patients with overlap syndrome of chronic obstructive pulmonary disease and obstructive sleep apnea. *Chin Med J (Engl).* 2019;132(14):1654–1659.
27. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med.* 1997;336(14):973–979.
28. Kaptoge S, Seshasai SRK, Gao P, et al. Inflammatory cytokines and risk of coronary heart disease: new prospective study and updated meta-analysis. *Eur Heart J.* 2014;35(9):578–589.
29. Baker RG, Hayden MS, Ghosh S. NF-κB, inflammation, and metabolic disease. *Cell Metab.* 2011;13(1):11–22.
30. López-Campos JL, Peces-Barba G, Soler-Cataluña JJ, et al; en nombre del grupo de estudio CHAIN. Chronic obstructive pulmonary disease history assessment in Spain: a multidimensional chronic obstructive pulmonary disease evaluation. Study methods and organization. *Arch Bronconeumol.* 2012;48(12):453–459.
31. Marin JM, Artal J, Martin T, et al. Epigenetics modifications and subclinical atherosclerosis in obstructive sleep apnea: the EPIOSA study. *BMC Pulm Med.* 2014;14(1):114.
32. Weaver TE, Grunstein RR. Adherence to continuous positive airway pressure therapy: the challenge to effective treatment. *Proc Am Thorac Soc.* 2008;5(2): 173–178.

33. Berry RB, Budhiraja R, Gottlieb DJ, et al; Deliberations of the Sleep Apnea Definitions Task Force of the American Academy of Sleep Medicine. Rules for scoring respiratory events in sleep: update of the 2007 AASM Manual for the Scoring of Sleep and Associated Events. *J Clin Sleep Med.* 2012;8(5):597–619.
34. Lloberes P, Durán-Cantolla J, Martínez-García MÁ, et al; Spanish Society of Pulmonology and Thoracic Surgery. Diagnosis and treatment of sleep apnea-hypopnea syndrome. *Arch Bronconeumol.* 2011;47(3):143–156.
35. Miller MR, Hankinson J, Brusasco V, et al; ATS/ERS Task Force. Standardisation of spirometry. *Eur Respir J.* 2005;26(2):319–338.
36. Ridker PM. Anticytokine agents: targeting interleukin signaling pathways for the treatment of atherothrombosis. *Circ Res.* 2019;124(3):437–450.
37. Shirazi LF, Bissett J, Romeo F, Mehta JL. Role of inflammation in heart failure. *Curr Atheroscler Rep.* 2017;19(6):27.
38. Ridker PM, Rane M. Interleukin-6 signaling and anti-interleukin-6 therapeutics in cardiovascular disease. *Circ Res.* 2021;128(11):1728–1746.
39. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol.* 2014;6(10):a016295.
40. Zahorec R. Neutrophil-to-lymphocyte ratio, past, present and future perspectives. *Bratisl Lek Listy.* 2021;122(7):474–488.
41. Paliogiannis P, Fois AG, Sotgia S, et al. The neutrophil-to-lymphocyte ratio as a marker of chronic obstructive pulmonary disease and its exacerbations: a systematic review and meta-analysis. *Eur J Clin Invest.* 2018;48(8):e12984.
42. Semenzato U, Biondini D, Bazzan E, et al. Low-blood lymphocyte number and lymphocyte decline as key factors in COPD outcomes: a longitudinal cohort study. *Respiration.* 2021;100(7):618–630.
43. Silvestre-Roig C, Braster Q, Ortega-Gomez A, Soehnlein O. Neutrophils as regulators of cardiovascular inflammation. *Nat Rev Cardiol.* 2020;17(6):327–340.
44. Winterbourn CC, Kettle AJ, Hampton MB. Reactive oxygen species and neutrophil function. *Annu Rev Biochem.* 2016;85(1):765–792.
45. Westerterp M, Fotakis P, Ouimet M, et al. Cholesterol efflux pathways suppress inflammasome activation, NETosis, and atherogenesis. *Circulation.* 2018;138(9): 898–912.
46. Döring Y, Manthey HD, Drechsler M, et al. Auto-antigenic protein-DNA complexes stimulate plasmacytoid dendritic cells to promote atherosclerosis. *Circulation.* 2012;125(13):1673–1683.
47. Marin-Oto M, Sanz-Rubio D, Santamaría-Martos F, et al. Soluble RAGE in COPD, with or without coexisting obstructive sleep apnoea. *Respir Res.* 2022;23(1):163.
48. Tsantikos E, Lau M, Castelino CM, et al. Granulocyte-CSF links destructive inflammation and comorbidities in obstructive lung disease. *J Clin Invest.* 2018; 128(6):2406–2418.
49. Armstrong J, Sargent C, Singh D. Glucocorticoid sensitivity of lipopolysaccharide-stimulated chronic obstructive pulmonary disease alveolar macrophages. *Clin Exp Immunol.* 2009;158(1):74–83.
50. Lavie L, Lavie P. Ischemic preconditioning as a possible explanation for the age decline relative mortality in sleep apnea. *Med Hypotheses.* 2006;66(6):1069–1073.
51. Zhen X, Moya EA, Gautane M, et al. Combined intermittent and sustained hypoxia is a novel and deleterious cardio-metabolic phenotype. *Sleep.* 2022;45(6):zsab290.
52. Marin JM, Carrizo SJ, Vicente E, Agusti AG. Long-term cardiovascular outcomes in men with obstructive sleep apnoea-hypopnoea with or without treatment with continuous positive airway pressure: an observational study. *Lancet.* 2005; 365(9464):1046–1053.
53. McEvoy RD, Antic NA, Heeley E, et al; SAVE Investigators and Coordinators. CPAP for prevention of cardiovascular events in obstructive sleep apnea. *N Engl J Med.* 2016;375(10):919–931.
54. Sánchez-de-la-Torre M, Sánchez-de-la-Torre A, Bertran S, et al; Spanish Sleep Network. Effect of obstructive sleep apnoea and its treatment with continuous positive airway pressure on the prevalence of cardiovascular events in patients with acute coronary syndrome (ISAACC study): a randomised controlled trial. *Lancet Respir Med.* 2020;8(4):359–367.
55. Köhnlein T, Windisch W, Köhler D, et al. Non-invasive positive pressure ventilation for the treatment of severe stable chronic obstructive pulmonary disease: a prospective, multicentre, randomised, controlled clinical trial. *Lancet Respir Med.* 2014;2(9):698–705.
56. Murphy PB, Rehal S, Arbane G, et al. Effect of home noninvasive ventilation with oxygen therapy vs oxygen therapy alone on hospital readmission or death after an acute COPD exacerbation: a randomized clinical trial. *JAMA.* 2017;317(21): 2177–2186.
57. Mediano O, González Mangado N, Montserrat JM, et al; el Spanish Sleep Network. Documento internacional de consenso sobre apnea obstructiva del sueño. *Arch Bronconeumol.* 2022;58(1):52–68.

ACKNOWLEDGMENTS

Author contributions: Study conception and design: AS-A, DS-R, JMM, AM, TSS. Acquisition of the data: AS-A, MM-O, PC, AVG, DS-R, JM-S, EAM. Interpretation of the data: AS-A, LAB, LCA, JMM, AM, JM-S, TSS, OM, WG, TM. Original draft preparation: AS-A, WG, LAB, JMM, JM-S, LCA, OM, AM, TSS, TM. Funding acquisition: JMM, AM, LCA, TSS.

SUBMISSION & CORRESPONDENCE INFORMATION

Submitted for publication December 14, 2022
Submitted in final revised form March 27, 2023
Accepted for publication March 27, 2023
Address correspondence to: Ana Sanchez-Azofra, MD, University of California San Diego, 9300 Campus Point Dr, Mail code 0623A, La Jolla, CA 92037; Email: asanchezazofra@health.ucsd.edu

DISCLOSURE STATEMENT

All authors have seen and approved this manuscript. Work for this study was performed at University of California, San Diego, La Jolla, CA, and Translational Research Unit, IIS Aragon, Hospital Universitario Miguel Servet, Zaragoza, Spain. Author's declarations: A.S.A.: Alfonso Martin Escudero Foundation grant. T.S.S.: NIH R01HL145470. O.A.M.: NIH K08HL143140. Dr. Malhotra is funded by NIH. He reports income related to medical education from LIvanova, Eli Lilly, Zoll and Jazz. ResMed provided a philanthropic donation to UCSD. L.C.A: NIH K24 HL155884, R01HL137052, R01HL147326, VA Merit 1I01BX004767, TRDRP T30IP0965. J.M.M.: Grants Number PI12/02175, PI15/01940 and PI18/01524 from the Instituto Salud Carlos III, Spanish Ministry of Health, Madrid, Spain and the European Regional Development Fund (FEDER) and by a Grant Number 01/2010 from the SADAR-Pneumo Aragón, Zaragoza, Spain. The other authors report no conflicts of interest.