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ORIGINAL RESEARCH

Sex-Specific Associations Between Leucocyte Measures and Obstructive Sleep Apnea in Han Chinese

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Background: White blood cell (WBC) and its subset counts are standard, inexpensive, direct markers of inflammation. Obstructive sleep apnea (OSA) is implicated in changes in inflammation markers, and sex differences are evident in both OSA and inflammation. It is unknown whether sex modulates the relationship between OSA severity and leukocyte measures.

Methods: 1222 patients (914 males, 308 females) underwent overnight laboratorial polysomnography and measurement of WBC and its subset (lymphocyte, neutrophil, monocyte) counts. Patients were divided into primary snoring and mild, moderate, and severe OSA groups, and differences in leukocyte parameters were analyzed separately by sex in multivariable analyses.

Results: In multiple regression models, higher apnea-hypopnea index (AHI) was independently associated with neutrophil counts only in men, and with higher total WBC, lymphocyte and monocyte counts both in women and men. Further ordinal logistic regression analysis revealed a significant association between AHI and total WBC (OR 1.87, 95% CI 1.09–3.23) and neutrophil (OR 1.77, 95% CI 1.02–3.07) counts in men only. Correlation analysis also revealed more robust relationships between leukocyte measures and cardiometabolic risk markers in men than in women.

Conclusion: This study provides novel data suggesting a significant association between neutrophil count and OSA severity only in men but not women. Similarly, the relationship between leukocyte parameters and cardiometabolic risk markers were more pronounced in men than women. Our findings suggest a sex-specific impact of OSA on leukocyte measures and on their relationship with indices of cardiometabolic risk.

Keywords: obstructive sleep apnea, leukocyte measures, neutrophil, sex differences, cardiometabolic risk factors

Introduction

Obstructive sleep apnea (OSA) is a common sleep disorder characterized by complete or partial upper airway obstruction and recurrent intermittent hypoxemia (IH) during sleep.[1] OSA can also be regarded as a low-grade inflammatory disease, with increased levels of serum inflammatory markers such as C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) detected in OSA patients compared with healthy controls.[2] In addition to these inflammatory markers, white blood cell (WBC) counts and leukocyte subsets have also been reported as direct, easily accessible, and inexpensive markers of inflammation. In the Multi-Ethnic Study of Atherosclerosis (MESA) cohort, apnea-hypopnea index (AHI) was independently positively associated with neutrophil counts, although neutrophil count was in the normal range,[3,4] and OSA severity and WBC count were also positively associated in a study of children with a normal weight range.[5] In another study, neutrophil and lymphocyte counts were independently associated with AHI.[6] The association between OSA severity and leukocyte counts has also been substantiated in a meta-analysis.[7] The potential mechanisms linking OSA and inflammation are multifactorial including oxidative stress, immune system activation, gut dysbiosis caused by ischemia-reperfusion injury and upper airway injury.[8]

The repetitive intermittent hypoxemia seen in OSA may induce cardiometabolic dysfunction, obesity, hypertension, and increased risk of cardiovascular disease (CVD) and sudden death.[9] The underlying mechanisms linking OSA to

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cardiometabolic dysfunction are complex, but systemic inflammation appears to play a pivotal role. As with OSA, blood-based inflammatory markers such as CRP, TNF-α, and IL-6 are associated with incident and prevalent CVD.[10] Elevated total WBC counts or their subpopulations (lymphocytes, neutrophils and monocytes) are also related to CVD,[11,12] and the hazard ratios for mortality in those with higher WBC count deciles are higher than those with lower deciles.[13] However, these studies did not investigate the potential effect of sex on these associations.

Sex differences have been reported with respect to both OSA and inflammatory markers. OSA is more common in young and middle-aged men than in their female counterparts.[14] It has been demonstrated that prevalence of OSA in men was higher than that in women with ratios ranging from 3:1 to 5:1 in the general population.[15] However, the morbidity gap narrows in older adults, as the risk of OSA increases markedly after the menopause in women.[16] With respect to inflammatory markers, a study of healthy volunteers demonstrated that circulating CRP, leptin, and adiponectin are naturally higher in women than in men[17] and, conversely, for all age groups, men have higher WBC and monocyte counts.[18] Importantly, the relationship between OSA and CVD appears to be modified by sex, even though there are discrepancies in the data. For instance, while many studies have reported that male OSA patients are more likely to have CVD than female counterparts,[19,20] Roca et al found that OSA was independently associated with incident heart failure or death only in women.[21] Moreover, sex-specific associations between leukocyte count and CVD have also been documented. A longitudinal analysis showed a greater increase in lymphocytes across the CVD continuum in women compared with men.[22]

Therefore, little is known about the sex differences in OSA severity and leukocyte measures. In a multiethnic cohort study of 1344 patients (47% males), higher AHI was associated with increased monocyte count in women but not in men,[4] but, of note, Chinese individuals comprised less than 1% of this cohort, despite its large size. OSA is a heterogeneous disorder, and people of Chinese ethnicity are more likely to have OSA at a younger age and with less obesity.[23] Additionally, while OSA and increased WBC counts are both associated with unfavorable cardiometabolic risk profiles, the relationship between leukocyte measures and cardiometabolic markers in OSA patients are not well understood. Given the established sex differences in OSA and leukocyte measures, we sought to examine whether the associations between OSA severity and leukocyte measures are modulated by sex in a large sample of male and female Han Chinese OSA patients. We further assessed the relationship between hematological measures and cardiometabolic risk indices separately in both sexes.

Methods

Study Design and Setting

This was a cross-sectional, retrospective study carried out in the Sleep Medicine Center, West China Hospital, Sichuan University, China. The study protocol was approved by the biomedical research ethics committee of West China Hospital, Sichuan University (No. 2022(1510)).

Participants

All participants were Chinese Han adults (>18 years old) with suspected OSA. Exclusion criteria for the present study included history of an immune disorder and infectious diseases, current scheduled use of non-steroidal anti-inflammatory agents, corticosteroids, or other immunomodulatory agents; history of exposure to an antibiotic in the past 2 weeks (based on subject's self-report and a review of their electronic medical record); living at high altitude (over 1500 m); total sleep time less than 4 hour on polysomnography.

Overnight Polysomnography

All participants underwent overnight polysomnography (PSG) between Jan 2014 and November 2021 in sleep laboratory. Electrode placement, calibration, recording and scoring were carried out by senior technicians and all techniques followed American Academy of Sleep Medicine (AASM) standards.[24] Regular technician training, equipment maintenance and scoring agreement tests were conducted every month to ensure reproducibility and reliability of the PSG and also to improve the agreement of sleep stage and respiratory events scoring among technicians.

Sleep data were collected with Alice 5 or Alice 6 Diagnostic Sleep Systems (Philips Respironics, Bend, OR, USA). Measures included electroencephalography, bilateral electrooculography, electrocardiography, electromyography (submental and anterior tibialis), nasal and oral thermal airflow, nasal pressure, thoracoabdominal movements, and peripheral arterial oxygen saturation. Senior technicians blinded to any diagnosis scored sleep data.

An oxygen desaturation event was defined as at least a 3% reduction in oxygen saturation. An apnea was defined as more than 90% reduction in airflow for at least 10s and hypopnea as 30% or more reduction in nasal pressure for at least 10s associated with at least a 3% reduction in oxygen saturation or arousal. AHI was computed as the sum of apnea and hypopnea divided by total sleep time. OSA was defined as AHI ≥5 events/h, while snorers with AHI <5 events/h were included in the primary snoring group. Sleep apnea severity was divided to mild (5–14.9 events/hour), moderate (15–29.9 events/hour), or severe (≥ 30 events/hour) according to AHI.

Clinical and Blood Measurements

All subjects completed a comprehensive questionnaire assessing history of sleep complaints, general health, and medication use. Clinical examinations included weight, height, and neck, waist, and hip circumference. Menopause status was ascertained based on the characteristics of menses or time since amenorrhea.[25] Post-menopause was defined as 12 or more months of amenorrhea occurring naturally or due to surgical interventions such as bilateral oophorectomy.

Supine blood pressure (BP) was measured in the evening prior to the beginning of PSG and in the morning at the end of the sleep study by a pneumoelectric microprocessor–controlled instrument (Nissei, DS-1902, Japan). Evening and morning BP values were averaged for analysis. Hypertension was defined as (1) systolic BP ≥ 140 mmHg or diastolic BP ≥ 90 mmHg, or (2) taking antihypertensive medication, or (3) diagnosis of hypertension by a physician.

Venous blood samples were drawn in the morning after overnight PSG examination. Leucocyte parameters were measured using an automated hematology analyzer (SYSMEXXE-5000, Sysmex, Hyogo, Japan). Based on peripheral blood cell counts, four systemic inflammation markers were calculated; SII, NLR, PLR, and LMR. Calculations were as follows; SII = (neutrophils * platelets)/lymphocytes, NLR = neutrophils/lymphocytes, PLR = platelets/lymphocytes, and LMR = lymphocytes/monocytes.

Statistical Analysis

Data are presented as mean and standard deviation for continuous variables and count and percentage for categorical variables. Comparisons across different OSA severity groups were performed separately by sex using one-way ANOVA and Mann–Whitney *U*-tests for normally and non-normally distributed data, respectively, and the χ^2 test for categorical variables. Crude and adjusted linear regression models were applied to assess the relationship between OSA and leukocyte measures and to test interactions between AHI and sex. Estimates were calculated for every 5-unit increment in AHI. Adjusted models were corrected for age, body mass index (BMI), neck circumference, waist-to-hip ratio, menopausal status, smoking, alcohol use, and hypertension. Covariates in regression models were chosen based on confounding factors and potential bias referred to previous studies.[3,4] We further computed tertiles of white blood cell, neutrophil, lymphocyte, and monocyte counts in the total sample. Ordinal logistic regression analysis was used to assess independent associations between tertiles of leukocyte measures and OSA severity, defined by AHI, in female and male subjects. Adjusted odds ratios (ORs) were estimated by correcting for the abovementioned list of covariates. Correlation analysis was used to explore the association between leukocyte and cardiometabolic measures.

During statistical analysis, data regarding to smoking, drinking and history of hypertension in 10 participants were missing and we excluded them in the final analysis.

Results

1483 individuals were assessed for eligibility, and 1222 individuals were available for final analyses including 914 men and 308 women. A participant flow chart is illustrated in Figure 1. Table S1 presents the demographic and clinical characteristics of the subjects by sex. Women were older, had lower BMI, and were less likely to smoke, drink alcohol, or have hypertension and OSA compared with men.

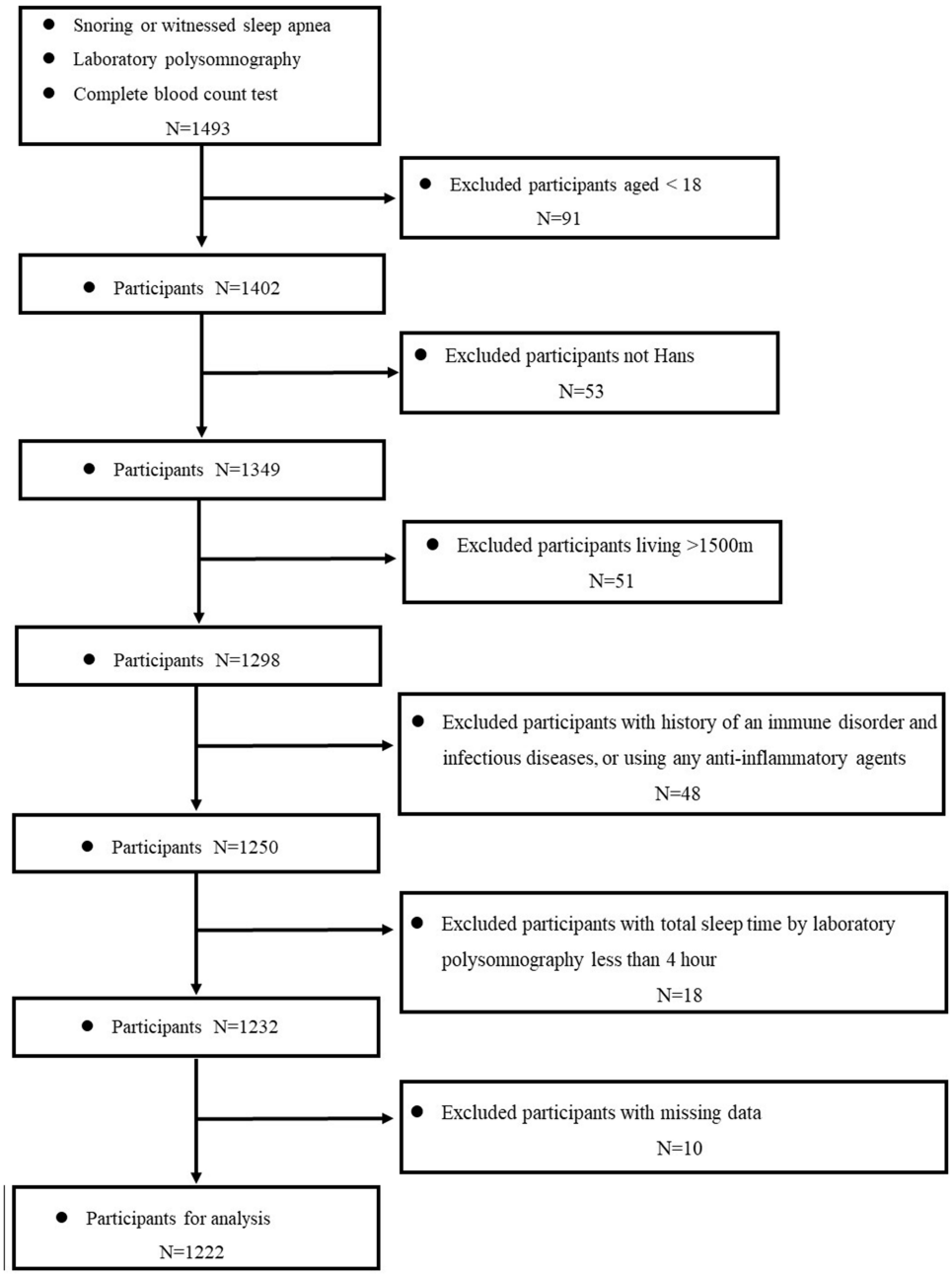

Figure 1 Flow chart for participant inclusion and exclusion.

Demographic, clinical, sleep, and inflammation characteristics of all subjects, men and women with primary snoring and OSA of different severity are described in Table S2, Table 1 and Table 2. Patients with severe OSA were older, with higher BMIs and a greater prevalence of hypertension, irrespective of sex. Women with higher AHI were also more likely to be post-menopausal. With respect to hematological measures, mean WBC, neutrophil, lymphocyte, and monocyte counts were in clinically accepted normal ranges in both men and women. Patients with severe OSA had higher WBC and neutrophil counts only in men, while lymphocyte and monocyte counts were higher in both women and men. There were no associations between OSA severity and NLR, monocyte to lymphocyte ratio, systemic inflammation index, or red cell distribution width in either men or women.

Figure S1 illustrates significant correlations between AHI and WBC counts, including its subsets. Specifically, when analyzed separately, there were stronger associations between AHI and WBC, as well as neutrophil count, in men. Conversely,

Table 1 Demographic, Clinical, Sleep, and Inflammation Characteristics of Primary Snoring and OSA Men

	Primary Snoring	OSA			p
		Mild	Moderate	Severe	
	(n=73)	(n=116)	(n=130)	(n=595)	
Age, years	35.91±11.72	42.60±12.58	42.61±10.36	44.66±10.96	**<0.001**
BMI, kg/m^2	22.95±2.75	24.96±3.20	25.59±2.87	27.52±3.43	**<0.001**
Neck circumference, cm	36.75±2.45	38.09±2.96	38.77±2.95	40.15±3.09	**<0.001**
Waist circumference, cm	84.01±8.79	89.53±8.84	91.76±8.60	96.98±9.46	**<0.001**
Hip circumference, cm	94.35±9.53	98.85±6.02	100.22±7.96	102.61±6.72	**<0.001**
Waist–to-hip ratio	0.89±0.08	0.90±0.06	0.92±0.06	0.94±0.06	0.903
Smoking, n (%)	23(31.51)	53(45.68)	60(46.15)	293(49.24)	**0.029**
Alcohol drinking, n (%)	21(28.77)	55(47.41)	56(43.08)	305(51.26)	**0.001**
Hypertension, n (%)	13(17.81)	24(20.67)	37(28.46)	299(50.25)	**<0.001**
Diabetes, n (%)	2(2.74)	4(3.45)	6(4.62)	31(5.21)	0.708
SBP, mmHg	118.82±12.95	120.47±12.35	123.70±13.89	128.52±14.54	**<0.001**
DBP, mmHg	76.91±9.89	77.30±8.59	81.67±10.48	85.46±10.98	**<0.001**
TST, min	436.15±64.15	433.34±67.19	437.93±56.01	451.76±59.48	0.761
N1, %	19.03±9.38	23.23±11.86	26.68±12.93	44.58±20.09	**<0.001**
N2, %	53.42±9.92	52.64±13.00	49.43±11.20	36.51±17.61	**<0.001**
N3, %	8.75±7.03	6.61±6.40	6.71±6.13	3.06±4.37	**0.001**
R, %	18.79±6.28	17.52±6.88	17.18±5.46	15.85±5.50	**0.001**
AHI, events/h	2.21±1.37	9.32±2.99	22.15±4.52	62.85±19.69	**<0.001**
ODI, events/h	2.11±1.53	8.37±8.23	19.89±8.00	61.64±23.12	**<0.001**
Mean SaO_2, %	95.99±1.02	95.44±1.22	95.03±1.80	91.06±4.51	**<0.001**
Nadir SaO_2, %	87.30±9.00	85.85±5.85	79.06±11.03	61.37±19.00	**<0.001**
Arousal index, events/h	18.83±9.92	19.34±10.07	21.86±9.91	43.35±21.71	**<0.001**
WBC, *10 ^{9}/L	6.12±1.35	6.47±1.49	6.78±1.66	7.23±1.70	**<0.001**
Neutrophil, *10 ^{9}/L	3.61±1.13	3.68±1.03	3.95±1.32	4.23±1.31	**<0.001**
Lymphocyte, *10^9/L	1.98±0.52	2.20±0.64	2.20±0.70	2.35±0.64	**<0.001**
Monocyte, *10^9/L	0.38±0.11	0.41±0.13	0.43±0.15	0.44±0.15	**0.001**
Eosinophil, *10^9/L	0.12±0.09	0.18±0.20	0.17±0.13	0.21±0.38	0.142
Basophil, *10 ^{9}/L	0.03±0.02	0.04±0.06	0.03±0.02	0.04±0.05	0.181
Plate count, *10^9/L	179.42±63.14	192.37±64.84	186.85±65.15	193.67±56.96	0.206
RDW, fl	13.18±0.99	13.33±1.28	13.31±0.95	13.32±1.07	0.788
NLR	1.98±1.02	1.77±0.58	1.99±1.23	1.91±0.78	0.187
PLR	96.74±42.47	90.35±29.09	90.97±37.27	87.06±31.26	0.082
MLR	0.20±0.07	0.19±0.07	0.21±0.09	0.20±0.08	0.443
SII	351.61±204.95	334.35±141.20	371.83±254.90	365.91±183.32	0.367

Notes: Categorical variables are presented as number and percentage, and continuous variables are presented as mean ± SD. P-values below 0.05 are highlighted in bold typeface.

Abbreviations: OSA, obstructive sleep apnea; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TST, total sleep time; N1-3, non-rapid eye movement sleep 1–3; R, rapid eye movement sleep; AHI, apnea-hypopnea index; ODI, oxygen desaturation index; SaO_2, oxygen saturation; WBC, white blood cell; RDW, red cell distribution width; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte ratio; SII, systemic inflammation index.

closer relationships were observed between AHI and lymphocyte, as well as monocyte count in women, as depicted in Figure 2. The results of linear regression models examining associations between AHI and hematological measures in men and women are presented in Table 3. In the univariate models, there were significant positive correlations between AHI and WBC, neutrophil, lymphocyte, and monocyte levels in both sexes. The relationship between AHI and total WBC, monocyte and lymphocyte counts remained significant in both Model 1 (adjusted for age and BMI) and Model 2 (also adjusted for neck circumference, waist-to-hip ratio, smoking, alcohol use, hypertension, and menopausal status for women), regardless of sex.

Table 2 Demographic, Clinical, Sleep, and Inflammation Characteristics of Primary Snoring and OSA Women

	Primary Snoring	OSA			p
		Mild	Moderate	Severe	
	(n=94)	(n=78)	(n=50)	(n=86)	
Age, years	37.64±11.50	45.50±13.69	50.54±10.82	54.42±10.83	**<0.001**
BMI, kg/m^2	21.68±3.37	23.49±3.62	24.82±3.26	26.67±4.35	**<0.001**
Neck circumference, cm	32.52±2.85	33.50±2.66	34.15±2.45	35.49±3.30	**<0.001**
Waist circumference, cm	76.96±9.58	82.38±10.14	85.69±10.18	93.66±12.53	**<0.001**
Hip circumference, cm cm	92.78±7.21	95.79±6.51	97.17±7.26	101.23±8.28	**<0.001**
Waist–to-hip ratio	0.83±0.07	0.86±0.08	0.88±0.07	0.92±0.07	**<0.001**
Post-menopause, n (%)	13(13.83))	26(33.33)	23(46.0)	55(63.95)	**<0.001**
Smoking, n (%)	4(4.26)	3(3.85)	0	6(6.98)	0.286
Alcohol use, n (%)	5(5.3)	4(5.12)	1(2)	10(11.63)	0.119
Hypertension, n (%)	14(14.89)	24(30.77)	20(40.0)	60(69.78)	**<0.001**
Diabetes, n (%)	1(1.06)	3(3.85)	1(2)	4(4.65)	0.498
SBP, mmHg	113.96±14.86	117.85±18.02	121.80±18.68	133.23±16.25	**<0.001**
DBP, mmHg	72.23±8.89	74.15±11.28	75.42±10.57	82.09±11.06	**<0.001**
TST, min	430.03±87.88	418.66±68.09	422.71±72.23	419.86±69.65	0.750
N1, %	18.36±12.71	20.18±11.01	23.18±12.49	41.63±19.76	**<0.001**
N2, %	54.31±12.69	55.16±10.98	54.97±10.49	38.24±15.81	**<0.001**
N3, %	8.83±7.82	7.04±6.53	3.67±5.18	4.42±5.04	**<0.001**
R, %	18.51±5.79	17.65±6.34	18.17±5.42	15.71±5.71	**0.01**
AHI, events/h	1.76±1.54	9.60±3.10	21.48±3.89	64.67±23.13	**<0.001**
ODI, events/h	2.16±4.39	8.54±4.48	17.84±6.73	63.63±24.92	**<0.001**
Mean SaO_2, %	96.49±1.18	95.47±1.36	94.78±1.27	91.64±1.46	**<0.001**
Nadir SaO_2, %	90.87±3.82	85.64±8.33	81.44±9.85	65.69±17.90	**<0.001**
Arousal index, events/h	12.73±8.04	15.48±10.17	19.93±10.09	36.35±20.64	**<0.001**
WBC, *10 ^{9}/L	6.02±1.56	5.85±1.44	6.11±1.69	6.60±1.77	**0.019**
Neutrophil, *10 ^{9}/L	3.54±1.19	3.39±1.14	3.64±1.37	3.72±1.38	0.384
Lymphocyte, *10 ^{9}/L	2.00±0.62	1.96±0.58	1.96±0.43	2.33±0.70	**<0.001**
Monocyte, *10 ^{9}/L	0.35±0.12	0.34±0.11	0.36±0.11	0.40±0.14	**0.006**
Eosinophil, *10 ^{9}/L	0.11±0.09	0.13±0.09	0.12±0.12	0.15±0.12	0.11
Basophil, *10 ^{9}/L	0.03±0.02	0.03±0.02	0.03±0.01	0.03±0.02	0.46
Plate count, *10 ^{9}/L	209.24±66.27	192.68±61.07	201.36±70.92	213.56±74.20	0.225
RDW, fl	13.47±1.29	13.39±1.16	13.39±1.17	13.64±1.19	0.517
NLR	1.92±0.92	1.88±0.86	1.90±0.71	1.70±0.74	0.285
PLR	112.92±42.92	107.06±49.58	106.59±43.90	96.39±39.16	0.091
MLR	0.18±0.07	0.18±0.07	0.18±0.06	0.17±0.05	0.689
SII	399.24±207.55	363.09±230.92	402.84±274.47	362.64±232.80	0.571

Notes: Categorical variables are presented as number and percentage, and continuous variables are presented as mean ± SD. P-values below 0.05 are highlighted in bold typeface.

Abbreviations: OSA, obstructive sleep apnea; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TST, total sleep time; N1-3, non-rapid eye movement sleep 1–3; R, rapid eye movement sleep; AHI, apnea hypopnea index; ODI, oxygen desaturation index; SaO_2, oxygen saturation; WBC, white blood cell; RDW, red cell distribution width; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte; SII, systemic inflammation index.

In the fully adjusted model, AHI was significantly associated with neutrophil count only in men. There was no interaction effect between sex and OSA for leukocyte measures (all $p > 0.05$).

When we examined the association between OSA severity and WBC parameters categorized as tertiles, we found that only men with AHI >30 had a higher odds of a higher WBC count (OR 1.87, 95% CI 1.09–3.23) and higher neutrophil count (OR 1.77, 95% CI 1.02–3.07) compared with those with primary snoring in the fully adjusted model (Figure 3).

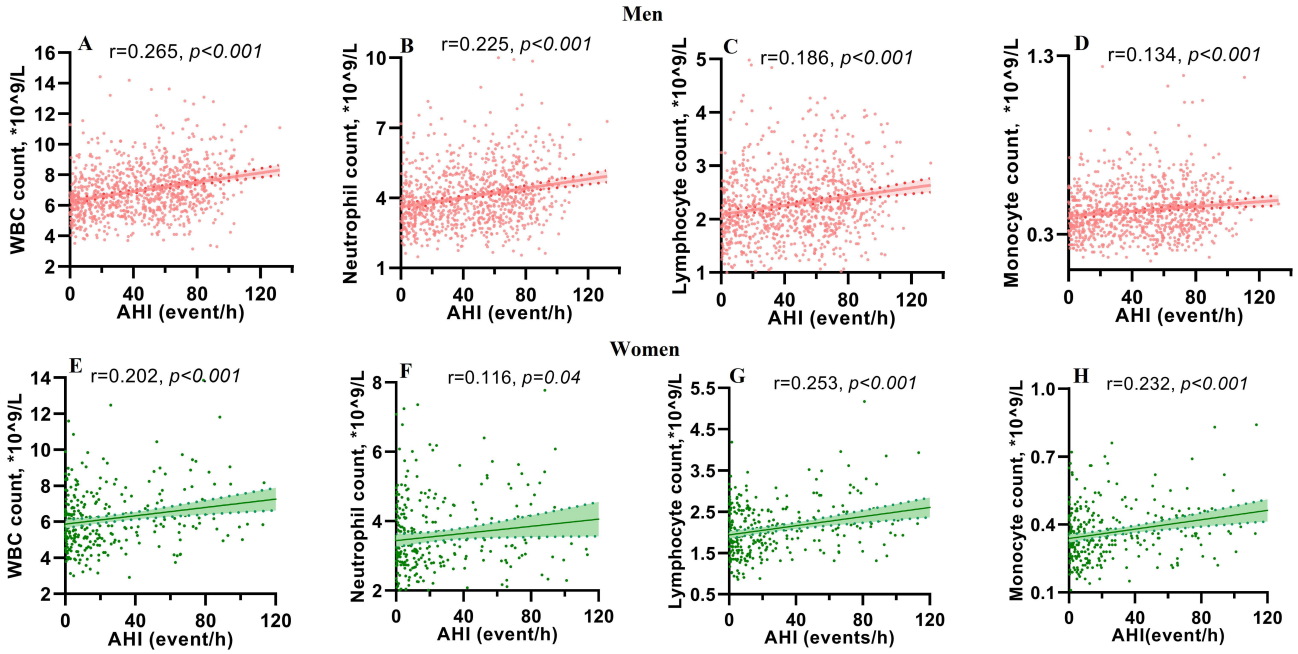

Figure 2 Scatter plots of apnea-hypopnea index vs total white blood cell, neutrophil, lymphocyte, and monocyte counts (**A-D** for men on the upper panel and **E-H** for women on the lower panel).
Abbreviation: WBC, white blood cell; AHI, apnea hypopnea index.

The odds for higher lymphocyte count and monocyte count with severe OSA in women were 2.21 (95% CI 1.05–4.62) and 2.67 (95% CI 1.26–5.68) respectively.

Table 4 shows the correlation analysis between leukocyte measures and cardiometabolic risk markers in all subjects and separately for men and women. In the entire sample, WBC and subset counts were positively associated with BMI, waist and hip circumference, waist-to-hip ratio, blood pressure, and comorbid hypertension. When examined separately, the relationship between subset counts and cardiometabolic risk factors were consistently observed and correlation coefficients were overall higher in men than in women.

Table 3 Relationship between AHI and inflammatory. markers in female and male subjects

	AHI					
	Crude Model		Model 1		Model 2	
	B (95% CI)	p	B (95% CI)	p	B (95% CI)	p
WBC						
Men	0.08(0.06, 0.10)	**<0.001**	0.05(0.03, 0.07)	**<0.001**	0.05(0.03, 0.07)	**<0.001**
Women	0.06(0.03, 0.09)	**0.001**	0.05(0.01, 0.08)	**0.01**	0.04(0.002, 0.09)	**0.038**
Neutrophil						
Men	0.03(0.001, 0.05)	**0.044**	0.04(0.02, 0.05)	**<0.001**	0.03(0.01,0.05)	**0.001**
Women	0.03(0.01, 0.05)	**0.04**	0.01(−0.01, 0.04)	0.314	0.01(−0.02, 0.05)	0.451
Lymphocyte						
Men	0.02(0.01, 0.03)	**<0.001**	0.01(0.004, 0.02)	**0.003**	0.01(0.004, 0.02)	**0.005**
Women	0.03(0.02, 0.04)	**<0.001**	0.03(0.01, 0.04)	**<0.001**	0.03(0.01, 0.04)	**0.001**

(*Continued*)

Table 3 (Continued).

	AHI					
	Crude Model		Model 1		Model 2	
	B (95% CI)	p	B (95% CI)	p	B (95% CI)	p
Monocyte						
Men	0.003(0.002, 0.005)	**<0.001**	0.002(0.0001, 0.004)	**0.039**	0.002(0.0002, 0.004)	**0.048**
Women	0.005(0.003, 0.008)	**<0.001**	0.005(0.002, 0.008)	**<0.001**	0.005(0.002, 0.008)	**0.003**

Notes: P-values below 0.05 are highlighted in bold typeface. Model 1: adjusted for age and body mass index; Model 2: adjusted for variables included in model 1 and for neck circumference, waist-to-hip ratio, smoking, alcohol use, and hypertension (plus menopausal status in women). Estimates are calculated for every 5-unit increase in apnea-hypopnea index (AHI).
Abbreviation: AHI, apnea-hypopnea index.

Discussion

In this cross-sectional study, we observed significant sex-specific associations between OSA severity and WBC and WBC subset counts. OSA severity was independently associated with total WBC and neutrophil counts in men but not in women after full adjustment. We showed that higher AHI was independently associated with higher lymphocyte and monocyte counts, irrespective of sex. Importantly, we found more robust relationships between leukocyte measures and cardiometabolic risk markers in men than in women with OSA. We previously reported sex differences in association between erythrocyte parameters and OSA.[26] Thus, OSA may induce different changes in hematologic parameters in different sexes.

OSA has been recognized as a chronic low-grade inflammation disease with increased IL-6, CRP and other inflammation markers. Total WBC or its subset counts were direct, more accessible and less expensive indicators of inflammation. Most prior studies have focused on associations between neutrophil-to-lymphocyte ratio and OSA.[27,28] Few have explored the association between OSA and WBC counts, and the results have been inconsistent.[3,4,6,29] It can be related to differences in study population. Previous studies has been demonstrated higher prevalence of OSA for Chinese due to anatomical features with a narrower airway,[30] and our Chinese are more likely to have OSA at younger age and lower BMI. In our study with Han Chinese, after full adjustment, we found that total WBC counts increased with OSA severity (as defined by AHI), which was consistent with a meta-analysis.[7] Furthermore, there was no significant interaction between sex and OSA for total WBC counts.

A few studies have been conducted on OSA and WBC subset in large samples of OSA patients. In the large MESA cohort, OSA was independently associated with an increase in neutrophil counts after full adjustment (covariates included

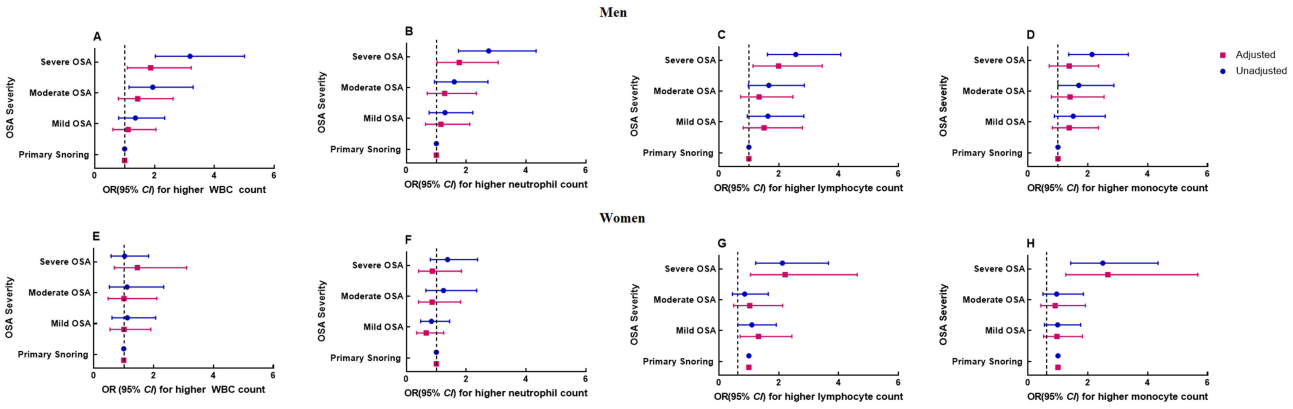

Figure 3 Odds Ratio (95% CI) for higher WBC, neutrophil, lymphocyte, and monocyte counts (**A-D** for men on the upper panel, **E-H** for women on the lower panel).
Abbreviations: OR, Odd ratio; CI, confidence interval; OSA, obstructive sleep apnea. Adjusted variables included age, body mass index, neck circumference, waist-to-hip ratio, smoking, alcohol use, and hypertension (plus menopausal status in women).

Table 4 Correlation coefficients between inflammatory markers and cardiometabolic markers in all subjects and separately by sex

	BMI	WC	HC	W/H	SBP	DBP	Hypertension
White blood cell							
Total	0.291**	0.310**	0.235**	0.262**	0.153**	0.204**	0.164**
Men	0.257**	0.273**	0.195**	0.230**	0.123**	0.162**	0.151**
Women	0.224**	0.219**	0.182**	0.147*	0.154**	0.138*	0.112*
Neutrophil							
Total	0.243**	0.258**	0.180**	0.209**	0.142**	0.185**	0.151**
Men	0.212**	0.231**	0.141**	0.160**	0.068*	0.108**	0.074**
Women	0.196**	0.167**	0.144*	0.081	0.135*	0.147*	0.091
Lymphocyte							
Total	0.209**	0.222**	0.203**	0.195**	0.093**	0.121**	0.090**
Men	0.199**	0.197**	0.192**	0.161**	0.068*	0.108**	0.056
Women	0.131*	0.174**	0.145*	0.174**	0.101	0.029	0.075
Monocyte							
Total	0.192**	0.225**	0.161**	0.208**	0.076**	0.120**	0.092**
Men	0.146**	0.162**	0.103**	0.149**	0.025	0.049	0.057
Women	0.137*	0.179**	0.146*	0.137*	0.124*	0.125*	0.108

Notes: *p < 0.05; **p < 0.01.
Abbreviations: BMI, body mass index; WC, waist circumference; HC, hip circumference; W/H, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure.

sex), while no associations between AHI and lymphocyte;[3,4] however, this study did not perform subgroup analysis according to sex. In another study of 1087 male patients, OSA severity was also independently related to higher neutrophil counts.[6] It is conceivable that such discrepant associations between WBC subset counts and OSA severity can be partly attributed to sex differences, although this hypothesis has not been systematically investigated in previous studies. Some studies only included male subjects, thus limiting the exploration of sex differences. In addition, subgroup analysis was not performed in previous studies, which may have weakened the effect of AHI on WBC levels in men. Our study also found that higher neutrophil counts were associated with AHI only in men but not in women. This observed sex discrepancy in OSA on neutrophil levels may partially be explained by age; in our study, male patients (mean age 43 years) were younger than female patients (mean age 47 years). Age affects WBC measures and could reasonably be implicated in our findings. For men, neutrophil count increased up to 55 years, whereas women appeared to have a bimodal distribution (peaked at 50 years then decreased until 60 years).[18] Another possible explanation for the neutrophil elevation only in men is that sex hormones play an important role in the maturation and metabolism of neutrophils.[31]

Neutrophils, a major category of leukocytes, are the main innate response cell acting as the first line of defense in acute inflammatory conditions, and there is also evidence supporting their role in chronic inflammatory conditions such as atherosclerosis and CVD.[32,33] Our findings suggest greater aggregation of cellular inflammatory markers in men than in women, a pattern that parallels several reports of a greater prevalence of CVD in men than in women.[34] Additionally, we found that AHI was associated with higher lymphocyte and monocyte counts in both men and women. This positive relationship between OSA and lymphocyte counts is consistent with another study with only male OSA patients.[6] However, the MESA cohort did not detect an association between AHI and lymphocyte or monocyte levels,[3] which might partially be explained by including CVD patients with lower lymphocyte counts.[3] In our study, we found evidence indicating that obstructive sleep apnea (OSA) could lead to elevated white blood cell counts in both men and women. Moreover, we observed a sex-specific differences in the increased subsets of white blood cells. Specifically, in men, there was a predominant increase in neutrophils, whereas in women, we observed elevated counts of lymphocytes and monocytes.

Although we detected significant increased leukocyte and composition counts, their average values both in women and men were within normal limits. Notwithstanding this, even within the normal spectrum, elevated leucocyte measures may have detrimental effects on health. We observed significant associations between leukocyte measures and cardiometabolic risk markers, such as BMI and blood pressure. In the general population, elevated WBC count is associated

with future development of hypertension and diabetes, higher blood pressure, higher glycated hemoglobin, and even CVD mortality.[13,35–37] For leukocyte subpopulations, subjects with the highest neutrophil counts were at higher risk of CVD mortality.[12] When we examined female and male OSA patients separately, we found more robust relationships between leukocyte measures and cardiometabolic risk markers in men than in women. These findings suggest that the increases in leukocyte measures are associated with greater cardiometabolic risk in men. Notably, among circulating leukocyte subpopulations, neutrophil counts in men have most consistently been associated with fatal and non-fatal CVD.[12] These findings also implicate a greater aggregation of cardiometabolic risk factors in men than in women with OSA. OSA and low-grade inflammation have been identified as risks for cardiometabolic disease. Therefore, our findings suggest potential therapies that focus on alleviating the inflammation linking OSA and cardiometabolic diseases.

Several limitations must be taken into account when interpreting our findings. First, the major limitation is the cross-sectional study design, we cannot draw causal inferences with regard to the relationships between OSA, leucocyte measures, and cardiometabolic risk markers. Second, the sample size of female patients was relatively small. The lower sample of women may be due to lack of recognition of the unique signs and symptoms of OSA presented uniquely in women. We have to acknowledge the smaller female sample size and sex imbalance reduces confidence to detect significant associations between OSA severity and leukocyte measures in women compared to men. Despite this, we conducted a sample estimation and the sample size of female subjects was sufficient to detect differences in WBC and subsets counts. Longitudinal studies with more equal numbers of male and female participants are needed to validate and extend our findings and assess whether OSA predicts future increases in leucocyte measures and whether such increases mediate the onset of adverse events in a sex-dependent manner. Therapeutic studies are also needed to determine whether OSA therapy contributes to lowering WBC measures and whether the anticipated positive effects are magnified in men. Third, our study lacked healthy control and used primary snoring group as comparison. In addition to this, unmeasured confounders and selection bias could influence the association between OSA and WBC measures in this clinical sample.

Conclusion

In conclusion, our study provides novel evidence of sex-dependent associations between OSA severity and elevated WBC subset counts. Specifically, neutrophil counts were only found to increase in male OSA patients, whereas in women, we observed predominant elevated counts of lymphocytes and monocytes. Importantly, leucocyte measures are more closely related to cardiometabolic risk markers in men than in women, suggesting that the impact of OSA on inflammation and cardiometabolic disease may be potentiated in men. Our study may contribute to tailored screening and recognition of OSA, especially for men with relatively high WBC or high neutrophil count and cardiometabolic risk factors. This may lead to early diagnosis and better management for OSA patients, and may stimulate further research into sex-dependent manners in OSA.

Abbreviations

AHI, apnea-hypopnea index; BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; MESA, the Multi-Ethnic Study of Atherosclerosis cohort; NLR, neutrophil-to-lymphocyte ration; OSA, obstructive sleep apnea; PSG, polysomnography; WBC, white blood cell.

Data Sharing Statement

The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request.

Ethics Approval and Informed Consent

This was a cross-sectional, retrospective study. The study was approved by the West China Hospital of Sichuan University Biomedical Research Ethics Committee (Number: 2022 (1510)). The requirement for individual informed consent was formally waived by the committee because the data were collected from medical records. Our study adheres to the principles of the Declaration of Helsinki. All identifying information has been anonymized to protect privacy of participants.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no competing interests.

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