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SLEEP

C-Reactive Protein Levels and the Risk of Incident Cardiovascular and Cerebrovascular Events in Patients with Obstructive Sleep Apnea

N. T. Ayas[1,2,3,6] · A. J. Hirsch Allen[1,3] · N. Fox[1] · B. Peres[3,4] · M. Mehrtash[1] · K. H. Humphries[5] · R. Jen[1,2] · C. M. Taylor[5] · S. F. van Eeden[2]

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Abstract

Purpose Patients with obstructive sleep apnea (OSA) are at increased risk of cardiovascular and cerebrovascular disease (CVD) but it is unclear who are at greatest risk. We determined whether the inflammatory marker, C-reactive protein (CRP), could be a useful prognostic biomarker.

Methods Adult patients referred for polysomnography (PSG) with OSA were studied. Serum CRP levels were measured using ELISA the morning after PSG. Validated CV events within 4 years of PSG were ascertained by linking to provincial research datasets.

Results 155 patients with OSA (AHI ≥ 5/h) had CRP measured. Median age was 53 and median AHI was 21/h. 10 patients (7.1%) suffered at least one event, but rates varied substantially by CRP (0/35 patients in the lowest quartile, and 7/39 in the highest CRP quartile). In the unadjusted analysis, patients in the highest CRP quartile (≥ 2.38 mg/L) were significantly more likely to suffer an event (odds ratio = 9.72 (95% CI 2.43–38.84), $p = 0.001$). CRP continued to be a significant predictor after controlling for multiple confounders. OSA severity and desaturation were not significantly associated with prospective events.

Conclusions In this small preliminary study, OSA patients with an elevated CRP were significantly more likely to suffer a CVD event in the 4 years after PSG. Although these findings need to be confirmed in larger prospective cohorts, CRP may be useful in risk stratifying OSA patients to guide therapy or to identify patients that might be most appropriate for clinical trials of CVD prevention.

Keywords Sleep apnea · Biomarkers · Cardiovascular

Introduction

Patients with obstructive sleep apnea (OSA) are at increased risk of cardiovascular and cerebrovascular disease (CVD) [1]. One potential mechanism for this increased risk is the systemic inflammatory response associated with the development of atherosclerosis [2]. C-reactive protein (CRP), an inflammatory biomarker, is an independent predictor value of CV events [3]. Also, a recent randomized controlled trial of an anti-inflammatory (interleukin-1β inhibitor) showed a reduction in CV events with treatment confirming the pivotal role of systemic inflammation [4]. Furthermore, rodents exposed to intermittent hypoxia and patients with OSA have increased systemic inflammation, including elevated levels of CRP, which in part is mediated by oxidative stress [5]. This suggests the systemic inflammatory response may be a pivotal pathway by which OSA leads to CVD [6].

Study was performed at the University of British Columbia, Vancouver, British Columbia, Canada.

✉ N. T. Ayas
nayas@providencehealth.bc.ca

1 Leon Judah Blackmore Centre for Sleep Disorders, University of British Columbia (UBC), Vancouver, Canada

2 Respiratory Division, Department of Medicine, UBC, Vancouver, Canada

3 Canadian Sleep and Circadian Network, Montreal, Canada

4 Department of Oral Health Sciences, Faculty of Dentistry, UBC, Vancouver, Canada

5 Division of Cardiology, Department of Medicine, UBC, Vancouver, Canada

6 Diamond Centre, 7th Floor, 2775 Laurel Street, Vancouver, BC V5Z 1M9, Canada

However, it is unclear which OSA patients are at particularly high risk, as factors such as sleep apnea severity are not particularly robust predictors. Identifying high risk patients would be valuable as more aggressive treatment of OSA and other CV risk factors could be considered. Furthermore, these patients could be preferentially enrolled in randomized controlled trials of CVD preventative therapy [7].

Circulating inflammatory biomarkers may be useful in identifying such a high-risk group. The purpose of this study was to determine if levels of CRP would be useful as a prognostic biomarker in predicting CV events in patients with OSA.

Methods

Study Participants

Adult patients (> 18 years of age) referred to the University of British Columbia Hospital Sleep Disorder Laboratory for inpatient polysomnography (PSG) for suspected OSA between January 2003 and July 2011 were eligible. Patients were excluded if they were medically unstable, had a mental disability or dementia, active psychiatric disease, or were unable to provide informed consent. The study was approved by the UBC Behavioural Research Ethics Board (H13-00346).

On the night of PSG, each patient was given a questionnaire that was completed in the laboratory which included self-reported information about medical history (including physician diagnosed cardiovascular disease, diabetes, hypertension, and use of lipid lowering medication), and smoking status. Sleepiness was measured by the Epworth Sleepiness Scale (ESS), an eight-question survey asking a patient his/her likelihood of falling asleep during different activities [8]. Body Mass Index (BMI) was calculated from height and weight measurements taken while patients were wearing light clothing.

CPAP prescription was determined by reviewing clinic records, and adherence defined as use of CPAP more than 4 h/night more than 70% [9] of nights extracted from physician reports. Adherence was predominately based on subjective reports rather than objective CPAP downloads, as this technology was not readily available during this time.

Measurement of CRP

All blood samples were collected the morning after PSG by venipuncture and centrifuged into aliquots of serum, plasma, and genetic material; these were stored in a − 80 °C freezer. Samples of serum were thawed and levels of C-reactive protein (CRP) were measured using a high sensitivity ELISA assay (R&D Systems, Minneapolis, MN, USA).

Ascertainment of OSA Severity

Inpatient full night attended polysomnogram (PSG) was performed using conventional instrumentation and scored according to the recommendations on syndrome definition and measurement techniques published by the American Academy of Sleep Medicine [10]. Patients were defined as having OSA and included in the analysis if AHI was ≥ 5/h of sleep. OSA severity was also classified according to standard threshold values (AHI < 15/h for mild, ≥ 15 and < 30 for moderate, ≥ 30/h for severe disease) [11]. Desaturation was defined as the percentage of sleep time spent below 90%.

Ascertainment of Events

The following outcomes were used to define the composite outcome of cardiovascular and cerebrovascular events: stroke, myocardial infarction, hospitalization for heart failure, acute coronary syndrome including myocardial infarction, ventricular tachycardia, atrial fibrillation, percutaneous coronary intervention, pacemaker, coronary artery bypass graft, cardioversion and/or ablation. These events were identified by linking patients to three different research databases in British Columbia through Population Data BC: the Cardiac Services of BC (CSBC) Database, Discharge Abstract Database (DAD), and Vital Statistics Database. Linkage thus captured BC residents who had an invasive CVD procedure, a CVD related hospitalization, and death from any cause. All events that happened within 4 years following PSG were obtained. Of note, the coding for these outcomes have been validated in previous studies [12].

Analysis

Patients' inclusion in the analysis was determined by BC residency, which was based on Medical Service Plan (MSP) registry data. Patients were excluded if they were absent from the registry for a period of > 6 consecutive months at any point in the 4 years following PSG, a criterion used in previous studies of CVD outcomes using these datasets [13].

For continuous variables, median and interquartile range values were reported. For categorical variables, counts and percentages were reported.

We assessed whether the highest quartile of CRP was associated with variables such as age, sex, OSA severity ((log (AHI + 1) was used as AHI distribution was skewed), desaturation, subjective sleepiness (Epworth Sleepiness Scale score), BMI, lipid lowering medication, hypertension, prior myocardial infarction or stroke, diabetes, and family history of early CVD (CVD in father prior to 55 years of age,

or CVD in mother prior to 65 years of age) using logistic regression.

The relationship between level of CRP and the incidence of CVD was assessed prospectively, using CRP quartiles. Because of relatively equal exposure time (only 6 patients (3.87%) did not have 4 years of follow-up available), relatively rare outcome events, and complete follow-up of all patients, logistic regression was selected as the model for analysis. We assessed whether other variables described above were associated with the composite outcome using logistic regression models.

Results

490 patients had blood taken and stored; 180 patients had CRP measured. Of note, only 6 patients were excluded because of absence from the registry for more than 6 months. Of these, 155 had OSA and were used in the analysis (Table 1). The majority were male, median age was 53 yrs, and median AHI was 21/h though degree of desaturation was modest (median of 7.4% of time spent below 90%).

Median CRP was 1.61 mg/L. Maximum value was 2.75 mg/L as exact levels above this were not available from the ELISA; 34 patients had a CRP at or above this level. Characteristics of patients classified by CRP are shown in Table 1. In univariate logistic regression analyses, being in the highest quartile of CRP was significantly associated with desaturation ($p=0.004$) and BMI ($p<0.0001$). Age, sex, ESS Score, diabetes, lipid lowering medications, previous MI/stroke, and log(AHI + 1) were not ($p>0.1$). However, when both BMI and desaturation were included in a logistic regression model, BMI remained a significant predictor ($p=0.001$) but desaturation was no longer significant ($p=0.17$). The interaction between the two variables (BMI and desaturation) was also tested and found to be not significant ($p=0.75$).

Prospective CVD

A total of 10 patients (7.1% of patients of the cohort) suffered 17 events in the 4 years after PSG (Characteristics shown in Table 2). The events were as follows: four myocardial infarctions, three cases of unstable angina, one stroke, one case of ventricular tachycardia, one case of atrial fibrillation, one coronary artery bypass graft, one cardioversion, four percutaneous coronary interventions (PCI) and one pacemaker placement. Of note, the pacemaker placement was in the context of a PCI and unstable angina. No deaths were reported. Rates varied substantially by CRP (Fig. 1) with no events in patients in the lowest CRP quartile, and a much greater rate in the highest quartile (7/39, 18%). In the unadjusted analysis, patients in the highest CRP quartile (≥ 2.38 mg/L) were significantly more likely to suffer an event compared to all other patients (odds ratio = 9.72 (95% CI 2.43–38.84), $p=0.001$).

OSA severity was not associated with events; 4/55 patients with mild OSA (7.27%), 2/57 patients with

Table 1 Baseline characteristics

Variable	All patients	Patients in first quartile of CRP levels	Patients in second quartile of CRP levels	Patients in third quartile of CRP levels	Patients in fourth quartile of CRP levels	*p* value
Gender (male)	112 (72.3%)	31 (88.6%)	30 (76.9%)	26 (61.9%)	25 (64.1%)	0.04
Age (years)	53.0 (44.0–60.0)	52.0 (43.0–57.0)	50.0 (38.5–58.0)	56.5 (46.0–62.0)	55.0 (46.0–61.0)	0.37
Body mass index (kg/m^2)	30.9 (26.6–36.4)	27.8 (24.3–31.2)	30.3 (24.8–34.2)	32.5 (28.1–37.6)	33.6 (30.4–43.4)	< 0.01
CRP levels (mg/L)	1.61 (0.66–2.38)	0.41 (0.24–0.55)	1.02 (0.72–1.31)	1.86 (1.68–2.13)	≥2.38	< 0.01
AHI (events/h)	21.0 (11.1–32.4)	20.2 (11.1–34.0)	16.1 (10.0–27.8)	21.7 (13.2–35.9)	21.4 (8.3–33.8)	0.16
Percent time with saturation below 90%	7.42 (0.98–31.66)	3.61 (4.50–17.07)	8.98 (1.22–28.10)	15.01 (1.56–26.00)	8.95 (0.84–49.03)	0.03
Use of lipid lowering drugs	31 (20.4%)	7 (20.0%)	8 (20.5%)	10 (25.0%)	6 (15.8%)	0.72
Previous myocardial infarction or stroke	9 (5.8%)	1 (2.9%)	4 (10.3%)	2 (4.8%)	2 (5.1%)	0.76
Diabetes	16 (10.3%)	2 (5.7%)	4 (10.3%)	6 (14.3%)	4 (10.26%)	0.45
Hypertension	29 (18.7%)	1 (2.9%)	9 (23.1)	12 (28.6%)	7 (17.95%)	0.06
Family history of premature CVD	20 (12.4%)	3 (8.6%)	6 (15.4%)	6 (14.3%)	5 (12.8%)	0.84
ESS	10 (6–14)	10 (8–13)	11 (5–14)	10 (6–15)	10 (6–16)	0.73

Continuous variables are presented as medians with interquartile ranges, and categorical variables as counts and percentages. For continuous variables group differences were tested using anova procedures and for categorical variables group differences were tested using the χ^2 test. Family history was defined as a father with CVD before the age of 55 or a mother with CVD before the age of 65

Table 2 Characteristics of patients with and without an event

Variable	Patients without event	Patients with event	*p* value
Gender (male)	*M* = 103 (71.03%) *F* = 42 (28.97%)	*M* = 8 (80.00%) *F* = 2 (20.00%)	0.55
Age (years)	52.0 (43.0–59.0)	62.0 (55.0–69.0)	< 0.01
Body mass index (kg/m^2)	30.84 (26.42–36.68)	31.67 (30.46–32.72)	0.73
CRP levels (mg/L)	1.55 (0.62–2.22)	2.75 (1.94–2.75)	< 01
AHI (events/h)	20.60 (11.4–31.5)	21.25 (8.2–55.3)	0.34
Percent time with saturation below 90%	8.33 (0.95–32.90)	3.33 (0.12–17.00)	0.98
Use of lipid lowering drugs	Yes = 29 (20.00%) No = 116 (80.00%)	Yes = 2 (20.00%) No = 8 (80.00%)	0.97
Previous myocardial infarction or stroke	Yes = 8 (5.51%) No = 137 (94.48%)	Yes = 1 (10.00%) No = 9 (90.00%)	0.55
Diabetes	Yes = 15 (10.34%) No = 130 (89.66%)	Yes = 1 (10.00%) No = 9 (90.00%)	0.98
Hypertension	Yes = 28 (19.31%) No = 117 (80.69%)	Yes = 1 (10.00%) No = 9 (90.00%)	0.47
Family history of premature CVD	Yes = 18 (12.41%) No = 127 (87.59%)	Yes = 2 (20.00%) No = 8 (20.00%)	0.48
ESS	10 (6–14)	13 (8–13)	0.72

Continuous variables are presented as medians with interquartile ranges, and categorical variables as counts and percentages. For continuous variables group differences were tested using anova procedures and for categorical variables group differences were tested using the χ^2 test

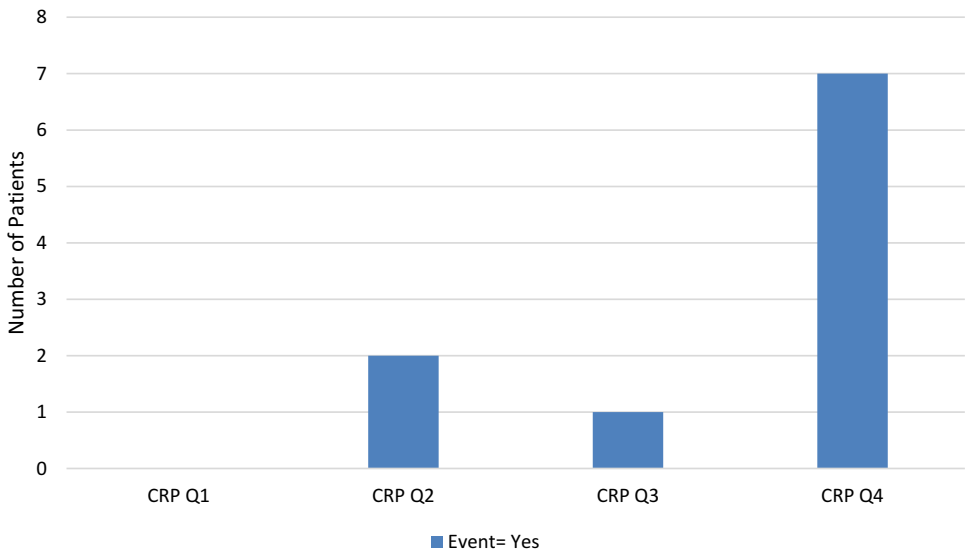

Fig. 1 Number of Patients with and without a cardio or cerebrovascular event in the 4 years after PSG by CRP Quartiles

moderate OSA (3.51%), and 4/43 patients with severe OSA (9.30%) had an event. When compared to patients with mild or moderate disease, patients with severe OSA did not have a significantly increased rate of events (OR 1.54 (95% CI 0.55–4.37), $p = 0.41$). Log(AHI + 1) and desaturation were not significant predictors ($p = 0.74$, 0.35 respectively).

We also performed logistic regression with each of the following variables individually to see if any were associated with prospective events (i.e., age, sex, lipid lowering drugs, diabetes, hypertension, BMI, family history, ESS, and prior MI or stroke). Only age was a significant predictor (OR 1.09, 1.02–1.16, $p = 0.01$); the others were not ($p > 0.5$).

In a multivariate logistic regression model with age and CRP, both age (OR 1.11, 1.02–1.22, $p = 0.015$) and the 4th CRP quartile (OR 12.5, 2.04–76.8, $p = 0.006$) were significant independent predictors. The interaction between the two variables was tested and found to be insignificant ($p = 0.97$). Because of the small number of events, we did not perform multivariate modelling with a large number of covariates. However, when we separately added the following confounders individually (i.e., sex, lipid lowering drugs,

diabetes, hypertension, BMI, family history, ESS, prior MI or stroke, log (AHI + 1) or desaturation), being in the 4th CRP quartile continued to be a significant risk factor for an event ($p < 0.02$) for each of the variables added.

Of these 155 patients, we were able to obtain the clinic records of 97 patients (some were unfortunately housed off site and impossible to obtain) for CPAP information. CPAP was prescribed in 85 of these patients and 50 were adherent. In these 97 patients, neither CPAP prescription nor adherence was significantly associated with CVD events.

Discussion

In this study, CRP was a strong, independent risk factor for CV events in patients with OSA, and this persisted after controlling for confounders of CVD risk. In univariate analyses, CRP at baseline was associated with desaturation, and is consistent with animal and human studies demonstrating that OSA may contribute to systemic inflammation [14, 15]. To our knowledge, this is the first study that has examined the potential utility of circulating biomarkers in predicting prospective CVD risk in OSA patients [16].

If these findings are confirmed in other larger cohorts, CRP could eventually be useful as a prognostic biomarker in OSA patients. Specifically, CRP could identify patients at high risk of incident CVD. In such a patient, one might consider more aggressive screening and management of risk factors such as hypertension, obesity, inactivity, and hyperlipidemia. In addition, instituting interventions to improve CPAP adherence might be considered, as CPAP can reduce CRP [17]. In addition, CRP may also be useful in enriching future randomized controlled trials in the area of CVD prevention; recruiting a high risk group may increase study power.

Strengths of our study included the prospective design and the fact OSA severity was assessed using the gold standard (PSG). Also, CVD events were based on validated administrative codes rather than self-reports. However, we acknowledge that there are a number of limitations to our study. The first is the relatively small size of the study, especially in terms of number of events. Second, this was a single centre study in one geographic location. As such, these findings may not be generalizable and should be verified in other OSA patient cohorts. Third, CRP was measured at only one time point, in the morning after their PSG. Whether CRP at other times of day would have the same predictive value is unclear. Fourth, we did not have measures of objective CPAP adherence in our study. Determining whether CPAP adherence modifies the association between CRP and risk of CVD would be useful to ascertain in future studies.

In conclusion, OSA patients in the highest quartile of CRP (≥ 2.38 mg/L) were significantly more likely to suffer a CVD event in the 4 years after PSG. Although these findings need to be confirmed in larger prospective clinic-based cohorts, these findings raise the potential clinical utility of CRP in risk stratifying OSA patients to guide therapy or to identify a group that might be most appropriate for clinical trials of interventions.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no competing interest.

References

1. Floras JS (2018) Sleep apnea and cardiovascular disease: an enigmatic risk factor. Circ Res 122:1741–1764
2. Hansson GK (2005) Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 352:1685–1695
3. Ridker PM (2003) High-sensitivity C-reactive protein and cardiovascular risk: rationale for screening and primary prevention. Am J Cardiol 92:17–22
4. Ridker PM, Everett BM et al (2017) Antiinflammatory therapy with canakinumab for atherosclerotic disease. N Engl J Med 377(12):1119–1131
5. Golbidi S, Badran M, Ayas N, Laher I (2012) Cardiovascular consequences of sleep apnea. Lung 190(2):113–132
6. Gozal D, Kheirandish-Gozal L (2008) Cardiovascular morbidity in obstructive sleep apnea: oxidative stress, inflammation, and much more. Am J Respir Crit Care Med 177:369–375
7. Kerr KF, Roth J, Zhu K et al (2017) Evaluating biomarkers for prognostic enrichment of clinical trials. Clin Trials 14(6):629–638
8. Johns MW (1991) A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 14(6):540–545
9. Schwab RJ, Badr SM, Epstein LJ et al (2013) An official American Thoracic Society statement: continuous positive airway pressure adherence tracking systems. The optimal monitoring strategies and outcome measures in adults. Am J Respir Crit Care Med 188:613–620
10. Berry RB, Budhiraja R, Gottlieb DJ, Gozal D, Iber C, Kapur VK, Marcus CL, Mehra R, Parthasarathy S, Quan SF, Redline S, Strohl KP, Davidson Ward SL, Tangredi MM, American Academy of Sleep Medicine (2012) Rules for scoring respiratory events in sleep: update of the 2007 AASM Manual for the Scoring of Sleep and Associated Events. Deliberations of the Sleep Apnea Definitions Task Force of the American Academy of Sleep Medicine. J Clin Sleep Med 8(5):597–619
11. Peppard PE, Young T, Barnet JH, Palta M, Hagen EW, Hla KM (2013) Increased prevalence of sleep-disordered breathing in adults. Am J Epidemiol 177(9):1006–1014
12. Humphries KH, Gao M, Lee MK, Izadnegahdar M, Holmes DT, Scheuermeyer FX, Mackay M, Mattman A, Grafstein E (2018) Sex differences in cardiac troponin testing in patients presenting to the emergency department with chest pain. J Womens Health 27(11):1327–1334
13. Ramanathan K, Abel JG, Park JE, Fung A, Mathew V, Taylor CM, Mancini GBJ, Gao M, Ding L, Verma S, Humphries KH, Farkouh ME (2017) Surgical versus percutaneous coronary

revascularization in patients with diabetes and acute coronary syndromes. J Am Coll Cardiol 70(24):2995–3006
14. McNicholas WT (2009) Chronic obstructive pulmonary disease and obstructive sleep apnea: overlaps in pathophysiology, systemic inflammation, and cardiovascular disease. Am J Respir Crit Care Med 180:692–700
15. Lavie L, Lavie P (2009) Molecular mechanisms of cardiovascular disease in OSAHS: the oxidative stress link. EurRespir J 33(6):1467–1484
16. Peres B, Hirsch Allen A, Fox N, et al (in press) Circulating biomarkers to identify cardiometabolic complications in patients with obstructive sleep apnea. Sleep Med Rev
17. Gottlieb DJ, Punjabi NM, Mehra R et al (2014) CPAP versus oxygen in obstructive sleep apnea. N Engl J Med 370(24):2276–2285