Association between C-reactive protein and unrecognised sleep-disordered breathing in the elderly

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ABSTRACT: Elevated levels of C-reactive protein (CRP) have been reported in patients with sleep-disordered breathing (SDB) and may represent an inflammatory marker of cardiovascular risk. However, the association of CRP with SBD in presumed healthy elderly subjects is unknown.

In total, 851 (58.5% females) 68-yr-old subjects, who were free of any known cardiac or sleep disorders, were prospectively examined. Subjects underwent unattended polygraphy, and the apnoea/hypopnoea index (AHI) and oxyhaemoglobin desaturation index (ODI) were assessed. Elevated levels of CRP were found on the morning after the sleep study in patients with more severe SDB. A significant correlation was found between CRP levels, time spent at night with arterial oxygen saturation $<$90% and ODI. No association was found between CRP levels and AHI. After adjustments for body mass index, smoking status, hypertension, diabetes and dyslipidaemia, a significant association remained between CRP levels and ODI $>$10 events·h^{-1}.

CRP levels were frequently increased in a large sample of elderly subjects free of major cardiovascular disease. CRP levels were not correlated with the AHI and the indices of sleep fragmentation; the ODI $>$10 events·h^{-1} was the strongest predictor of raised CRP level.

The present results suggest that, in the elderly, intermittent hypoxaemia may underlie inflammatory processes leading to cardiovascular morbidity.

KEYWORDS: C-reactive protein, elderly, hypoxaemia, inflammation, sleep apnoea

Obstructive sleep apnoea syndrome (OSAS) is a highly prevalent disorder affecting 2–4% of the general population and is considered an independent risk factor for cardiovascular diseases [1–3], particularly hypertension, coronary artery disease, heart failure and stroke [4, 5]. Furthermore, newly diagnosed OSAS patients, free of classical cardiovascular risk factors, such as hypertension, diabetes and smoking, may have early signs of atherosclerosis [6]. Although the pathophysiology of cardiovascular risk is mutifactorial, sympathetic hypertonia [7], endothelial dysfunction [8, 9] and insulin resistance [10] have been postulated as factors initiating and sustaining inflammatory microvascular alterations and therefore atherosclerosis [11, 12]. In middle-aged OSAS patients, C-reactive protein (CRP), a marker of inflammation in atherosclerotic lesions [13], is elevated in severe cases [14] and decreases after treatment with nasal continuous positive airway pressure [15]. Despite the putative role of CRP in cardiovascular risk in OSAS, studies conducted to date have yielded contradictory results, with some showing an independent association with disease severity in adults [16–19] and children [20, 21], and others showing no relationship [22, 23]. Moreover, the association between obesity and CRP [24] raised the question as to whether elevated CRP reflects the effects of obesity or whether it is specific to OSAS itself.

In the elderly, the prevalence of sleep-disordered breathing (SDB) is estimated to be higher than in middle-aged subjects. ANCOLI-ISRAEL et al. [25] reported that, in a community-dwelling elderly population, 62% had a respiratory disturbances index $>$10 and 44% $>$20. The prevalence rate of SDB was 15% in the general population when the criterion of an apnoea/hypopnoea index (AHI) of $\geqslant$15 was used [26]. Despite the high incidence of SDB, the clinical spectrum of the disease appears to be different, elderly patients have shown a lower incidence of sleepiness and hypertension compared with middle-aged patients [27], and have a different prognosis when treated [28, 29].

Therefore, an interesting, but still unanswered question is whether CRP levels are increased in elderly subjects having undiagnosed OSAS and whether higher CRP levels predict the risk of future cardiovascular morbidity among persons without known cardiovascular disease.

The aim of the current study was to assess the association between SDB and CRP levels in a large prospective population-based cohort of elderly subjects examined to evaluate the possible role of SDB in cardiovascular risk generation. Specifically, the present authors focused on the relationship between apnoea density, sleep fragmentation, hypoxaemia and CRP as a marker of inflammatory processes.

METHODS

Population

The present investigation is part of the PROOF study, a population-based cohort study of 1,011 (61% females) volunteers aged 65 ± 1 yrs living in the town of Saint-Etienne (France) who were enrolled to assess whether an age-related decrease in autonomic nervous system activity could represent an independent risk for cardiovascular mortality, silent and symptomatic ischaemic stroke, myocardial infarction and new-onset heart failure [30]. An ancillary study addressing the association between SDB and cardiovascular and cerebrovascular morbidity during a 7-yr follow-up was proposed to participants (SYNAPSE study). Exclusion criteria for entry in the present study were history of myocardial infarction, heart failure, stroke, pacemaker therapy, previously diagnosed or treated OSAS, type 1 diabetes mellitus, symptoms or signs of acute or chronic inflammation and use of corticosteroids and/ or antibiotics for the 3 weeks preceding recruitment. Participants with hypertension were kept in the study if their condition was controlled under anti-hypertensive medications.

Of the original sample, 851 (58.5% females) subjects participated in the SYNAPSE study between March 2003 and June 2005. The PROOF and SYNAPSE studies were approved by the local ethics committee (CCPRB Rhone-Alpes Loire, France) and all subjects gave written consent to study participation.

Clinical data

All subjects underwent a clinical assessment including a questionnaire on demographics, medical history and medication, measurements of body mass index (BMI) and neck circumference and an evaluation of sleepiness using the Epworth sleepiness scale. Detailed clinical assessment was specially focused on cardiac and cerebrovascular disease, hypertension, obstructive or restrictive lung disease, metabolic disorders, psychiatric diseases and current medication. Subjects were defined as normotensive if they did not report history of hypertension and antihypertensive treatment and they did not have a mean systolic ambulatory blood pressure (ABP) <135 mmHg (Diasoft, Novacor, Rueil Malmaison, France) and a mean diastolic ABP <85 mmHg.

Sleep study

A nocturnal unattended sleep study was performed at-home in all subjects using a polygraphic system (HypnoPTT; Tyco Healthcare, Puritan Bennett, Boulder, CO, USA), which included the following parameters: sound measurement, electrocardiography, pulse transit time, heart rate, airflow by nasal pressure, respiratory effort and body position. Arterial oxygen saturation was measured by pulse oximetry (S_{p,O_2}). A software package was used for downloading and analysing tracings. A recording duration of $\geqslant 5$ h was required for validation, and a second night of monitoring was performed when subjective sleep latency exceeded 2 h on the first night, or when respiratory parameters were missing. All recordings were visually validated and manually scored for respiratory events and nocturnal S_{p,O_2} according to standard criteria [31]. Hypopnoea was defined as $\geqslant 50\%$ reduction in airflow from the baseline value lasting $\geqslant 10$ s associated with $\geqslant 3\%$ oxygen desaturation. Apnoeas were defined as the absence of airflow on the nasal cannula lasting for $\geqslant 10$ s. The absence of rib cage movements associated with an apnoea was defined as central, while a progressive increase in pulse transit time was defined as obstructive. The AHI was established as the ratio of the number of apnoea and hypopnoea events per hour of recording. Indices of nocturnal hypoxaemia were as follows: mean S_{p,O_2}; percentage of recording time S_{p,O_2} <90%; minimal S_{p,O_2} value recorded during sleep and the oxygen desaturation index (ODI), *i.e.* the number of episodes of oxygen desaturation per hour of recording time during which blood oxygen fell by $\geqslant 3\%$. Pulse transit time was continuously monitored and, according to previously defined criteria [32], an autonomic respiratory-related arousal index (AAI) was calculated (arousals following a defined respiratory event). To minimise potential overestimation of sleep duration, subjects completed a sleep diary to exclude wakefulness before turning lights off from the analysis. An AHI >15 events·h^{-1} [26] with $\geqslant 85\%$ of events scored as obstructive or an ODI >10 events·h^{-1} [33] was considered a diagnostic of SDB. An AHI >15–30 events·h^{-1} indicated mild OSAS and >30 events·h^{-1} indicated moderate-to-severe OSAS.

CRP levels

Blood samples for measurement of plasma CRP levels were collected in all subjects on the morning after the sleep study. Plasma CRP levels were measured with a flex reagent cartridge, which is based on a particle-enhanced, turbidimetric immunoassay technique (Roche Diagnostic GmbH, Mannheim, Germany) allowing a detection level of 0.2 mg·L^{-1} and exhibiting linear behaviour up to 25 mg·L^{-1} with intra-assay and inter-assay coefficients of variability of 9% and 18%, respectively. Fasting glycaemia, serum lipid levels, including triglycerides and total high-density lipoprotein and calculated low-density lipoprotein cholesterol, were also assessed.

Statistical analysis

Results are presented as mean $\pm$ SD, unless otherwise indicated. All reported p-values are two-tailed, with a statistical significance of <0.05. Anthropometric, clinical and sleep study data are presented using descriptive statistics (mean, SD, range and percentage of subjects). ANOVA and *post hoc* analyses were performed to compare CRP levels with groups of subjects according to AHI, ODI and AAI quartiles.

Since plasma CRP levels were not normally distributed, logarithmic transformation was used to achieve a distribution close to normality. Linear regression analysis was used to explore the relationship between log CRP and SBD severity defined by the ODI, AAI and AHI, all variables considered are

TABLE 1	Demographic, anthropometric and clinical data of the study group
Clinical data	
Age yrs	68.1±1.1
BMI kg·m^{-2}	25.3±3.7
Neck circumference cm	37.1±4.0
Systolic ABP mmHg	118.8±13.8
Diastolic ABP mmHg	74.0±8.4
CRP serum level mg·L^{-1}	2.26±1.83
ESS	5.7±3.6
Sleep study data	
AHI events·h^{-1}	20.4±14.8
ODI events·h^{-1}	9.4±9.5
Time S_{p,O_2} <90% %	2.0±6.7
Mean S_{p,O_2} %	95.4±1.6
Minimal S_{p,O_2} %	89.8±4.1
AAI events·h^{-1}	15.4±10.5

Data are presented as mean±SD. BMI: body mass index; ABP: ambulatory blood pressure; CRP: C-reactive protein; ESS: Epworth Sleepiness Scale; AHI: apnoea/hypopnoea index; ODI: oxygen desaturation index; S_{p,O_2}: arterial oxygen saturation measured by pulse oximetry; AAI: autonomic respiratory-related arousal index.

shown in quartile distribution. Multiple logistic regression models were constructed to test for independent associations between the presence of SDB (independent variable: AHI), autonomic sleep fragmentation (independent variable: AAI), or hypoxaemic load (independent variable: ODI) and log CRP. Unadjusted odds ratios (ORs) and 95% confidence intervals (CIs), as well as adjusted ORs and 95% CIs, were calculated.

RESULTS
Clinical and sleep study data
Demographic, anthropometric and nocturnal parameters are summarised in table 1. The age of the total sample of 851 subjects was 68±1.1 yrs and 41.5% were male.

In total, 12.3% of subjects met the criteria for obesity defined as a BMI >30 kg·m^{-2}, whereas 38.5% were classified as overweight (BMI >25 kg·m^{-2}). The prevalence of treated hypertension was 43.1% and 76 (8.7%) subjects had clinical newly diagnosed systolic and/or diastolic hypertension (incident hypertension). In treated patients, 64% received β-blockers and/or diuretics and/or calcium channel blockers and 58% received angiotensin-converting enzyme-inhibitors and/or angiotensin II receptor inhibitors. Hypercholesterolaemia was

found in 35.1% of subjects, 201 subjects were receiving statins. A clinical history of diabetes was reported by 5.3% of subjects.

SDB was identified in 482 (56.8%) subjects, with 315 (37.1%) having a mild form (AHI 15–30 events·h^{-1}) and 167 (19.7%) having a moderate-to-severe form (AHI >30 events·h^{-1}). Based on the oxygen desaturation values, 296 (34.8%) had an ODI >10 events·h^{-1}. Time spent with S_{p,O_2} <90% reached 2.01±6.6% (range 0–100%), with a 90th percentile threshold of 4.44%. Autonomic sleep fragmentation was moderate in 306 (36.2%) and severe in 79 (9.4%) cases.

In the SYNAPSE study subjects, the mean CRP level was 2.26±1.83 mg·L^{-1}. In adults, CRP levels <1 mg·L^{-1} are considered low risk, 1–3 mg·L^{-1} medium risk and >3 mg·L^{-1} high risk. In the current study, 29.3% of subjects were low risk, 50% of subjects had a medium risk and 20.7% had a high risk CRP level. CRP levels >10 mg·L^{-1} were found in 38 participants. Thus, 813 participants were included in the final analysis that focused on sub-clinical inflammation (CRP level ⩽10 mg·L^{-1}).

Variation of the CRP levels by participant characteristics
In unadjusted analyses, CRP levels did not vary significantly with sex (2.24±1.95 versus 2.27±1.74 mg·L^{-1} in males and females, respectively; p=0.83). In contrast, current/past smoking (2.50±2.19 versus 2.18±1.69 mg·L^{-1}; p=0.04) and hypertension (2.54±1.96 versus 2.06±1.70 mg·L^{-1}; p=0.002) were associated with higher CRP levels. CRP levels also varied significantly with BMI (ρ=0.27, p<0.0001) for all subjects (4.09±3.84 versus 2.62±5.45 mg·L^{-1} in obese and normal BMI participants, respectively; p=0.003) and in the overweight group (4.09±3.84 versus 2.99±3.36 mg·L^{-1} in obese and overweight subjects, respectively; p=0.03). Linear regression analysis between CRP levels and blood pressure showed no significant correlation between log CRP and systolic or diastolic blood pressure in hypertensive subjects. A slight relationship was found between log CRP levels and systolic (ρ=0.102, p=0.03) and diastolic (ρ=0.108, p=0.02) blood pressure in normotensive cases. CRP levels by subgroups of participants categorised by ODI quartiles, AHI quartiles and AAI quartiles are shown in table 2.

Association between log CRP and nocturnal variables
Table 3 reports Spearman correlation coefficients between nocturnal variables and log CRP. While log CRP levels were strongly correlated with all indices of nocturnal hypoxaemia, no significant relationship was found with AAI and AHI.

TABLE 2	Mean C-reactive protein (CRP) levels according to the severity of oxygen desaturation index (ODI), apnoea/hypopnoea index (AHI) and autonomic respiratory-related arousal index (AAI)				
ODI quartiles	**CRP mg·L^{-1}**	**AHI quartiles**	**CRP mg·L^{-1}**	**AAI quartiles**	**CRP mg·L^{-1}**
<2.6	2.04±1.63	<9.2	2.13±1.82	<7.7	2.25±1.83
2.6–6.2	2.21±1.83	9.2–17.1	2.15±1.72	7.7–13.6	2.17±1.79
6.3–13.1	2.25±1.95	17.2–28.2	2.19±1.91	13.6–20.6	2.34±1.93
>13.1	2.56±1.91*	>28.2	2.41±1.90	>20	2.29±1.76

ANOVA and post hoc tests. Data are presented as mean±SD, unless otherwise stated. *: p<0.05 lowest quartile versus the highest quartile disorders.

| TABLE 3 | Spearman correlation coefficients relating log C-reactive protein (CRP) with nocturnal parameters |

	Log CRP	AHI	ODI	AAI
AHI				
Coefficient	0.060	1		
p-value	0.09			
ODI				
Coefficient	0.121	0.803		
p-value	0.006	<0.0001		
AAI				
Coefficient	0.037	0.799	0.652	1
p-value	0.301	<0.0001	<0.0001	
S_{P,O_2} <90% time %				
Coefficient	0.078	0.162	0.295	0.074
p-value	0.027	<0.0001	<0.0001	0.036
Minimal S_{P,O_2}				
Coefficient	-0.082	-0.408	-0.571	-0.291
p-value	0.020	<0.0001	<0.0001	<0.0001
Sleep duration				
Coefficient	-0.013	-0.103	-0.023	-0.131
p-value	0.704	0.003	0.519	0.001

AHI: apnoea/hypopnoea index; ODI: oxygen desaturation index; AAI: autonomic arousals index; S_{P,O_2}: arterial oxygen saturation measured by pulse oximetry; AAI: autonomic respiratory-related arousal index.

Compared with patients without OSAS (first quartile for AHI <9.2), CRP levels were higher in more severe cases (fourth quartile), although the differences did not reach statistical significance (p=0.06). Using logistic regression analysis before and after adjustments for sex, BMI, smoking habits, diabetes and hypertension (table 4), no significant association between AHI severity (<15, 15–30 or >30 events·h^{-1}) and log CRP levels was found. When indices of sleep fragmentation were considered, AAI did not show any significant relationship with log CRP, its values being similar even if AAI was higher (table 2).

Mean CRP values according to ODI quartiles are shown in table 2. There was a trend towards a progressive increase in CRP levels according to the occurrence of oxygen desaturation, with higher values found in subjects having an ODI >13.1 events·h^{-1} (p<0.05). Using logistic regression analysis, ODI >10 events·h^{-1} was associated with increased log CRP levels after adjustments (OR 1.33, 95% CI 1.08–1.64; p=0.0063; table 5).

DISCUSSION

To the present authors' knowledge, this is the first epidemiological investigation of the relationship between SDB and CRP serum levels in a large population-based study performed in the elderly. In the current investigation of healthy 68-yr-old subjects without diagnosed OSAS, it was found that after adjustments for relevant covariates affecting CRP levels, the presence of an ODI >10 events·h^{-1} was associated with higher levels of serum CRP, the frequency of respiratory events and the indices of sleep fragmentation had no significant effect. These findings suggest that, in OSAS patients, increased levels of CRP may reflect the key role of intermittent hypoxaemia on oxidative stress and, consequently, on cardiovascular risk.

Among mechanisms mediating cardiovascular morbidity and mortality in SDB, CRP has gathered the greatest attention; several investigations demonstrated a link between elevated values of CRP, SDB severity [20–23] and cardiovascular morbidity [19, 24]. CRP has pro-inflammatory, pro-adhesive and pro-thrombotic effects, properties participating in the formation of atheromatous lesions through reduction of nitric oxide synthesis and induction of particular adhesion molecules in endothelial cells [34]. The link between inflammatory responses, including CRP levels, and SDB may be related to several factors such as frequency of apnoea, sleep fragmentation and hypoxaemia, all of which up-regulate inflammation and atherosclerotic processes. Recent studies in animals emphasised [35] that intermittent hypoxia is a more potent stimulus for sympathetic activation and hypertension related to episodic re-oxygenation, the latter representing oxidative stress [36] similar to that implicated in ischeamia-reperfusion [37]. Oxidative stress would result in the activation of inflammatory pathways [38, 39], such as those mediated by nuclear factor-κB, tumour necrosis factor-α, interleukin (IL)-6 and IL-8, all parameters that are found to be elevated in OSAS patients and modified by therapy [21, 25]. Studies in humans [40] have confirmed the key role of chronic intermittent hypoxia, more than that of the AHI, in the development of atherosclerosis in SDB and on partial reversal by efficacious therapy [41]. Therefore, atherosclerotic lesions and inflammatory processes, induced by intermittent hypoxia, contribute to long-term increased cardiovascular morbidity and mortality described in patients with SDB [42, 43].

In line with this hypothesis, the present authors found in healthy elderly subjects, free of major cardiovascular risk, an increased level of CRP associated with ODI without any effect on markers of chronic hypoxaemia, mean S_{P,O_2} and time spent at <90% S_{P,O_2}, as well as apnoea frequency and sleep

| TABLE 4 | Logistic model coefficients for log C-reactive protein (CRP) by apnoea/hypopnoea index (AHI) severity |

	AHI h^{-1}	Coefficient	SE	Chi-squared	p-value	OR (95% CI)
Unadjusted model	15–30	-0.045	0.086	0.25	0.605	0.96 (0.81–1.13)
	>30	0.190	0.103	3.39	0.065	1.21 (0.99–1.48)
Adjusted model#	15–30	-0.140	0.092	2.35	0.129	0.87 (0.72–1.04)
	>30	0.043	0.115	0.141	0.708	1.04 (0.83–1.31)

OR: odds ratio; CI: confidence interval. #: for sex, body mass index, hypertension, type 2 diabetes and current smoking status.

TABLE 5	Logistic likelihood ratio tests table for oxygen desaturation index >10 events·h^{-1}	
	Chi-squared	**p-value**
Sex male	31.22	<0.0001
BMI$^{\#}$	24.28	<0.0001
Type 2 diabetes	9.93	0.0016
Log CRP mg·L^{-1}	7.45	0.0064
Hypertension	4.80	0.028
Current/past smoker	1.06	0.305

BMI: body mass index; CRP: C-reactive protein. Odds ratio for log CRP: 1.33 (95% confidence interval 1.08–1.64) with $\rho=0.071$, coefficient 0.249 and SE 0.091. $^{\#}$: <25 versus 25–30 versus >30 kg·m^{-2}.

fragmentation. This association persisted after adjustment for hypertension, obesity and diabetes, stressing the link between the hypoxia-re-oxygenation factor and increased circulation levels of atherogenic inflammatory mediators. One interesting finding was that the strongest independent factor associated with CRP levels was the ODI, even if the degree of overnight hypoxaemic load was mild. Although speculative, these results may be explained by two factors. First, the recurrence of hypoxaemia in itself, independent of the severity of hypoxaemia [35, 36, 40] and, secondly, the age-related vascular changes inducing a greater reaction to the ischaemia-re-oxygenation process in the elderly even when the hypoxaemic level is moderate.

The key role of mild-to-moderate intermittent hypoxaemia on CRP levels and the lack of AHI and sleep fragmentation effect in the current elderly subjects may explain some of the reported age-related differences on cardiovascular risk. The mortality risk in SDB is greater in patients with an AHI >20 events·h^{-1} [44, 45], an effect, however, significant only for middle-aged patients. Moreover, the morbidity for hypertension, heart failure and stroke [44, 45] is related to the AHI in 50-yr-old patients, an association, however, not significant or acting in the reverse direction when older patients are considered [46, 47]. Therefore, the present authors can conclude that in the elderly, atherosclerotic and inflammatory processes are dependent on intermittent hypoxaemic load, a more potent stimulus for sympathetic activation and hypertension than the AHI and sleep fragmentation. The age-related remodelling of the vascular system may affect this association and mild-to-moderate hypoxaemia is sufficient, in itself, to activate inflammatory processes.

Even though the strength of the current study lies in the analysis of a large number of elderly subjects free from stroke and heart failure, some limitations should be considered. First, that an unknown sub-clinical cardiovascular disease cannot be ruled out in the present study subjects. However, the importance of such findings in the association between CRP levels and ODI was limited by the exclusion of subjects with prevalent medical conditions and by adjustment for covariates such as diabetes, hypercholesterolaemia and BMI. Secondly, despite a wide spectrum of SDB being present in the current population, mild cases were prevalent, the absence of severe cases may reduce the relationship between CRP and AHI. However, this finding is common in clinical and epidemiological studies; severe cases are more common in young patients [48]. Thirdly, the examined elderly subjects were noninstitutionalised and therefore they might constitute a survivor group more resistant to vascular risk and stress. This latter possibility could be suggested by the different clinical aspects of SDB in the elderly, in which neither sleepiness nor common predisposing factors appear strongly related to apnoea density [49]. Finally, in the present population, the nocturnal sleep study was performed by polygraphy, which could give both an overestimation of the incidence of positive SDB cases and a crude estimate of the real sleep fragmentation. Since autonomic arousals are actually considered as sensitivity markers of sleep fragmentation [32] the lack of association between CRP levels, the AHI and AAI exclude a primary key role of apnoea density and sleep fragmentation on the activation of inflammatory processes in SDB.

In conclusion, the current study shows that intermittent nocturnal hypoxaemia is associated with elevated C-reactive protein levels in healthy elderly subjects with sleep apnoea. After adjustment for confounding factors, oxyhaemoglobin desaturations, i.e. recurrent hypoxaemia/reoxygenation events, are the only significant factors associated with inflammatory processes in sleep-disordered breathing, apnoea/hypopnoea density and sleep fragmentation showed no such association. The eventual cardiovascular consequences of such observations need to be explored by prospective studies examining the long-term outcomes of such patients and the usefulness of nocturnal ventilatory support.

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