

Original Article

Full title:

The additive impact of periodic limb movements during sleep on inflammation in obstructive sleep apnea patients

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Contribution:

KM contributed to the study design, collection of data, analysis and interpretation of data and writing the manuscript. KC contributed to the study design, collection of data and editing the draft. MM contributed to study supervision. All other authors contributed to the collection of data.

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Abstract

Rationale: Both periodic limb movements during sleep (PLMS) and obstructive sleep apnea (OSA) are major causes of sleep disorders and have been associated with systemic inflammation and cardiovascular events. However, it is uncertain whether in combination they promote a higher inflammatory response and greater risk of cardiovascular events than each condition alone.

Objectives: To investigate whether the presence of PLMS is associated with increased inflammation in patients suspected of having OSA.

Methods: In 342 patients who underwent polysomnography to diagnose OSA, plasma C-reactive protein (CRP) and fibrinogen levels were measured.

Measurements and Main Results: OSA was found in 254 patients, with 46 also having PLMS. Among the 88 patients who did not have OSA, 8 had PLMS. Plasma CRP and fibrinogen levels in the group with both PLMS and OSA were higher than in patients with neither OSA nor PLMS and in patients with OSA only (CRP: 0.20 ± 0.48 vs. 0.09 ± 0.15 vs. 0.13 ± 0.18 mg/dl, $p=0.03$; fibrinogen: 298.2 ± 76.1 vs. 269.0 ± 57.1 vs. 270.0 ± 52.6 mg/dl, $p < 0.01$) Multivariate analysis showed that the presence of PLMS was associated with higher plasma CRP levels ($\beta=0.1401$, $p<0.01$) and fibrinogen levels ($\beta=0.1359$, $p=0.01$) independently from other clinical variables such as body mass index and the severity of OSA.

Conclusions: PLMS were positively associated with plasma CRP and fibrinogen levels in patients suspected of having OSA. Since plasma levels of these proteins have been established as predictive factors of future cardiovascular events, the presence of PLMS

may be a useful clinical sign to identify OSA patients at high risk of cardiovascular events.

(253 words)

Introduction

Periodic limb movements during sleep (PLMS) are involuntary, repetitive, stereotypic, short-lasting, segmental movements of the lower and sometimes upper extremities. They occur in 5-8% of the general population and prevalence increases with age (1, 2). PLMS are identified in the vast majority of patients with restless leg syndrome (RLS), and both PLMS and RLS were reported to be associated with cardiovascular disease (CVD) and mortality (3-8). Although the causal relationship between PLMS and CVD remains uncertain, an association between PLMS and systemic inflammation has been shown, and this relationship is considered to be a factor in the increased risk of CVD in patients with PLMS (9-11). In addition, obstructive sleep apnea (OSA) syndrome is a highly prevalent sleep disorder, affecting about 4-20% of adults (12-14). OSA is characterized by repetitive episodes of partial or complete obstruction of the upper airway during sleep associated with transient oxygen desaturation. Accumulating clinical evidence suggests that OSA is an independent risk factor for CVD through impaired endothelial dysfunction and increased platelet aggregability caused by nocturnal intermittent hypoxia and subsequent chronic inflammation (15-17).

PLMS are commonly seen during polysomnography (PSG) in OSA patients, and their prevalence in OSA patients has been reported to be significantly higher than in the general population (18-20). The underlying mechanisms for the association of OSA with PLMS have not been fully elucidated nor has it been investigated whether the coexistence of OSA and PLMS promotes a greater inflammatory response than the presence of either one alone. Therefore, we hypothesized that patients with both OSA

and PLMS would have a higher inflammatory response than patients with OSA only and that comorbid PLMS is an independent risk factor for a high inflammatory response in patients suspected of having OSA. Since we have routinely measured plasma levels of inflammatory proteins such as C-reactive protein (CRP) and fibrinogen in patients in our sleep laboratory to assess patients' general condition (21), we attempted to verify these hypotheses by evaluating the data accumulated in our clinical practice.

Methods

Subjects

We examined data on all patients who underwent a diagnostic full overnight PSG at the sleep unit of Kyoto University Hospital between 2008 and 2011. All had been referred to our sleep unit under suspicion of OSA with symptoms such as habitual snoring or daytime sleepiness. Data were systematically extracted by a single investigator (KM) from patients' clinical records and PSG reports, after which they were entered into a software database for later analysis. This study protocol was approved by the Kyoto University Graduate School and Faculty of Medicine Ethics Committee.

For a patient's data to be included in the analysis, the patient had to meet the following criteria: 1) age at least 30 years and less than 80 years and 2) no prior treatment for OSA and/or PLMS. Patients with diseases that have been reported to cause RLS and PLMS were excluded. Specifically, data on patients with Parkinson's disease, collagen diseases,

renal failure (serum creatinine level >1.3 mg/dl), anemia (hemoglobin level <12 g/dl), severe intervertebral hernia, pregnancy and with any history of heart or cerebrovascular diseases were excluded. Patients with malignancy and acute and/or chronic infection were also excluded from analysis because these conditions could possibly affect the inflammatory protein levels. Lastly, patients who were regularly taking any antidepressant, anxiolytic, anticoagulant and anti-inflammatory medications were also excluded because these medications might change the patient's PLMS status and plasma CRP and fibrinogen levels.

The definitions of the comorbid diseases are shown in the online supplement.

Procedures

Polysomnography

The diagnoses of OSA and PLMS were confirmed by PSG (SomnoStar pro, Cardinal Health, Dublin, OH, USA or Alice 4, Philips Respironics, Inc., Murrysville, PA, USA), which started at 22:00 and ended at 6:00 the following morning. A detailed description of materials and methods used for performance of polysomnography is provided in the online supplement.

In the present study, the PSG studies were scored by four certified sleep laboratory technicians. To assess intra- and inter-scorer agreement, we randomly selected 20 patients (Apnea Hypopnea Index (AHI): 24.0 ± 17.4 /h, PLMS index: 17.0 ± 27.1 /h) from the cohort whose PLMS index was not zero. Then, intra-class correlation

coefficient (ICC) values for AHI and PLMS index scored by these technicians were calculated. The ICC values for intra-scorer agreement were more than 0.99 for AHI and 0.96 for PLMS index. The ICC value representing inter-scorer agreement was 0.98 for AHI and 0.88 for PLMS index. Because a high level of agreement for AHI and PLMS index among the technicians was found, we adopted the values for AHI and PLMS index scored by one of these four technicians for the statistical analysis in the present study. We defined $\text{AHI} \geq 15/\text{h}$ as 'OSA positive' and a $\text{PLMS index} \geq 15/\text{h}$ as 'PLMS positive' according to a previous study (1).

Anthropometric measurements were performed in the evening before PSG. In the morning following PSG, blood pressure (BP) was measured five times at one-minute intervals with the patient in the sitting position after resting for at least five minutes. The average of the latter two recordings was calculated.

Blood sampling and measurement of plasma fibrinogen level

Since OSA has been reported to be associated with various diseases such as metabolic syndrome and CVD, we have routinely recommended that patients undergo a blood test to check their status for diabetes, dyslipidemia and hypercoagulation. If patients consented, blood samples were drawn at 7:00 in the morning following a 12-h overnight fast and PSG. Thrombocheck Fib (L) (Sysmex Corporation, Kobe, Japan) is a liquid type reagent for use with the Clauss method and was employed for fibrinogen measurement. Measurements were performed using a fully automated coagulation analyzer (Coagrex 800, Shimazu Corporation, Kyoto, Japan). Aside from the plasma

fibrinogen level, we simultaneously measured blood counts, biochemistry, CRP levels and indexes of metabolic syndrome such as HbA1c and cholesterol levels.

Statistical Analysis

First, we categorized the patients into four groups according to the presence and/or absence of OSA and PLMS: “neither OSA nor PLMS”, ‘PLMS only’, ‘OSA only’ and ‘both PLMS and OSA’. The significance of intergroup differences in patients’ background was determined by an analysis of variance. Because the number of patients in the PLMS-only group was too small (n=8), that group was excluded from this intergroup analysis. When a significant difference was found, we used the Tukey’s honestly significant difference procedure to identify where the difference was significant. A chi-square test was used to compare categorical variables. Second, we used Pearson’s coefficient test to evaluate the relationships between plasma CRP or fibrinogen levels and other clinical variables for the entire cohort. In this analysis, the dichotomous variables were converted to dummy variables (‘Male’=0, ‘Female’=1 and ‘PLMS negative’=0, ‘PLMS positive’=1).

Based on results of this analysis, multivariate regression analyses were performed to clarify the contribution rate of PLMS, OSA and other comorbidities to CRP and fibrinogen levels. The variables entered into the multivariate analysis were those yielding a p value <0.10 by univariate analysis, and when two independent variables had strong collinearity ($\gamma > 0.7$), one was selected. Third, we performed the same analyses only for the cohort that was OSA positive. Data were expressed as means $\pm$

standard deviation. Two-tailed p-values <0.05 were considered statistically significant.

All statistical analyses were performed using JMP 7.0.2 statistical software (SAS Institute Inc., Cary, NC, USA).

Results

Of 841 eligible patients, 471 patients were excluded from the analysis and blood tests were not undertaken in 28 patients. Therefore, 342 patients were enrolled in the analysis (Figure 1). OSA was found in 254 patients, with 46 having PLMS. Among the 88 patients who did not have OSA, PLMS was found in just 8 patients. The prevalence rate of PLMS in the OSA-positive cohort was significantly higher than that in the OSA-negative cohort (46/254 (18.1%) vs. 8/88 (9.1%), $p=0.04$). Tables 1 and 2 show the clinical backgrounds of the study patients and their sleep parameters, respectively. Compared to patients with OSA only, patients with both PLMS and OSA were older and had a lower body mass index, lower diastolic blood pressure, lower hemoglobin level and milder OSA. Plasma CRP and fibrinogen levels in the group with both PLMS and OSA were higher than in patients with neither OSA nor PLMS and in patients with OSA only (CRP: 0.20 ± 0.48 vs. 0.09 ± 0.15 vs. 0.13 ± 0.18 mg/dl $p=0.03$; fibrinogen: 298.2 ± 76.1 vs. 269.0 ± 57.1 vs. 270.0 ± 52.6 mg/dl, $p<0.01$).

All patients had three or more hours of total sleep time (TST) during PSG recording. While TST in patients with only OSA was significantly longer than that in patients with both PLMS and OSA (390.8 ± 74.6 vs. 363.9 ± 78.1 m, $p=0.03$), the indexes of severity of OSA such as the AHI and 3% oxygen desaturation index (ODI) in patients with only

OSA were significantly higher than those in patients with PLMS and OSA (AHI: 40.8 ± 19.9 vs. $33.7 \pm 15.1/h$, $p=0.02$, 3% ODI: 38.2 ± 21.2 vs. $31.0 \pm 19.0/h$, $p=0.04$) (Table 2).

Table 3 shows results of univariate and multivariate analyses of plasma CRP and fibrinogen levels for the entire cohort. Strong collinearities were found between the AHI and 3% ODI ($\gamma=0.97$) and between the PLMS index and being PLMS positive ($\gamma=0.76$). For the multiple regression analysis, we chose 3%ODI and being PLMS positive as the representative variable for OSA and PLMS severity, respectively, as these had better correlation with the CRP or fibrinogen levels in the simple correlation analysis (Table E1 in online supplement). The multivariate analyses demonstrated that being PLMS-positive was associated with plasma CRP or fibrinogen levels (CRP: $\beta=0.1401$, $p<0.01$; fibrinogen: $\beta=0.1359$, $p=0.01$) independently of other clinical variables such as body mass index (BMI) and HbA1c.

Next, as we previously noted, we performed the same analyses for the OSA-positive cohort. Table 4 shows the results of these analyses. In the OSA-positive cohort also, being PLMS positive was associated with plasma CRP or fibrinogen levels independently of clinical variables. (CRP: $\beta=0.1466$, $p=0.0192$; fibrinogen: $\beta=0.1844$, $p=0.0036$)

Discussion

The results of the present cross-sectional study indicated that patients with both OSA and PLMS had the highest plasma CRP or fibrinogen levels of the four cohorts with

suspected OSA. Furthermore, multivariate analysis showed that the presence of PLMS contributed, although weakly, to elevated plasma CRP and fibrinogen levels independently of other clinical variables. Both CRP and fibrinogen are known as acute phase proteins involved in inflammation, and elevated plasma levels of these proteins were reported to be independent risk factors for future CVD events through several mechanisms, such as a contribution to platelet aggregation, promotion of fibrin formation and increase in plasma viscosity (22-26). Therefore, the results of the present study suggested that high CRP and fibrinogen levels in patients with PLMS could be a key in clarifying why PLMS are associated with CVD. In addition, the results of this study suggest that PLMS can be a useful clinical sign to identify OSA patients at high risk of CVD. In patients with both PLMS and OSA, plasma CRP and fibrinogen levels were about 0.07 mg/dl and 30 mg/dl higher, respectively, than in patients with OSA only. Based on a previous meta-analysis that investigated the impact of elevated inflammatory protein levels on CVD, patients with both PLMS and OSA could be estimated to be 1.1-1.3 times more likely to develop CVD events than patients with OSA only (24, 27). The multivariate analysis for the entire cohort showed a significant association between the BMI and CRP level and between HbA1c and fibrinogen levels. Some previous studies showed similar relationships between these factors (28-30).

Underlying mechanism for the relationship between PLMS and the inflammatory status

Even though the results of this study did not confirm a causal relationship between

PLMS and high plasma inflammatory protein levels, several mechanisms may explain a relationship between them. Pennestri et al. reported that PLMS, whether or not associated with arousals, were correlated with repetitive nocturnal BP increments (31). Based on indications by results of some previous studies that plasma levels of inflammatory proteins were significantly associated with BP variability (32-34), BP surges provoked by PLMS may cause a heightened inflammatory status. However, in the present study, the patients with both PLMS and OSA had lower morning DBP than those with OSA only. As a possible explanation for this apparently contradictory finding, it was previously demonstrated that in OSA patients BP while awake did not always reflect BP surges during sleep (35, 36). Furthermore, the BP response to OSA and PLMS events appeared to vary depending on age (31, 37). An investigation including nocturnal BP monitoring in age-matched cohorts would help in clarifying the underlying mechanism.

In addition, as another mechanism, physical inactivity is a possible intermediary between PLMS and a heightened inflammatory state. Physical activity was reported to be inversely correlated with RLS severity and exercise was reported to decrease RLS/PLMS severity (38-40). Blood inflammatory protein levels were also reported to be inversely associated with physical activity (41-43). Furthermore, it is possible that other undetected common clinical conditions might also be responsible for an association between PLMS and elevated inflammation. According to our literature survey, the association between fibrinogen and PLMS has never been investigated in OSA patients nor in the general population. The precise mechanisms for the association between PLMS and an enhanced inflammatory response remain to be elucidated.

Relationship between OSA and PLMS

Previous studies showed that PLMS are more common in patients with sleep disordered breathing than in the general population (18, 19). In fact, in the present cohort, the prevalence rate of PLMS in OSA-positive patients was significantly higher than that in non-OSA patients. However, the mechanisms for the associations between PLMS genesis and OSA also remain to be elucidated. One possible mechanism is through obesity and dysfunction of the dopaminergic pathway. Obesity is a major risk factor for OSA, and obese people had lower dopamine D2 receptor availability in their brain striatum than normal weight individuals (44). Because a dysfunctional dopaminergic pathway is involved in the genesis of RLS or PLMS (45), OSA and PLMS could be connected with each other. However, Manconi et al. reported that dopamine agonists do not decrease the number of PLMS-associated sleep disordered breathing episodes and suggested that primary dopaminergic dysfunction may not play a major role in the relationship between OSA and PLMS (46). In fact, patients with both PLMS and OSA had a lower BMI than those with OSA only in the present study. The proposed mechanism for PLMS genesis through obesity and dopaminergic dysfunction may not be applicable in the present cohort.

The direct causal relation between PLMS and OSA is poorly understood. Exar et al. performed PSG in subjects with PLMS and monitored intrathoracic pressure during sleep by a pressure transducer catheter that was transnasally placed in the esophagus. They demonstrated that PLMS may occur in association with subtle hypopneic episodes and episodes of increased upper airway resistance that could not be identified by

conventional PSG without a transnasally placed pressure transducer (47). In addition, others indicated that moderate to severe OSA masks PLMS, which may be more fully manifested during OSA treatment because of the amelioration of frank apneas to respiratory effort-related arousals (48, 49). In contrast, other studies indicated that the severity of PLMS had decreased with treatment of OSA (50, 51). The precise mechanism for the associations among PLMS, OSA and treatment of OSA has not been elucidated.

Most previous studies that investigated the relationship between OSA and inflammatory protein levels did not include PLMS as a confounding factor (52, 53). Because the present study showed that the contribution rate of PLMS to inflammatory protein levels was similar or larger than that of OSA, taking the contribution of PLMS into consideration might lead to a more precise evaluation of the relationships between sleep disorders and inflammatory response.

Limitations

We recognized several limitations in the present study. First, the subjects were only those under suspicion of OSA. Therefore, it is likely that if the initial cohort had been from the general population a greater number of patients with PLMS might have been identified. However, with the current study design, the number of patients identified with PLMS only was too small to perform meaningful statistical analyses. Whether we can extrapolate the present results to the general population should be examined in further studies. In addition, all of the subjects in this study were Japanese. Since the

clinical characteristics of patients with OSA and/or PLMS did vary depending on their ethnicity (54, 55), whether our findings can be applied to a cohort comprised of different races should be investigated. Second, we did not evaluate the symptoms of RLS, such as dysesthesias and unpleasant sensations in the legs. Therefore, we could not identify the precise prevalence of RLS in the present cohort. Third, iron deficiency anemia is also considered as a possible cause of PLMS and RLS (56-58). In fact, in the present study the patients with both PLMS and OSA had lower hemoglobin levels than those with OSA only. Because of the retrospective design, we could not evaluate serum iron and ferritin levels. These results might lead us to undertake more sophisticated evaluations of the associations of PLMS, OSA and inflammatory protein levels. Fourth, we did not exclude patients with components of metabolic syndrome such as diabetes and dyslipidemia in order to reflect the situation encountered in actual clinical practice. In addition, to exclude patients with severe renal failure, we chose the serum creatinine level as the index of renal function in this study because values of the estimated glomerular filtration rate vary significantly depending on which predictive formula is adopted (59). The precise evaluation of renal function is of clinical concern and it was possible that this cohort included individuals with moderate renal failure. Although the presence of these comorbid diseases could possibly affect the results, we believe that the possibility was minimized because we took these factors into consideration in the statistical analysis. Lastly, this was a cross-sectional study, and we did not have data on levels of inflammatory proteins after treatment for OSA and/or PLMS. A longitudinal investigation may clarify the more precise underlying mechanisms among OSA, PLMS and an elevated inflammatory response.

Conclusion

In summary, the present study provides the first clinical evidence demonstrating that PLMS were positively associated with plasma CRP and fibrinogen levels in patients under suspicion of OSA. Because the levels of these proteins are established predictive factors of future CVD events, PLMS can be a useful clinical sign to identify OSA patients at high risk for CVD. Since the precise pathophysiologic mechanisms among OSA, PLMS and an elevated inflammatory response remain to be elucidated, further studies are warranted. Moreover, whether we can extrapolate these results to the general population should be examined in further studies to clarify the reasons why PLMS are associated with CVD.

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Reference

- (1) Scofield H, Roth T, Drake C. Periodic limb movements during sleep: population prevalence, clinical correlates, and racial differences. *Sleep* 2008;31:1221-1227.
- (2) Ancoli-Israel S, Kripke DF, Klauber MR, Mason WJ, Fell R, Kaplan O. Periodic limb movements in sleep in community-dwelling elderly. *Sleep* 1991;14:496-500.
- (3) Montplaisir J, Boucher S, Poirier G, Lavigne G, Lapierre O, Lesperance P. Clinical, polysomnographic, and genetic characteristics of restless legs syndrome: a study of 133 patients diagnosed with new standard criteria. *Mov Disord* 1997;12:61-65.
- (4) Li Y, Walters AS, Chiuve SE, Rimm EB, Winkelman JW, Gao X. Prospective study of restless legs syndrome and coronary heart disease among women. *Circulation* 2012;126:1689-1694.
- (5) Winkelman JW, Shahar E, Sharief I, Gottlieb DJ. Association of restless legs syndrome and cardiovascular disease in the Sleep Heart Health Study. *Neurology* 2008;70:35-42.
- (6) Koo BB, Blackwell T, Ancoli-Israel S, Stone KL, Stefanick ML, Redline S, Osteoporotic Fractures in Men (MrOS) Study Group. Association of incident cardiovascular disease with periodic limb movements during sleep in older men: outcomes of sleep disorders in older men (MrOS) study. *Circulation* 2011;124:1223-1231.
- (7) Yumino D, Wang H, Floras JS, Newton GE, Mak S, Ruttanaumpawan P, Parker JD, Bradley TD. Relation of periodic leg movements during sleep and mortality in patients with systolic heart failure. *Am J Cardiol* 2011;107:447-451.

- (8) Li Y, Wang W, Winkelman JW, Malhotra A, Ma J, Gao X. Prospective study of restless legs syndrome and mortality among men. *Neurology* 2013;81:52-59.
- (9) Trotti LM, Rye DB, De Staercke C, Hooper WC, Quyyumi A, Bliwise DL. Elevated C-reactive protein is associated with severe periodic leg movements of sleep in patients with restless legs syndrome. *Brain Behav Immun* 2012;26:1239-1243.
- (10) Bekci TT, Kayrak M, Kiyici A, Ari H, Teke T, Maden E, Akilli H. The relation between Lp-PLA2 levels with periodic limb movements. *Sleep Breath* 2012;16:117-122.
- (11) Weinstock LB, Walters AS, Paueksakon P. Restless legs syndrome--theoretical roles of inflammatory and immune mechanisms. *Sleep Med Rev* 2012;16:341-354.
- (12) Punjabi NM, Sorkin JD, Katzel LI, Goldberg AP, Schwartz AR, Smith PL. Sleep-disordered breathing and insulin resistance in middle-aged and overweight men. *Am J Respir Crit Care Med* 2002;165:677-682.
- (13) Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S. The occurrence of sleep-disordered breathing among middle-aged adults. *N Engl J Med* 1993;328:1230-1235.
- (14) Nakayama-Ashida Y, Takegami M, Chin K, Sumi K, Nakamura T, Takahashi K, Wakamura T, Horita S, Oka Y, Minami I, Fukuhara S, Kadotani H. Sleep-disordered breathing in the usual lifestyle setting as detected with home monitoring in a population of working men in Japan. *Sleep* 2008;31:419-425.
- (15) 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;365:1046-1053.

(16) von Kanel R, Dimsdale JE. Hemostatic alterations in patients with obstructive sleep apnea and the implications for cardiovascular disease. *Chest* 2003;124:1956-1967.

(17) Kraiczi H, Caidahl K, Samuelsson A, Peker Y, Hedner J. Impairment of vascular endothelial function and left ventricular filling : association with the severity of apnea-induced hypoxemia during sleep. *Chest* 2001;119:1085-1091.

(18) Al-Alawi A, Mulgrew A, Tench E, Ryan CF. Prevalence, risk factors and impact on daytime sleepiness and hypertension of periodic leg movements with arousals in patients with obstructive sleep apnea. *J Clin Sleep Med* 2006;2:281-287.

(19) Chervin RD. Periodic leg movements and sleepiness in patients evaluated for sleep-disordered breathing. *Am J Respir Crit Care Med* 2001;164:1454-1458.

(20) Mendelson WB. Are periodic leg movements associated with clinical sleep disturbance? *Sleep* 1996;19:219-223.

(21) Chin K, Ohi M, Kita H, Noguchi T, Otsuka N, Tsuboi T, Mishima M, Kuno K. Effects of NCPAP therapy on fibrinogen levels in obstructive sleep apnea syndrome. *Am J Respir Crit Care Med* 1996;153:1972-1976.

(22) Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO,3rd, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC,Jr, Taubert K, Tracy RP, Vinicor F, Centers for Disease Control and Prevention, American Heart Association. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2003;107:499-511.

(23) Emerging Risk Factors Collaboration, Kaptoge S, Di Angelantonio E, Pennells L, Wood AM, White IR, Gao P, Walker M, Thompson A, Sarwar N, Caslake M, Butterworth AS, Amouyel P, Assmann G, Bakker SJ, Barr EL, Barrett-Connor E, Benjamin EJ, Bjorkelund C, Brenner H, Brunner E, Clarke R, Cooper JA, Cremer P, Cushman M, Dagenais GR, D'Agostino RB S, Dankner R, Davey-Smith G, Deeg D, Dekker JM, Engstrom G, Folsom AR, Fowkes FG, Gallacher J, Gaziano JM, Giampaoli S, Gillum RF, Hofman A, Howard BV, Ingelsson E, Iso H, Jorgensen T, Kiechl S, Kitamura A, Kiyohara Y, Koenig W, Kromhout D, Kuller LH, Lawlor DA, Meade TW, Nissinen A, Nordestgaard BG, Onat A, Panagiotakos DB, Psaty BM, Rodriguez B, Rosengren A, Salomaa V, Kauhanen J, Salonen JT, Shaffer JA, Shea S, Ford I, Stehouwer CD, Strandberg TE, Tipping RW, Tosoetto A, Wassertheil-Smoller S, Wennberg P, Westendorp RG, Whincup PH, Wilhelmsen L, Woodward M, Lowe GD, Wareham NJ, Khaw KT, Sattar N, Packard CJ, Gudnason V, Ridker PM, Pepys MB, Thompson SG, Danesh J. C-reactive protein, fibrinogen, and cardiovascular disease prediction. *N Engl J Med* 2012;367:1310-1320.

(24) Fibrinogen Studies Collaboration, Danesh J, Lewington S, Thompson SG, Lowe GD, Collins R, Kostis JB, Wilson AC, Folsom AR, Wu K, Benderly M, Goldbourt U, Willeit J, Kiechl S, Yarnell JW, Sweetnam PM, Elwood PC, Cushman M, Psaty BM, Tracy RP, Tybjaerg-Hansen A, Haverkate F, de Maat MP, Fowkes FG, Lee AJ, Smith FB, Salomaa V, Harald K, Rasi R, Vahtera E, Jousilahti P, Pekkanen J, D'Agostino R, Kannel WB, Wilson PW, Tofler G, Arocha-Pinango CL, Rodriguez-Larralde A, Nagy E, Mijares M, Espinosa R, Rodriguez-Roa E, Ryder E, Diez-Ewald MP, Campos G, Fernandez V, Torres E, Marchioli R, Valagussa F, Rosengren A, Wilhelmsen L, Lappas

G, Eriksson H, Cremer P, Nagel D, Curb JD, Rodriguez B, Yano K, Salonen JT, Nyyssonen K, Tuomainen TP, Hedblad B, Lind P, Loewel H, Koenig W, Meade TW, Cooper JA, De Stavola B, Knottenbelt C, Miller GJ, Cooper JA, Bauer KA, Rosenberg RD, Sato S, Kitamura A, Naito Y, Palosuo T, Ducimetiere P, Amouyel P, Arveiler D, Evans AE, Ferrieres J, Juhan-Vague I, Bingham A, Schulte H, Assmann G, Cantin B, Lamarche B, Despres JP, Dagenais GR, Tunstall-Pedoe H, Woodward M, Ben-Shlomo Y, Davey Smith G, Palmieri V, Yeh JL, Rudnicka A, Ridker P, Rodeghiero F, Tosetto A, Shepherd J, Ford I, Robertson M, Brunner E, Shipley M, Feskens EJ, Kromhout D, Dickinson A, Ireland B, Juzwishin K, Kaptoge S, Lewington S, Memon A, Sarwar N, Walker M, Wheeler J, White I, Wood A. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. *JAMA* 2005;294:1799-1809.

(25) Danesh J, Collins R, Peto R, Lowe GD. Haematocrit, viscosity, erythrocyte sedimentation rate: meta-analyses of prospective studies of coronary heart disease. *Eur Heart J* 2000;21:515-520.

(26) Lowe GD. Fibrinogen and cardiovascular disease: historical introduction. *Eur Heart J* 1995;16 Suppl A:2-5.

(27) Emerging Risk Factors Collaboration, Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, Collins R, Danesh J. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *Lancet* 2010;375:132-140.

(28) Mansfield MW, Heywood DM, Grant PJ. Circulating levels of factor VII, fibrinogen, and von Willebrand factor and features of insulin resistance in first-degree

relatives of patients with NIDDM. *Circulation* 1996;94:2171-2176.

(29) Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. *JAMA* 1999;282:2131-2135.

(30) Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol* 1999;19:972-978.

(31) Pennestri MH, Montplaisir J, Colombo R, Lavigne G, Lanfranchi PA. Nocturnal blood pressure changes in patients with restless legs syndrome. *Neurology* 2007;68:1213-1218.

(32) Abramson JL, Lewis C, Murrah NV, Anderson GT, Vaccarino V. Relation of C-reactive protein and tumor necrosis factor-alpha to ambulatory blood pressure variability in healthy adults. *Am J Cardiol* 2006;98:649-652.

(33) Kim KI, Lee JH, Chang HJ, Cho YS, Youn TJ, Chung WY, Chae IH, Choi DJ, Park KU, Kim CH. Association between blood pressure variability and inflammatory marker in hypertensive patients. *Circ J* 2008;72:293-298.

(34) Gupta AK, Cornelissen G, Greenway FL, Dhoopati V, Halberg F, Johnson WD. Abnormalities in circadian blood pressure variability and endothelial function: pragmatic markers for adverse cardiometabolic profiles in asymptomatic obese adults. *Cardiovasc Diabetol* 2010;9:58-2840-9-58.

(35) Sekizuka H, Kida K, Akashi YJ, Yoneyama K, Osada N, Omiya K, Miyake F. Relationship between sleep apnea syndrome and sleep blood pressure in patients without hypertension. *J Cardiol* 2010;55:92-98.

- (36) Wright JT,Jr, Redline S, Taylor AL, Aylor J, Clark K, O'Malia B, Graham G, Liao GS, Morton S. Relationship between 24-H blood pressure and sleep disordered breathing in a normotensive community sample. *Am J Hypertens* 2001;14:743-748.
- (37) Haas DC, Foster GL, Nieto FJ, Redline S, Resnick HE, Robbins JA, Young T, Pickering TG. Age-dependent associations between sleep-disordered breathing and hypertension: importance of discriminating between systolic/diastolic hypertension and isolated systolic hypertension in the Sleep Heart Health Study. *Circulation* 2005;111:614-621.
- (38) De Mello MT, Esteves AM, Tufik S. Comparison between dopaminergic agents and physical exercise as treatment for periodic limb movements in patients with spinal cord injury. *Spinal Cord* 2004;42:218-221.
- (39) Aukerman MM, Aukerman D, Bayard M, Tudiver F, Thorp L, Bailey B. Exercise and restless legs syndrome: a randomized controlled trial. *J Am Board Fam Med* 2006;19:487-493.
- (40) Daniele TM, de Bruin VM, E Forte AC, de Oliveira DS, Pompeu CM, de Bruin PF. The relationship between physical activity, restless legs syndrome, and health-related quality of life in type 2 diabetes. *Endocrine* 2012.
- (41) Tomey K, Sowers M, Zheng H, Jackson EA. Physical functioning related to C-reactive protein and fibrinogen levels in mid-life women. *Exp Gerontol* 2009;44:799-804.
- (42) Zanettini R, Bettega D, Agostoni O, Ballestra B, del Rosso G, di Michele R, Mannucci PM. Exercise training in mild hypertension: effects on blood pressure, left ventricular mass and coagulation factor VII and fibrinogen. *Cardiology*

1997;88:468-473.

(43) Hamer M. The relative influences of fitness and fatness on inflammatory factors.

Prev Med 2007;44:3-11.

(44) Wang GJ, Volkow ND, Logan J, Pappas NR, Wong CT, Zhu W, Netusil N, Fowler JS. Brain dopamine and obesity. *Lancet* 2001;357:354-357.

(45) Clemens S, Rye D, Hochman S. Restless legs syndrome: revisiting the dopamine hypothesis from the spinal cord perspective. *Neurology* 2006;67:125-130.

(46) Manconi M, Vitale G, Ferri R, Zucconi M, Ferini-Strambi L. Periodic leg movements in Cheyne-Stokes respiration. *Eur Respir J* 2008;32:1656-1662.

(47) Exar EN, Collop NA. The association of upper airway resistance with periodic limb movements. *Sleep* 2001;24:188-192.

(48) Baran AS, Richert AC, Douglass AB, May W, Ansarin K. Change in periodic limb movement index during treatment of obstructive sleep apnea with continuous positive airway pressure. *Sleep* 2003;26:717-720.

(49) Fry JM, DiPhillipo MA, Pressman MR. Periodic leg movements in sleep following treatment of obstructive sleep apnea with nasal continuous positive airway pressure. *Chest* 1989;96:89-91.

(50) Yamashiro Y, Kryger MH. Acute effect of nasal CPAP on periodic limb movements associated with breathing disorders during sleep. *Sleep* 1994;17:172-175.

(51) Nosedá A, Nouvelle M, Lanquart JR, Kempenaers C, De Maertelaer V, Linkowski R, Kerkhofs M. High leg motor activity in sleep apnea hypopnea patients: efficacy of clonazepam combined with nasal CPAP on polysomnographic variables. *Respir Med* 2002;96:693-699.

- (52) Mehra R, Xu F, Babineau DC, Tracy RP, Jenny NS, Patel SR, Redline S. Sleep-disordered breathing and prothrombotic biomarkers: cross-sectional results of the Cleveland Family Study. *Am J Respir Crit Care Med* 2010;182:826-833.
- (53) Yao M, Tachibana N, Okura M, Ikeda A, Tanigawa T, Yamagishi K, Sato S, Shimamoto T, Iso H. The relationship between sleep-disordered breathing and high-sensitivity C-reactive protein in Japanese men. *Sleep* 2006;29:661-665.
- (54) Ohayon MM, O'Hara R, Vitiello MV. Epidemiology of restless legs syndrome: a synthesis of the literature. *Sleep Med Rev* 2012;16:283-295.
- (55) Sutherland K, Lee RW, Cistulli PA. Obesity and craniofacial structure as risk factors for obstructive sleep apnoea: impact of ethnicity. *Respirology* 2012;17:213-222.
- (56) O'Brien LM, Koo J, Fan L, Owusu JT, Chotinaiwattarakul W, Felt BT, Chervin RD. Iron stores, periodic leg movements, and sleepiness in obstructive sleep apnea. *J Clin Sleep Med* 2009;5:525-531.
- (57) O'Keeffe ST, Gavin K, Lavan JN. Iron status and restless legs syndrome in the elderly. *Age Ageing* 1994;23:200-203.
- (58) Silber MH, Richardson JW. Multiple blood donations associated with iron deficiency in patients with restless legs syndrome. *Mayo Clin Proc* 2003;78:52-54.
- (59) Pedone C, Corsonello A, Incalzi RA, GIFA Investigators. Estimating renal function in older people: a comparison of three formulas. *Age Ageing* 2006;35:121-126.

Table 1. Clinical backgrounds of study patients.

	Neither OSA nor PLMS (n=80)	OSA only (n=208)	PLMS only (n=8)	Both PLMS and OSA (n=46)	p*
Clinical background					
Age (y)	51.9±13.0	55.7±12.6 ^a	62.9±9.6	66.1±8.5 ^{a,b}	<0.0001
Female, n(%)	37 (46.3%)	46 (22.1%)	7 (87.5%)	13 (28.3%)	0.0004
Body mass index (kg/m ²)	25.2±4.1	27.7±5.9 ^a	22.9±4.8	25.6±4.6 ^b	0.0005
Brinkman index	185.2±423.9	282.8±366.0	75.0±116.5	377.6±622.3 ^a	0.0439
Hypertension, n(%)	25 (31.3%)	108 (51.9%)	2 (25.0%)	26 (56.5%)	0.0027
Diabetes, n(%)	11 (13.8%)	51 (24.5%)	1 (12.5%)	14 (30.4%)	0.0514
Dyslipidemia, n(%)	45 (56.3%)	95 (45.7%)	5 (62.5%)	18 (39.1%)	0.1331
SBP (mmHg)	119.7±15.9	128.4±15.2 ^a	120.6±16.3	125.4±15.7	0.0001
DBP (mmHg)	73.7±11.7	80.1±11.0 ^a	69.0±12.1	75.5±11.0 ^b	<0.0001
Laboratory profiles					
Hemoglobin (g/dl)	14.3±1.6	14.6±1.6 ^a	13.2±1.0	14.0±1.3 ^b	0.0160
Creatinine (mg/dl)	0.73±0.16	0.79±0.17 ^a	0.66±0.15	0.79±0.18	0.0229
HbA1c (%)	5.4±0.9	5.8±1.1 ^a	5.5±0.6	5.8±0.8 ^a	0.0079
Total protein (g/dl)	6.8±0.5	6.7±0.4	6.7±0.3	6.8±0.4	0.4431
LDL cholesterol (mg/dl)	112.9±27.0	115.7±32.1	116.0±33.2	114.0±23.4	0.7662
HDL cholesterol (mg/dl)	52.6±13.9	49.8±12.3	56.5±6.9	52.7±16.4	0.1760
Triglyceride (mg/dl)	133.2±89.0	143.2±85.2	91.8±22.8	122.2±59.1	0.2530
C-reactive protein (mg/dl)	0.09±0.15	0.13±0.18	0.14±0.25	0.20±0.48 ^a	0.0259
Fibrinogen (mg/dl)	269.0±57.1	270.0±52.6	289.6±56.5	298.2±76.1 ^{a,b}	0.0082

Data are expressed in mean ± SD. PLMS: periodic limb movements during sleep; OSA: obstructive sleep apnea; SBP: systolic blood pressure; DBP: diastolic blood pressure; LDL: low-density lipoprotein; HDL: high-density lipoprotein.

* p value determined by analysis of variance among groups of patients.

However, the PLMS-only group was excluded from this inter-group analysis because of the small number of patients.

When a significant difference was found among three groups, post hoc analysis was performed to identify where the difference was significant. ^a: p<0.05 vs. Neither OSA nor PLMS, ^b:p<0.05 vs. OSA only.

Table 2. Sleep parameters of study patients

Data are expressed in mean $\pm$ SD. OSA: obstructive sleep apnea, PLMS: periodic limb movements during

	Neither OSA or PLMS (n=80)	OSA only (n=208)	PLMS only (n=8)	Both PLMS and OSA (n=46)	p*
Time in bed (m)	529.1 $\pm$ 53.1	526.0 $\pm$ 53.1	503.6 $\pm$ 46.8	538.4 $\pm$ 48.2	0.3442
Total sleep time (m)	420.2 $\pm$ 67.2	390.8 $\pm$ 74.6 ^a	410.1 $\pm$ 81.1	363.9 $\pm$ 78.1 ^{a,b}	0.0001
AHI (/h)	7.9 $\pm$ 4.3	40.8 $\pm$ 19.9 ^a	7.9 $\pm$ 4.0	33.7 $\pm$ 15.1 ^{a,b}	<0.0001
3%ODI (/h)	5.8 $\pm$ 4.4	38.2 $\pm$ 21.2 ^a	5.9 $\pm$ 4.7	31.0 $\pm$ 19.0 ^{a,b}	<0.0001
Apnea index (/h)	1.9 $\pm$ 2.5	20.6 $\pm$ 18.4 ^a	2.0 $\pm$ 1.1	15.4 $\pm$ 14.0 ^a	<0.0001
SpO ₂ <90% time (m)	4.5 $\pm$ 10.0	94.8 $\pm$ 120.7 ^a	0.7 $\pm$ 0.8	61.6 $\pm$ 103.4 ^a	<0.0001
Minimum SpO ₂ (%)	88.4 $\pm$ 4.6	76.9 $\pm$ 10.9 ^a	89.4 $\pm$ 5.6	78.1 $\pm$ 10.0 ^a	<0.0001
Non slow wave sleep (%)	73.8 $\pm$ 9.1	80.5 $\pm$ 9.9 ^a	82.3 $\pm$ 4.3	81.7 $\pm$ 7.9 ^a	<0.0001
Slow wave sleep (%)	7.8 $\pm$ 7.5	4.8 $\pm$ 7.1 ^a	2.3 $\pm$ 4.1	4.5 $\pm$ 5.5 ^a	0.0028
REM sleep (%)	18.4 $\pm$ 5.7	14.7 $\pm$ 6.1 ^a	15.4 $\pm$ 2.8	13.8 $\pm$ 5.3 ^a	<0.0001
PLMS index (/h)	1.5 $\pm$ 3.5	1.4 $\pm$ 3.3	36.1 $\pm$ 14.9	41.9 $\pm$ 31.9 ^{a,b}	<0.0001
PLMS with arousal index (/h)	0.2 $\pm$ 0.6	0.2 $\pm$ 0.8	5.2 $\pm$ 4.8	3.5 $\pm$ 5.5 ^{a,b}	<0.0001

sleep; AHI: apnea hypopnea index; ODI: oxygen desaturation index; REM; rapid eye movements.

*: p value determined by analysis of variance among groups of patients. However, the PLMS-only group was excluded from the intergroup analysis because of the small number of patients.

When a significant difference was found among three groups, post hoc analysis was performed to identify where the difference was significant. ^a: p<0.05 vs. Neither OSA nor PLMS, ^b:p<0.05 vs. OSA only.

Table 3. Univariate and multivariate regression analyses for the entire cohort (n=342) using the C-reactive protein or fibrinogen level as the dependent variables

	CRP				Fibrinogen			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	r	p	β	p	r	p	β	p
Age (y)	0.0223	0.6808	-	-	0.1703	0.0016	-	0.2848
Female	<0.001	0.9841	-	-	0.1364	0.0115	-	0.2904
Body mass index (kg/m ²)	0.2191	<0.0001	0.2090	0.0005	0.1229	0.0229	-	0.2738
Brinkman index	0.1034	0.0562	-	0.0629	0.1673	0.3219	-	-
SBP (mmHg)	0.1034	0.0563	-	0.5497	0.1323	0.0141	-	0.1142
Hemoglobin (g/dl)	<0.0001	0.8770	-	-	-0.1786	0.0009	-	0.0610
Creatinine (mg/dl)	<0.0001	0.8670	-	-	-0.0837	0.1446	-	-
HbA1c (%)	0.1170	0.0306	-	0.4262	0.1729	0.0014	0.1181	0.0296
Total sleep time (m)	0.0282	0.5933	-	-	-0.2358	0.1831	-	-
3%ODI (/h)	0.1526	0.0047	-	0.4284	0.1442	0.0076	-	0.0646
PLMS positive	0.1183	0.0291	0.1401	0.0085	0.1712	0.0015	0.1359	0.0128

r: correlation efficient; β : standard regression coefficient; SBP: systolic blood pressure; ODI: oxygen desaturation index; PLMS: periodic limb movements during sleep.

Table 4. Univariate and multivariate regression analyses for the OSA cohort (n=254) using the plasma C-reactive protein or fibrinogen level as the dependent variables.

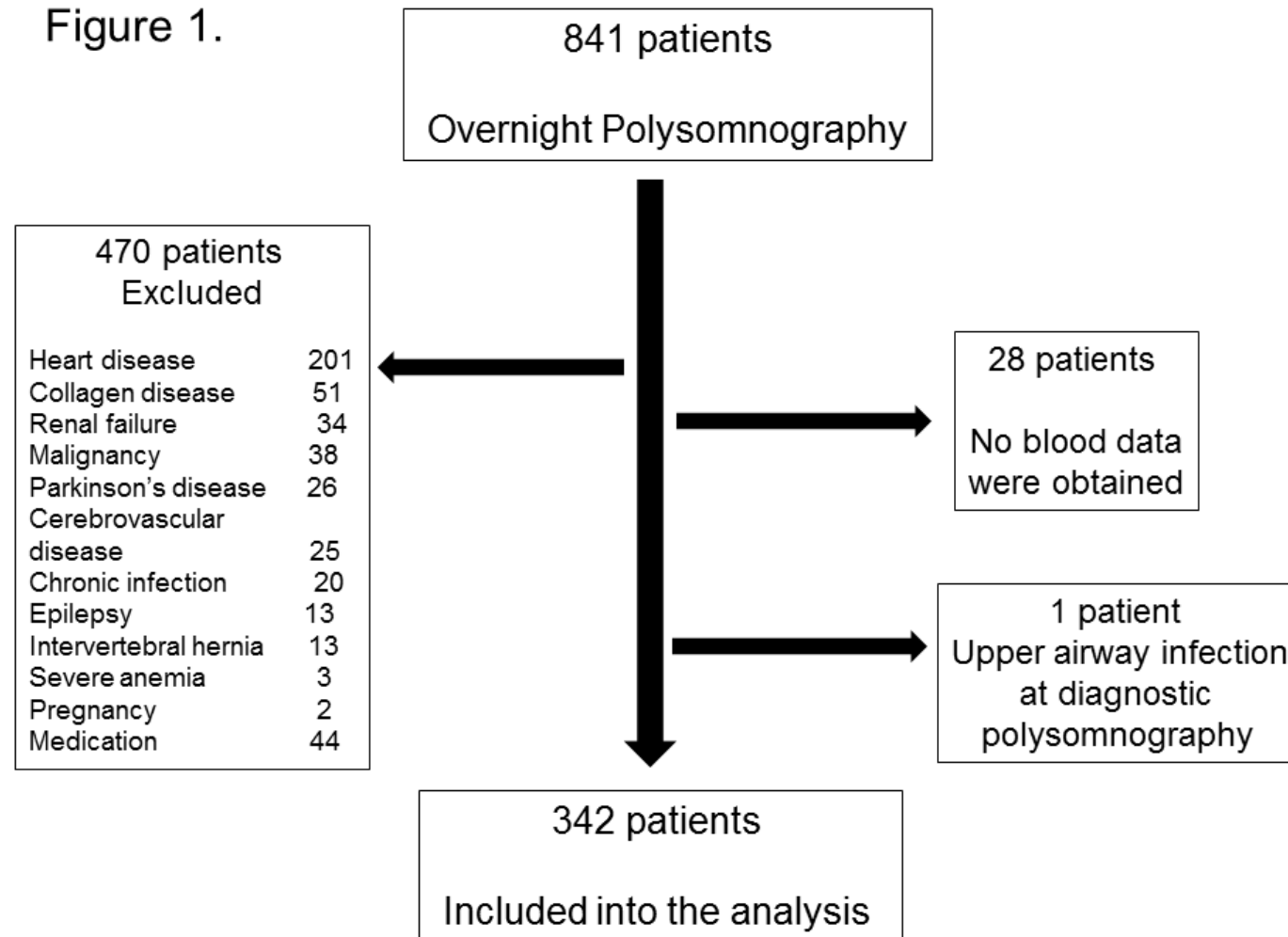
	CRP				Fibrinogen			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	r	p	β	p	r	p	β	p
Age (y)	0.0400	0.5264	-	-	0.1345	0.0322	-	0.5280
Female	0.0420	0.5047	-	-	0.1507	0.0162	-	0.5029
Body mass index (kg/m ²)	0.1863	0.0029	0.1794	0.0086	0.1414	0.0242	-	0.4011
Brinkman index	0.1000	0.1109	-	-	<0.0001	0.8851	-	-
SBP (mmHg)	0.1034	0.1006	-	-	0.1432	0.0223	-	0.0815
Hemoglobin (g/dl)	<0.0001	0.9118	-	-	-0.2020	0.0012	-	0.0530
Creatinine (mg/dl)	0.0678	0.2839	-	-	-0.1030	0.1009	-	-
HbA1c (%)	0.0520	0.4130	-	-	0.1389	0.0268	-	0.1377
Total sleep time (m)	0.0100	0.5430	-	-	-0.2156	0.3373	-	-
3%ODI (/h)	0.1225	0.0509	-	0.3183	0.1715	0.0062	0.1855	0.0071
PLMS positive	0.1118	0.0749	0.1466	0.0192	0.1860	0.0029	0.1844	0.0036

r: correlation efficient; β : standard regression coefficient; SBP: systolic blood pressure; ODI: oxygen desaturation index; PLMS: periodic limb movements during sleep.

Figure legend

Figure 1. Flow chart of patient selection.

Figure 1.



ONLINE DATA SUPPLEMENT

The additive impact of periodic limb movements during sleep on inflammation in obstructive sleep apnea patients

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Methods

The definition of comorbid diseases

Hypertension was defined by a systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg or previous treatment for hypertension. Diabetes mellitus was defined by HbA1c $\geq 6.0\%$ or previous treatment. Dyslipidemia was defined by triglycerides ≥ 150 mg/dl, high-density lipoprotein cholesterol level < 40 mg/dl, low-density lipoprotein cholesterol level ≥ 140 mg/dl or specific treatment for these lipid abnormalities. Smoking status was evaluated by the Brinkmann index, which represents the number of cigarettes smoked per day multiplied by the number of years of smoking.

Polysomnography

The diagnoses of obstructive sleep apnea (OSA) and periodic limb movements during sleep (PLMS) were confirmed by polysomnography (PSG) (SomnoStar pro, Cardinal Health, Dublin, OH, USA or Alice 4, Philips Respironics, Inc., Murrysville, PA, USA), which started at 22:00 and ended at 6:00 the following morning. Surface electrodes were attached using standard techniques to obtain an electrooculogram, electromyogram (EMG) of the chin and 12-lead electroencephalogram (EEG). Sleep stages were defined according to the criteria of Rechtschaffen and Kales.⁽¹⁾ Ventilation was monitored by inductive plethysmography (Respirtrace QDC, Viasys Healthcare, Palm Springs, CA, USA). Airflow was monitored by a nasal pressure transducer and supplemented by an oronasal thermal sensor. Arterial oxygen saturation (SpO_2) was monitored continuously with a pulse oximeter. Apnea was defined as the complete cessation of airflow and hypopnea as a clear decrease in airflow of 50% lasting more than 10 s and followed by

either a decrease in SpO₂ of at least 3% or EEG arousal.(2) All apnea hypopnea index (AHI) values were expressed as the number of episodes of apnea and hypopnea per hour over the total sleep time. 3% oxygen desaturation index (ODI) values were defined as the number of desaturations $\geq 3\%$ per hour of sleep. The length of time of SpO₂<90% during sleep was calculated in each patient.

All movements of the left and right legs were recorded independently from the anterior tibialis EMG using surface electrodes. We scored PLMS based on the standard American Academy of Sleep Medicine criteria in which individual movements were scored as PLMS if the duration was between 0.5 and 5 s and when there was a clear increase in amplitude from baseline in leg channels.(3) To be considered periodic, at least 4 movements needed to occur in succession no less than 5 s and no more than 90 s apart. Leg movements that occurred at resolution of an apnea or hypopnea were not scored as PLMS. The PLMS index was the total number of periodic leg movements per hour of sleep. The PLMS arousal index was determined as the total number of periodic leg movements per hour of sleep in which EEG arousal occurred within 1 s of movement termination.

Blood sampling and measurement of plasma fibrinogen level

Blood samples were drawn at 7:00 in the morning following a 12-h overnight fast and PSG. Thrombocheck Fib (L) (Sysmex Corporation, Kobe, Japan) is a liquid type reagent for use with the Clauss method and was employed for fibrinogen measurement. Measurements were performed using a fully automated coagulation analyzer (Coagrex 800, Shimazu Corporation, Kyoto, Japan). The intra- and inter-assay coefficients of variation for this method of measurement were less than 15% and 6%, respectively.

Reference

(E1) Rechtschaffen A, Kales A. A Manual of Standardized Terminology, techniques and Scoring system for sleep stages of human subjects. Washington, DC: National Institutes of Health; 1968.

(E2) Iber C, Ancoli-Israel S, Chesson A, Quan S. The AASM manual for the Scoring of sleep and associated Events: Rules, Terminology and Technical Specifications. Westchester, IL, USA: American Academy of Sleep Medicine; 2007.

(E3) Recording and scoring leg movements. The Atlas Task Force. *Sleep* 1993;16:748-759.

Table E1. The correlation coefficients between plasma inflammatory protein levels and indexes of obstructive sleep apnea or PLMS.

(A)

	r	p
Apnea Hypopnea Index, /h	0.1449	0.0073
3% oxygen desaturation index, /h	0.1526	0.0045
PLMS index, /h	0.0656	0.2236
PLMS positive	0.1187	0.0284

(B)

	r	p
Apnea Hypopnea Index, /h	0.1315	0.0149
3% oxygen desaturation index, /h	0.1442	0.0076
PLMS index, /h	0.1327	0.0142
PLMS positive	0.1712	0.0015

(A) Simple correlations between plasma C reactive protein levels and indexes of

obstructive sleep apnea or PLMS.

(B) Simple correlations between plasma fibrinogen levels and indexes of obstructive

sleep apnea or PLMS.

r: correlation coefficient; PLMS: periodic limb movements during sleep.