Published in final edited form as:

Brain Behav Immun. 2012 November ; 26(8): 1239–1243. doi:10.1016/j.bbi.2012.06.003.

Elevated C-reactive protein is associated with severe periodic leg movements of sleep in patients with restless legs syndrome

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Abstract

Background—Restless legs syndrome (RLS) is a common sleep disorder in which urges to move the legs are felt during rest, are felt at night, and are improved by leg movement. RLS has been implicated in the development of cardiovascular disease. Periodic leg movements (PLMs) may be a mediator of this relationship. We evaluated systemic inflammation and PLMs in RLS patients to further assess cardiovascular risk.

Methods—137 RLS patients had PLM measurements taken while unmedicated for RLS. Banked plasma was assayed for high sensitivity C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-alpha).

Results—Mean (SD) PLM index was 19.3 (22.0). PLMs were unrelated to TNF-a and IL-6, but were modestly correlated with log CRP ($r(129) = 0.19$, $p = 0.03$). Those patients with at least 45 PLMs/hour had an odds ratio of 3.56 (95% CI 1.26 to 10.03, $p = 0.02$, $df = 1$) for having elevated CRP compared to those with fewer than 45 PLMs/hour. After adjustment for age, race, gender, diabetes, hypertension, hyperlipidemia, inflammatory disorders, CRP-lowering medications, and body mass index, the OR for those with ≥ 45 PLMs/hour was 8.60 (95% CI 1.23 to 60.17, $p = 0.03$, $df = 10$).

Conclusions—PLMs are associated with increased inflammation, such that those RLS patients with at least 45 PLMs/hour had more than triple the odds of elevated CRP than those with fewer PLMs. Further investigation into PLMs and inflammation is warranted.

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Conflict of Interest Statement: Drs. Trotti and Rye have been consultants for UCB Pharma, and Dr. Rye has given CME lectures sponsored by UCB Pharma. Dr. Bliwise is a consultant for Ferring. The remaining authors declare that they have no conflicts of interest.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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Keywords

C-reactive protein; periodic leg movements of sleep; restless legs syndrome; interleukin-6; tumor necrosis factor alpha; cardiovascular disease

Introduction

Restless legs syndrome (RLS) is a common sleep disorder in which urges to move the legs are bothersome during rest and at night, and are improved by leg movement. In addition to sensory symptoms, the majority of RLS patients also experience periodic leg movements (PLMs). PLMs are repetitive movements occurring every 5–90 seconds. Over 90% of RLS patients demonstrate at least 5 PLMs/hour when monitored by leg actigraphy for five nights (Trotti et al., 2009).

Both RLS and PLMs have been implicated in the development of cardiovascular disease. RLS has been shown to be associated with prevalent cardiovascular disease in several large population-based studies (Innes et al., 2011). PLMs, either in association with RLS or as an isolated finding, have been associated with changes in heart rate and heart rate variability (Ferri et al., 2007; Guggisberg et al., 2007; Manconi et al., 2011; Sforza et al., 2005), beat to beat increases in blood pressure (Pennestri et al., 2007; Siddiqui et al., 2007a), and prevalent hypertension (Billars et al., 2007), and predict incident vascular events in elderly normotensive men (Koo et al., 2011).

RLS is strongly associated with systemic inflammation, in that nearly 90% of the medical conditions known to be associated with RLS are inflammatory or infectious (Weinstock et al., 2012), and systemic inflammation is associated with and predictive of cardiovascular disease (Casas et al., 2008; Danesh et al., 2008; Libby, 2006; Sarwar et al., 2009; Venugopal et al., 2002; Yudkin et al., 2000; Zhang et al., 2009). Systemic inflammation may be measured with a variety of markers, including C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-alpha). While these markers are known to be associated with other causes of sleep disruption, especially sleep apnea (Ciftci et al., 2004; Jelic et al., 2008; Ryan et al., 2006; Shamsuzzaman et al., 2002; Steiropoulos et al., 2010; Svatikova et al., 2003; Vgontzas et al., 1997) and chronic partial sleep deprivation (Lange et al., 2010), they have not been evaluated with respect to PLMs in RLS patients. We sought to determine the relationship between PLMs and markers of systemic inflammation in such a population.

Methods

Subjects

RLS patients (diagnosed by clinical interview) evaluated before December 2009, with PLM measurements taken while free of dopaminergic, opiate, or gabapentin-derived medications, were included. RLS mimickers (e.g., diabetic neuropathy, leg cramps, arthritis, positional leg discomfort) were excluded during face-to-face interview by a sleep physician with experience in the diagnosis of RLS. Information about the resulting 137 patients' demographic and clinical features was collected from existing research and clinical databases. Subjects provided informed consent for use of information and blood samples through a protocol approved by the Emory Institutional Review Board.

Measurements

Inflammatory markers—Banked, frozen plasma was assayed for high sensitivity CRP by nephelometry (Dade-Behring, Deerfield, IL) and for IL-6 and TNF-alpha by fluorokine

multianalyte profiling (R&D Systems, Minneapolis, MN). Assays were performed by investigators blinded to all other data collected from subjects.

PLMs—PLM measurements were obtained using the PAM-RL tri-axial accelerometer (Philips Respironics, Murrysville, PA) worn on one ankle for five consecutive nights. The mean PLM index (PLMI, number of PLMs/hour) of recorded nights was used for analysis. The major rest period was used as a surrogate for sleep period (Stefansson et al., 2007).

Clinical and demographic features—We collected demographic (age, gender, race) and clinical information. This included conditions associated with cardiovascular disease (hypertension, diabetes, smoking history, body mass index (BMI)), stroke, coronary artery disease, heart failure, or cardiomyopathy, and systemic inflammation (observed inflammatory conditions included asthma, COPD or chronic bronchitis, malignancy, arthritis other than osteoarthritis, chronic sinusitis, eczema, Crohn's disease, ulcerative colitis, celiac sprue, hepatitis C, recurrent bladder infections, human immunodeficiency virus, endometriosis, chronic inflammatory demyelinating polyneuropathy, and dermatomyositis). Clinical suspicion for obstructive sleep apnea (OSA) was based on a measured respiratory disturbance index ((RDI, from overnight, in-laboratory polysomnography performed on a different night) 5/hour of sleep, or a history of snoring or witnessed apneas in the absence of polysomnography. We also collected data on medications known or suspected to lower CRP levels (HMGCoA reductase inhibitors, ezetimibe, fenofibrate, niacin, beta-antagonists, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, vitamin E, rosiglitazone, pioglitazone, omega-3 fatty acids, and cod liver oil (Ebrahimi et al., 2009; Muhlestein, 2010; Prasad, 2006)). We collected data regarding common secondary forms of RLS (pregnancy, iron deficiency, and end-stage renal disease).

Statistical analyses

Relationships between severity of PLMs and demographic and clinical features were evaluated with t-tests and Spearman correlation coefficients. TNF-alpha, IL-6, and log CRP levels were correlated with PLMs using Spearman correlation coefficients. TNF-alpha and IL-6 were then divided into quartiles and PLMs in lowest versus highest quartiles compared using t-tests. Based on published standards, CRP was categorized as low-intermediate (<3 mg/L) or high (3–10 mg/L) and values greater than 10 were excluded from CRP-related analyses as potentially representing acute rather than chronic inflammation (n = 6) (Pearson et al., 2003). Univariate analyses comparing clinical and demographic features in those with low- intermediate or high CRP were performed using t-tests for continuous variables and Chi-square tests or Fisher exact tests for categorical variables, as appropriate.

The relationship between PLMs and CRP was modeled using unadjusted logistic regression. We first evaluated whether the relationship between PLMs and elevated CRP was log-linear by categorizing PLMs by groups of 15 per hour (i.e., 15–30, 30–45, and > 45), using PLMs < 15 as the reference group as this is the threshold above which PLMs are generally considered to be elevated (AASM, 2005). The subsequent regression model did not show a linear trend in ORs with increasing PLMS. Instead, elevated odds were apparent only for the highest PLM group (i.e., those with PLMs > 45). Therefore, PLM index was dichotomized at 45.

To control for potential confounders, a multivariate logistic regression analysis was then performed. Because age and BMI were also not log-linear with respect to CRP, age was divided into quartiles and BMI categorized following standard values (i.e., <25, 25–30, 30–40, >40). All analyses were performed using SAS software (version 9.2, SAS Institute, Cary, NC).

Results

Mean age of subjects was 42.3 (21.2) years. Mean PLMI was 19.3 (22.0). Iron deficiency was the most common form of secondary RLS observed, with 59 patients having a serum ferritin < 50 ng/mL (54% of subjects for whom ferritin measurements were available). Two women were pregnant at the time of enrollment and none of the patients had end-stage renal disease. Thirty-nine percent of subjects were men and 15% were African-American. Medical comorbidities were common, with hypertension being the most common (Table 1). However, diagnosed cardiovascular disease (coronary artery disease, cardiomyopathy, or congestive heart failure) and cerebrovascular disease (stroke or transient ischemic attack) were uncommon, each occurring in only four percent of subjects.

Periodic leg movements were significantly associated with many covariates (Table 2). PLMI was significantly higher in subjects with co-morbid hypertension, diabetes, hyperlipidemia, and cerebrovascular disease. Men and patients on medications with CRP-lowering effects also had higher mean PLMIs. Higher levels of PLMs were also significantly correlated with age, BMI, and RDI.

PLMs were unrelated to TNF-alpha levels (Spearman correlation $r(135) = -0.05$, $p = 0.54$). Those with the highest quartile of TNF-alpha values had similar mean PLMs to those with the lowest quartile (20.9 vs 16.7, $t(53) = -0.91$, $p = 0.36$). PLMs were unrelated to IL-6 (Spearman correlation $r(135) = 0.04$, $p = 0.63$). Those patients with the highest quartile IL-6 values did not have higher mean PLMs than those with the lowest quartile (20.6 vs 18.8, $t(67) = -0.31$, $p = 0.76$).

In contrast, PLMs were modestly but significantly correlated with log-CRP values ($r(129) = 0.19$, $p = 0.03$, Figure 1). In the unadjusted logistic model, the odds of elevated CRP were significantly higher for those with PLMs ≥ 45 /hour (OR 3.56, 95% CI 1.26 to 10.03, $p = 0.02$, $df = 1$, $n = 131$ for model). Relative to the low-intermediate CRP patients, those with high CRP were more likely to be female, had higher mean BMI, were more likely to have clinically-suspected OSA, and tended toward having higher measured RDIs (Table 1). Adjusting the regression model for age, gender, race, diabetes, hypertension, hyperlipidemia, CRP-lowering medication, inflammatory conditions, and body mass index, PLMs ≥ 45 /hour remained a significant predictor of CRP (OR 8.60, 95% CI 1.23 to 60.17, $p = 0.03$, $df = 10$, $n = 101$). Female gender (OR 32.6, $p = 0.0007$) and body mass index (OR = 3.7, $p = 0.0006$) were the only other significant predictors of high CRP in the multivariate model. Including serum ferritin level as a dichotomous variable (less than or greater than 50 ng/mL) resulted in a model that ran on fewer subjects, but PLMs, female gender, and body mass index remained the only significant predictors of elevated CRP (data not shown). Similarly, when including clinically-suspected OSA in the multivariate model, the same three variables (PLMs, gender, BMI) remained significant predictors of elevated CRP.

Discussion

This study demonstrated that among patients with RLS, those with high numbers of PLMs (i.e., at least 45 per hour) were significantly more likely to demonstrate systemic inflammation, as measured by CRP. This relationship remained apparent after controlling for demographic features (i.e., gender, age, and race) and clinical conditions. This is consistent with the finding by Bekci et al that in patients with PLMs, not selected for the presence of RLS, CRP levels were significantly higher in those with PLMs ≥ 15 /hour; in a multivariate analysis, this relationship became marginally non-significant ($p = 0.07$) (Bekci et al., 2011).

Despite the observed relationship between PLMs and CRP in these two studies, several prior studies have determined that the presence of RLS itself is not associated with elevated CRP levels (Benediktsdottir et al., 2010; Berger et al., 2002). There are several possible explanations for these apparently discrepant findings regarding the relationship of CRP with PLMs but not with RLS. First, the RLS-CRP studies were population-based while the PLM studies derived from clinic populations, so it is likely that the underlying disease severity of both RLS and number of PLMs was different (i.e., more severe in clinic patients). Because number of PLMs correlates with RLS severity (Aksu et al., 2007; Garcia-Borreguero et al., 2004) and the risk of elevated CRP appears to be driven by those with the most PLMs, differences in disease severity in the underlying populations may account for these different findings. Alternatively, it may be that it is the disruption of sleep or associated sympathetic arousals related to PLMs, rather than the sensory component of RLS, that is most strongly associated with systemic inflammation as assessed with CRP.

In contrast to the significant relationship between CRP and PLMs, the other tested inflammatory markers (IL-6, TNF-alpha) were not associated with PLMs in our subjects. This may reflect the increased variability in measurements of these markers. CRP has a nineteen hour half-life and does not have substantial circadian variability, so a single measurement is considered to be an accurate reflection of true CRP levels (Bajpai et al., 2010; Danesh et al., 2004; Meier-Ewert et al., 2001). In contrast, both IL-6 and TNF-alpha have been shown to have circadian variability (Petrovsky, 2001). IL-6 additionally has substantial intra-subject variability with repeated measures (Ho et al., 2005). Furthermore, levels of both IL-6 and TNF-alpha, but not CRP, may be affected by high fat content meals (Payette et al., 2009). Plasma samples for all inflammatory markers were collected at a single point without respect to time of day or food consumption which may have reduced our ability to see a true association, if any, with PLMs. As with CRP, IL-6 and TNF-alpha do not appear to be associated with RLS (Siddiqui et al., 2007b), although many of the inflammatory diseases that are associated with RLS are also associated with increases in IL-6 and TNF-alpha (Weinstock et al., 2012).

One limitation of this study was the lack of a control group free of both RLS and PLMS. Thus, our results are applicable to PLMs in patients with RLS, but should not necessarily be extrapolated to people having PLMs without RLS. Due to the retrospective nature of this study, data were missing on several potentially important variables. Obstructive sleep apnea is one such condition, which is potentially relevant here for several reasons. It has been shown that in the case of hypercholesterolemia, the apparent association with RLS is fully explained by comorbid sleep apnea (Cosentino et al., 2012). Furthermore, because leg actigraphy cannot distinguish between idiopathic PLMs and periodic leg movements accompanying respiratory events, it is possible that some of the PLMs detected in our study were movements co-occurring with sleep apnea that were incorrectly classified because of the lack of polysomnographic monitoring. However, the fact that the association remained significant after controlling for BMI (which would be expected to be elevated in those patients with sleep apnea) and a measure of clinical likelihood of OSA reduces but does not eliminate this important confounding factor.

Our overall sample size was small relative to the number of potential confounders evaluated in the multivariate model, which may have affected the precision of the odds ratio estimates (Vittinghoff and McCulloch, 2007). For some conditions (e.g., celiac sprue), diagnosis was predicated on a formal diagnosis within the medical record, such that undiagnosed or silent cases would have been missed. Despite these limitations, our preliminary finding of an association between high PLM counts and elevated CRP levels suggests that systemic inflammation might be involved in the association between cardiovascular disease and RLS/

PLMs. Prospective investigation of inflammation in subjects with PLMs, fully evaluating potentially relevant confounders such as sleep apnea, is warranted.

Acknowledgments

Supported by PHS Grant KL2RR025009 from the Clinical and Translational Science Award program (LMT), 2P50AG025688, and P30NS055077.

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Figure 1. Patients with restless legs syndrome who have frequent periodic leg movements of sleep are more likely to have elevated levels of C-reactive protein, but not IL-6 or TNF-alpha.

Table 1

Demographic and clinical features

	All ^A (n = 137)	Low-intermediate CRP (n = 104)	High CRP (n = 27)	p-value (df)
Age (n = 137)	42.3 (21.2)	40.6 (22.0)	46.4 (18.1)	0.21 (129)
Male Gender (n = 137)	53 (38.7%)	48 (46.2%)	4 (14.8%)	0.003 (1)
African-American Race (n = 133)	20 (15.0%)	15 (15.0%)	4 (14.8%)	1.00 (1)
Hypertension (n = 136)	40 (29.4%)	29 (28.2%)	8 (29.6%)	0.88 (1)
Diabetes (n = 136)	12 (8.8%)	9 (8.7%)	3 (11.1%)	0.71 (1)
Hyperlipidemia (n = 131)	16 (12.2%)	13 (13.1%)	3 (11.5%)	1.00 (1)
Inflammatory diseases (n = 131)	31 (23.7%)	21 (21.2%)	9 (34.6%)	0.15 (1)
CRP-lowering medications (n = 137)	50 (36.5%)	38 (36.5%)	9 (33.3%)	0.76 (1)
Current or past smoking (n = 92)	13 (14.1%)	9 (14.1%)	3 (13.6%)	1.00 (1)
RDI (n = 45)	10.2 (13.1)	7.7 (10.7)	16.2 (12.8)	0.06 (40)
Clinically-suspected OSA (n = 121)	39 (32.2%)	25 (27.5%)	12 (50%)	0.036 (1)
BMI (n = 109)	27.4 (7.3)	25.5 (5.6)	31.8 (7.7)	0.0007 (31)
Serum ferritin less than 50 ng/mL (n = 109)	59 (54.1%)	47 (57.3%)	11 (52.4%)	0.68 (1)

Abbreviations: CRP = C-reactive protein, df = degrees of freedom, RDI = respiratory disturbance index (a measure of sleep apnea), BMI = body mass index

^AIncludes those patients with CRP > 10

Table 2

Relationships between PLMs and covariates (univariate analyses)

	Mean (SD) PLMs (movements per hour)		<i>t</i> -test <i>p</i> -value (<i>df</i>)
	Presence of covariate	Absence of covariate	
Hypertension	29.0 (23.9)	15.4 (20.0)	0.0008 (134)
Diabetes	36.1 (24.4)	17.8 (21.2)	0.006 (134)
Hyperlipidemia	31.5 (26.8)	18.2 (21.3)	0.03 (129)
Inflammatory diseases	28.2 (28.1)	17.2 (19.7)	0.05 (40)
CRP-lowering medications	28.4 (24.9)	14.0 (18.4)	0.0006 (80)
Current or past smoking	35.3 (32.5)	19.0 (21.4)	0.10 (14)
African-American	16.9 (19.4)	20.3 (22.6)	0.52 (131)
Male	24.6 (27.8)	15.9 (16.8)	0.045 (76)
Clinically suspected OSA	35.3 (25.4)	28.0 (21.1)	0.23 (119)
Serum ferritin < 50	27.6 (19.8)	37.3 (27.4)	0.12 (107)
	Spearman correlation coefficient		<i>p</i> -value (<i>df</i>)
Age	0.48		<0.0001 (135)
BMI	0.21		0.03 (109)
RDI	0.32		0.03 (43)

Abbreviations: PLMs = periodic limb movements, SD = standard deviation, df = degrees of freedom, CRP = C-reactive protein, BMI = body mass index, RDI = respiratory distress index