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Jonathan S. Schultz April 21th 2011

Determinants of Periodic Limb Movements in Sleep within Patients with Restless Legs Syndrome

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Patients with Restless Legs Syndrome

By

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Bachelor of Science, Iowa State University, 2005

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Abstract

Determinants of Periodic Limb Movements in Sleep within Patients with Restless Legs Syndrome

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Purpose: Clinico-epidemiological surveys disagree on the correlations between the measurements of iron metabolism and Periodic Leg Movements in Sleep (PLMS) in patients with Restless Leg Syndrome (RLS). PLMS are a feature of RLS patients that coincide temporally with sympathetically mediated heart rate and blood pressure elevations, and could represent a plausible biological mechanism for some RLS-comorbidities. This study evaluated the clinical determinants of PLMS, specifically measurements of iron metabolism in a clinical cohort of patients enriched for RLS.

Methods: RLS patients seen at the Emory Sleep Center with measured PLMS (n = 452) were given a detailed questionnaire by a physician assessing health habits, RLS symptoms, and co-morbid conditions. PLMS were measured over 5 nights with an accelerometer, one night polysomnograph, or both. A retrospective chart review was conducted to retrieve patient data and determine significant clinical predictors and to explore how medications affect PLMS.

Results: Total Iron Binding Capacity (TIBC) was found to be a significantly correlated with increased number of PLMS after controlling for potential confounders. However, TIBC was not a robust determinant in predicting categorical PLMS when taken as a binary predictor. All other iron metabolism measures were not significantly correlated with PLMS, after controlling for potential confounders. Exploratory analyses of medication effects showed that dopamenergics and both groups of selective serotonin reuptake inhibitors (SSRIs), those with minimal and those with higher norepinephrine reuptake inhibition, were significant predictors of a 4 level categorical PLMS index, while controlling for potential confounders.

Conclusions: Prior studies have suggested that PLMS are associated with anemia and that iron deficiency might be one way to explain increased PLMS in RLS patients. TIBC was found to be weakly associated with increased number of PLMS, however this was not found to be a very robust finding. The blood iron panel was not associated with the severity of PLMS in patients with RLS. Future studies should investigate the potential of TIBC, but different biological mechanisms should be considered to understand the underlying pathology of PLMS in patients with RLS.

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Table of Contents

Introduction

In the mid-17th century, Thomas Willis first described a disease that would become known as "Restless Legs Syndrome" [1]. Restless legs syndrome (RLS) is characterized by a compelling, often insatiable, need to move the legs, accompanied by unpleasant sensations located mainly in the ankles and calves. Everyday activities such as plane travel, car rides, attending school, meetings, or the theatre, and sleep, tend to increase these unpleasant sensations. RLS is conventionally defined by the International Restless Legs Syndrome Study Group (IRLSSG) as a symptom complex that includes: a) an intense urge to move the legs that is often uncomfortable or painful; b) a worsening at rest; c) relief with movement; and d) a circadian preference to emerge in the evening and at night [2]. The prevalence of RLS in populations of European descent ranges from 3-15% [3-9]. RLS symptoms that are deemed frequent or severe enough to require clinical treatment are less common, occurring in about 1.6% to 2.8% of these populations [4, 5, 10]. In addition to the four criteria defined by the IRLSSG other common features of RLS include Periodic Limb Movements in Sleep (PLMS)[11], sleep disturbances[12], and involuntary movements while awake[13]. Variability in symptom expressivity is common [14-18]. PLMS are a typical feature of RLS patients, occurring in 80-88% of patients meeting all four criteria for RLS [19].

PLMS originally coined "nocturnal myoclonus" by Symonds, are involuntary, highly stereotyped, and regularly occurring [20]. PLMS are classically described as repetitive and rhythmic movements of the lower legs, often an extension of the big toe (*i.e.*, extensor hallicus longus) and dorsiflexion of the ankle, with occasional flexion at

the knee and hip [11]. According to the American Sleep Disorders Association (ASDA), PLMS are defined as a series of at least four consecutive leg movements lasting 0.5–5 seconds for each movement, and a gap of 4–90 seconds between each movement in the series [21]. They are evident at a rate of greater than 5/hour from anterior tibialis surface electrodes (employed in routine polysomnography (PSG)) in 80% of RLS subjects, increasing to 88% on two consecutive recording nights [19].

PLMS are of scientific interest because they are a quantitative, measurable and objective RLS endophentoype [22]. A PLMS index (PLMI; PLMs per hour of recumbency) ≥ 5/h has been shown in 90% of subjects diagnosed with RLS on any single night of polysomnography, if at least two consecutive nights are recorded [18]. When PLMS measurements are combined with self-reported RLS symptoms, the positive predictive value for RLS is > 95% when compared to the gold standard examination administered by a clinician[23]. The PLMI also allows for the reasonable discrimination of RLS from insomnia at a threshold of 10/hour [19]. Correlations of PLMI with the IRLSSG Rating Scale, a measure of RLS severity, are not robust (Pearson's correlations r = 0.22-0.46) [24-26], but nonetheless PLMS are quite sensitive, as are subjective measures, to medication effects [26]. The occurrence of PLMS supports a diagnosis of RLS, but must be accompanied by the sensory and motor symptoms defined by the IRLSSG to be certain of an accurate RLS diagnosis.

A PLMI > 5/hour is generally considered pathological and was first set by Coleman in 1982 [27], and in 1997 was recommended by the International Classification of Sleep Disorders (ICSD-1) recommendations for clinical significance, whereas a PLMI < 5/hour is regarded as a benign sleep-associated phenomenon [28]. The 5/hour

threshold is also of genetic importance; the frequency of the RLS-associated BTBD9 gene variant conferring risk for PLMs differs significantly between RLS cases and population controls [29]. A PLMI of 5 to 15 per hour was defined as moderate severity defined in the ICSD-1. In 1999, the moderate severity threshold was increased to a PLMI of 15/hour in the International Classification of Sleep Disorders 2nd Edition: Diagnostic and coding manual (ICSD-2). The ICSD-2 recommends for a diagnosis of PLM disorder when associated with a clinical sleep disturbance or daytime fatigue [30]. The range of PLMI from 15 to 30 per hour was defined as severe in the ICSD-2. A PLMI of greater than 30 per hour is regarded as very severe and has been associated with poor cardiovascular health outcomes, such as hypertension [Rye, Unpublished Data].

Although PLMS are not specific for RLS, they have attracted considerable attention as they are linked to the biology of RLS [29], contribute to some of the disorder's morbidity and mortality, and are typically sensitive to treatments that also bring relief from the distressing sensory components of RLS. PLMS are thought to reflect disinhibition of spinal cord somatomotor and autonomic pathways that originate from brain networks responsive to dopamine and opiate receptor agonists [31]. PLMS are one component of a repetitive, sleep-related phenomenon that also includes cardiovascular and cerebrocortical arousals occurring at regular intervals of approximately 20-30 seconds. Elevations in heart rate [32-34] and robust systolic and diastolic blood pressure upsurges [35, 36] temporally coincide with PLMS in RLS and exceed those observed with volitional leg movements or random, non-periodic movements in sleep. The magnitude these sympathetically mediated cardiovascular responses are similar to the elevations in blood pressure that accompany the

hyperventilatory phase of sleep apnea [37-39], and have been associated with hypertension [Rye, Unpublished Data], cardiovascular disease [40] and stroke [41]. Further understanding of the determinants of PLMS could lend insight on the mechanisms and possible prevention strategies or interventions among patients with RLS.

However, despite the growing knowledge of the pathophysiology and potential vascular consequences of PLMS, the clinical determinants of PLMS occurring in patients with RLS have not been well characterized. Specifically, the role of factors such as the measurements of iron metabolism, duration of RLS sensory symptoms, prior pregnancies and medications affecting the severity of PLMS have not been fully evaluated while controlling for potential confounders of age, sex, race, and alcohol use. Only a few small cross-sectional studies and clinical case reviews have investigated the role of certain measurements of iron metabolism and PLMS [25, 42-46]. Some of these observational clinical studies have reported associations of ferritin and other measurements of iron metabolism and PLMS, but the results have been inconsistent and due to their small size were unable to control for potential confounders.

In 2003, Simakajornboon et al. looked at the potential role of serum iron and ferritin in children with PLMS (n = 39). Patients with serum ferritin concentrations less than 50 μg/L were prescribed supplemental iron for 3 months. At the end of treatment, serum iron and ferritin levels and PSGs were repeated. They found that ferritin was not associated with PLMS, but that serum iron was associated with the PLMI ($r = -0.43$, $p < 0.01$). Among the responders to iron therapy, PLMI decreased from 27.6 ± 14.9 PLM per hour to 12.6 ± 5.3 PLM per hour after the 3 months of iron supplement ($P < 0.001$) and coincided with significant increases in serum ferritin [43]. This study used one night

PSG data for measuring the PLMI and has limited validity due to the recognized high night to night variability in the measurements of PLMs [18].

In 2007, Earley el al. conducted an experimental randomized double blinded clinical trial to ascertain if high dose iron sucrose could improve RLS symptoms, however this study was underpowered (n = 36) to find significant differences in PLMS, which was a secondary outcome [47]. At an interim analysis, the underpowered nature of this study was discovered and they stopped the study, since there was no indication of benefit in RLS symptoms.

In 2009, O'Brien et al. have provided some evidence that low ferritin is not significantly associated with increased PLMS, while controlling for potential confounders [48]. However, again this study looked at PSG data to where the PLMI from only one night was recorded and did not consider other iron metabolism measurements.

To address the lack of sufficient evidence on the determinants of PLMS in adults with RLS, we report the results of a cross sectional study evaluating the determinants of PLMS in a clinical RLS patient population, which is the first of our knowledge to use a 5-night PLMI mean obtained from validated actigraphy methods and to be of sufficient size to control for potential confounders and medications.

Methods

Medical records from all patients seen at the Emory Sleep Center with diagnoses of RLS, PLMS, or myoclonus (used as a billing code for PLMS) from February 2002 to September 2010 were reviewed. All patients for whom results of the measured PLMI (either by single-night polysomnography, 5-night leg actigraphy, or both) and a

comprehensive clinical RLS questionnaire were available were included in the initial analyses.

Other relevant clinical and demographic information was extracted from the electronic medical record, including the patients age at PLMI measurement, age at onset of RLS symptoms, sex, race, measurements of iron metabolism at time of PLMI measurement, family history of RLS, comorbid medical conditions, medication use at the time of PLMI, number of pregnancies, and RLS symptoms (including characteristics of the symptoms and symptom severity). If measurements listed above were not available for the same day that PLMI measurement was obtained, data from the closest date to the PLMI measurement date were obtained.

The measurements of iron metabolism routinely included four parameters: ferritin, serum iron, percent transferrin saturation and the total iron binding capacity (TIBC). Ferritin is a ubiquitous intracellular protein that stores iron and releases it in a controlled fashion. It acts as a buffer against iron deficiency and iron overload by controlling the amount of free iron in the blood. [49] Serum iron measures the amount of circulating iron that is bound to transferrin in the blood. Percent transferrin saturation is the ratio of serum iron and total iron binding capacity x 100. TIBC is the laboratory test which measures the bloods capacity to bind iron with the transferrin blood protein. The test measures the maximum amount of iron that transferrin can carry, which indirectly measures transferrin since transferrin is the most dynamic carrier. [50]

PLMs were analyzed using a tri-axial accelerometer with 10Hz sampling (PAM-RL device, Philips Respironics, Murrysville, PA). The accelerometer is a small (65gm), wristwatch-sized device that is affixed via a hook-and-loop strap to the most affected

ankle (or, as a default position, the non-dominant ankle) for five consecutive nights. The PAM-RL accelerometer provides an accurate assessment of polysomnographically derived PLMs (Pearson's correlation $r = 0.87$, $P < 0.0001$) [51]. It discriminates between PLMs and random nocturnal motor activity [52], and provides a valid estimate of a RLS subject's PLMs count when used over five nights [18].

Unfortunately, the PAM-RL accelerometer cannot discriminate between PLMs that occur while subjects are awake and those that occur during sleep; however it can discriminate between movements that occur during recumbency versus those occurring when upright. Quantification of PLMs occurring during recumbency was performed using a software algorithm adhering to standards of practice for PLMs scoring that accompanied the PAM-RL accelerometer (version 7.5.70) [21]. In the Emory Sleep Center, intra-rater reliabilities for PLMs derived from a sample of 191 subject nights were very high (Spearman $\rho = 0.987$; $P < 0.0001$) [Rye, unpublished data].

The PLMI from each available night of actigraphy monitoring was used to calculate a mean PLMI, using data from the maximum number of nights available. Over 90% patients had 5-nights of available PLMs data. If fewer than 5 nights were available, the nightly mean was calculated from the number of nights available. This mean PLMI was used to examine correlations between PLMs and potential predictors. Furthermore, this 5-night mean was used to classify patients into four PLMI severity categories: 0 to less than 5, 5 to less than 15, 15 to less than 30, and greater than 30, for the previously discussed reasons, including the ICSDs and current research.

Based on current literature and the available medical data, the following variables were assessed as potential confounders: age at PLMI measurement, age at onset of RLS

symptoms, duration of RLS symptoms, sex, race, alcohol use, and medications used at the time of PLMI measurements.

Age was treated as a continuous variable calculated from the difference between the patient date of birth and the date of PLMI measurement. Age at onset of RLS symptoms was a categorical variable based on 10 year groups of when reported RLS symptoms first appeared. Duration of RLS symptoms was created as a continuous variable as the difference from the midpoint of the categorical age at onset of RLS symptoms to the date of PLMs measurement. Sex was coded as 0 for females and 1 for males in the analysis. Current alcohol use was obtained from a question in the RLS questionnaire determining if alcohol improved or worsened RLS symptoms, or if patients did not participate in drinking alcohol. Alcohol was coded as 1, if the patient reported participating in this activity regardless if it improved or worsened RLS symptoms. Alcohol was coded as 0, if the patient reported to not participate in drinking alcohol.

Participant medications during the measurement of PLMs were obtained from the electronic medical record. Medications were categorized into 7 groups by mechanism of action as a: dopaminergic (pramipexole, ropinirole, or carbidopa/levodopa), opiate (any opiate derived medication), gabapentinoid (gabapentin, pregabalin), iron supplement (oral or intravenous), selective serotonin reuptake inhibitors (SSRIs) with minimal norepinephrine reuptake inhibition (NESSRIs: Sertraline, Citalopram, Escitalopram, Fluoxetine, Fluvoxamine, Paroxetine), SSRIs with higher norepinephrine reuptake inhibition (NESSRIs: effexor, pristiq, cymbalta), dopamine antagonists (antipsychotics and antiemetics with anti-dopaminergic action), and diphenhydramine containing

mediations. All medications at the time of PLMs measurement were grouped into the above categories and were coded as individual binary predictors.

All statistical analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC). A P-value < 0.05 was used for statistical significance. Differences in proportions were compared by the Chi-square test and differences in means compared using a two-sample t-test. A non-parametric version of the two-sample t-test, the Wilcoxon-Mann-Whitney test, was used when variables did not satisfy the normality requirement, or a small sample size. ANOVA tests were used to look for differences between continuous variables across multiple categorical groups. Correlations between iron panel parameters and the log-transformed 5-night average PLMI values were performed on completely untreated patients, those not on medications suspected to alter PLMS a priori, and all patients. Furthermore, partial correlation coefficients were determined by using a linear regression models predicting the log-transformed 5-night average PLMI, controlling for potential confounders. Each of the measurements of iron metabolism (ferritin, serum iron, percent transferrin saturation and the TIBC) were individually entered into the linear regression model to determine each individual partial correlation, because they are highly collinear. Highly correlated predictors, such as the determinants of iron metabolism, in the same model create can create unreliable predictors and a very unstable model.

A multinomial logistic regression was used to determine the effect of the potential determinants of PLMI, including those grouped medications that had sufficient number of patients using <5 PLMI as the categorical reference group. The odd ratios (ORs) were obtained for the four other categorical PLMI groupings from the multinomial logistic

regression model. A corresponding proportional odds model (ordinal logistic regression) was fit to test for trend of the ORs across the different categorical groupings of PLMI. The score test for the proportional odds assumption was satisfied for all ordinal logistic regression models. [53]

This study was approved by the Emory University Institutional Review Board (IRB).

Results

The study population consisted of 452 patients (58% women) with a mean (SD) age of 53.19 (15.89) years and with mean (SD) duration of RLS symptoms of 13.38 (15.18) years. The 5-night PLMI mean (SD) using actigraphy was 20.40 (20.95) among the 218 patients with actigraphy measurements. Patients with a PSG, 5-night actigraphy or both, were included for descriptive analyses but further analyses were conducted on only the 5-night average due to the high night-to-night variability of PLMS [18]. As expected, ferritin levels were significantly lower among women (62.9 ng/mL, n = 237) compared to men (107.5 ng/mL, n = 165) (T-Test for ln(Ferritin), $p < 0.001$). Significant differences in serum iron, percent transferrin saturation, and TIBC, between men and women for were also observed. Among the 228 women with pregnancy data, the mean (SD) number of pregnancies was 2.1 (1.6). Further descriptive data are presented in table 1 by type of PLMs measurement.

In the univariate analysis, ANOVA and chi-squared tests were used to look for significant differences in variables among PLMI categories (table 2); only sex and age were each significantly associated with differences in the number of PLMs. These

univariate analyses only looked for overall differences among the categorical groups and not for trends or correlations in the variables. The measurements of iron metabolism were analyzed for correlations (table 3). As suspected, these four measurements were highly correlated. The median number of days between the blood draw for the measurements of iron metabolism and the PLMI measurement was zero days, with an interquartlie range of three days.

Due to the non-normal distribution of the PLMI the log-transformed 5-night average PLMI was used in linear regression analyses; the log-PLMI was not significantly correlated with any of the measurements of iron metabolism, among un-medicated patients, patients not taking medications expected to influence PLMS, and among all patients (table 4). Among all patients (n = 200), the Pearson correlation coefficient of PLMS with ferritin was r = -0.012 (p = 0.87), with serum iron was r = -0.005 (p = 0. 0.95), with percent transferrin saturation at r = -0.023 (p = 0.75), and with TIBC at r = 0.120 (p = 0.09).

After controlling for sex, age, duration of RLS symptoms, and alcohol use, none of the individual measures of iron metabolism significantly predicted the categorical outcome for PLMS, among completely untreated RLS patients, and among all patients (table 4). However, TBIC was nearly a significant predictor (p = 0.07 and p = 0.06 for these two groups, respectively). Furthermore, when this analysis was conducted on patients not taking medications expected to influence PLMS, the partial correlation coefficient for TIBC was significant (p = 0.01). In this group, all the other measures of iron metabolism remained insignificant. Among patients not taking medications expected to influence PLMS (n = 97), the partial correlation coefficient of PLMI

correlated with ferritin was r = -0.091 (p = 0.39), with serum iron was r = -0.034 (p = 0.74), with percent transferrin saturation at r = -0.101 (p = 0.34), and with TIBC at r = 0.254 (p = 0.01). Although the partial correlations between TIBC and PLMI were weak, they were significant among patients not taking medications expected to influence PLMS. Of all the four determinants of iron metabolism we investigated, TIBC is the only potential predictor of PLMS and is positively correlated with increased number PLMS.

To evaluate the robustness of TIBC as a potential predictor of PLMS, a proportional odds logistic regression model was used on the categorical PLMs while controlling for sex, age, duration of RLS symptoms, and alcohol consumption. A dichotomous TIBC parameter (greater than 450 μg/d) was not a significant predictor of increased categorical PLMI among untreated patients (p = 0.13), among patients on medications suspected not to affect PLMS (p = 0.06), and among all patients (p = 0.41), (table 5). The absence of a strong association between high levels of TIBC and PLMI suggests that the weak correlation between a clinically high TIBC level and PLMI may be a chance finding.

We used multinomial logistic regression to obtain the odds ratio for the categories of PLMI (reference < 5/hour) and a proportional odds model for a trend test (table 6). Age was the most significant predictor and can be seen graphically in figures 1 and 2, along with figures 3 and 4 for sex specific findings. The fitted regression lines show different slopes of increasing 5-night mean PLMI by age for each sex, men had a slope = 0.60 PLMI/age (years) (n = 87) and women had a slope = 0.28 PLMI/age (years) (n = 131). Men have a greater increase in the observed PLMI with increasing age compared to women (figures 3 and 4).

Furthermore, among women the number of pregnancies was not a significant predictor of PLMS when controlling for other potential confounders, and thus the number of pregnancies was not further considered as a significant predictor in the following regression analyses.

With respect to medications, we evaluated dopaminergics (pramipexole, ropinirole, or carbidopa/levodopa), opiates (any opiate derived medication), selective serotonin reuptake inhibitors (SSRIs) with minimal norepinephrine reuptake inhibition (NESSRIs: Sertraline, Citalopram, Escitalopram, Fluoxetine, Fluvoxamine, Paroxetine), SSRIs with higher norepinephrine reuptake inhibition (NESSRIs: effexor, pristiq, cymbalta) and they were entered into the multinominal logistic regression model. Taking a dopaminergic medication (pramipexole, ropinirole, or carbidopa/levodopa) decreased the odds of having a PLMI greater than 30 compared to the referent group of less than 5 PLMI (OR = 0.32 (95% CI 0.10 – 1.05)). Taking an opiate medication (any opiate derived medication) decreased the odds of having a greater than 30 PLMI (OR = 0.68 (95% CI 0.12 – 3.95)), compared to the referent group of less than 5 PLMI. Taking a selective serotonin reuptake inhibitors (SSRIs) medication with minimal norepinephrine reuptake inhibition (NESSRIs: Sertraline, Citalopram, Escitalopram, Fluoxetine, Fluvoxamine, Paroxetine), increased the odds of having a greater than 30 PLMI (OR = 6.02 (95% CI 1.38 – 26.24)), compared to the referent group of less than 5 PLMI. Taking a SSRIs medication with higher norepinephrine reuptake inhibition (NESSRIs: effexor, pristiq, cymbalta) increased the odds of having a greater than 30 PLMI (OR = 12.11 (95% CI 1.85 – 79.44)), compared to the referent group of less than 5 PLMI. In summary, dopamenergic and opiate derived medications decreased the amount of

observed PLMs, while any type of SSRI increased the amount of observed PLMs among RLS patients (table 7).

Discussion

Although iron deficiency has been implicated in the development of RLS and has been suspected to play a part in the presentation of PLMs among RLS patients, we found that PLMS are most likely not associated with iron deficiency. Correlations on untreated patients showed that ferritin, serum iron and percent saturation and TIBC were not significantly correlated with PLMS while controlling for potential confounders. However, increased TIBC showed a significant correlation with increased PLMS, among patients not taking medications expected to influence PLMS when controlling for potential confounders. However this result was not observed when PLMS were categorized into 4 groups or when high TIBC (TIBC > 450 μg/dL) was defined as a dichotomous predictor. These observations suggest that the correlation between high TIBC and increased number of PLMS is not a very robust finding.

This study is the first to provide strong evidence among a large number of untreated patients that PLMI is not associated with the measurements of iron metabolism within a RLS patient population. This study shows that PLMS are not likely due to iron or anemia and that other biologic pathways mechanisms should be considered. PLMS are important and easily measured sleep parameter that can be used to help diagnose RLS, but the pathophysiology of PLMS needs further study.

Dopaminergic medications have been shown to be one of the most effective treatments of PLMS [54]. Furthermore, nocturnal dopamine urinary excretion has been

shown to be reduced in patients with PLMS [55], further implicating the dopaminergic system in the pathophysiology of PLMS. We also observed that patients taking dopaminergic medications had less severe PLMS.

Antidepressant medications also have been shown to play a role in the pathophysiology of PLMS, however the pathophyisiology behind why certain SSRI's used for the treatment of depression might affect PLMS is less clear. Winkelman et al, have shown that SSRIs are associated with an increased PLMI when compared to non-medicated controls [56]. This finding

is hypothesisized to be due to increased serotonergic availability and secondarily to a decrease dopaminergic effects. In our study, we saw similar findings where both SSRIs with minimal norepinephrine reuptake inhibition (NESSRIs: Sertraline, Citalopram, Escitalopram, Fluoxetine, Fluvoxamine, Paroxetine), and SSRIs with higher norepinephrine reuptake inhibition (NESSRIs: effexor, pristiq, cymbalta) were both individually associated with a significant increase in the number of PLMs, in patients with RLS.

The strengths of this study include the large number of patients with 5-night mean PLMI measurements from a verified actigraphy methods, detailed medical information from electronic medical records and an additional physician administered RLS questionnaire. Furthermore the potential medications that affect the PLMS were considered and controlled for in the various regression models. The high variance of night to night PLMs [18], make it difficult to accurately obtain PLMI measurements in a single PSG. This is the first study to our knowledge to use a 5-night PLMI mean for all correlation and regression analysis on the predictors of PLMS. This is also the first study

to our knowledge to examine the various medication effects on PLMS, common to patients with RLS, in a patient population of this size. Certain medications, such as dopamine and opiates, have been suspected to alter the amount of PLMS in patients with RLS, and this study adds to the evidence supporting which medications affect PLMS. This study is in agreement with previous studies that showed that an increased PLMI in certain patient groups can be associated with sex, advanced age, duration of disease, and alcohol use, strengthening the validity of our study.

Limitations of this study include the fact that some patients had iron panel measurements that were not on the same date as the PLMI measurement. However there was no correlation between that window of time and the measured PMLI, and most patients had blood draws on the same day of starting the actigraphy measurements. Also this length of time was not a significant predictor of PLMI in any of the regression models.

Understanding how medication groups influence PLMS is a difficult task because patients could be on more than one medication. These medications could have opposing or synergistic effects on PLMS, and thus decrease or increase the amount of PLMS. Ideally, a randomized clinical trial (RCT) would be used to determine if increased PLMS result from the medication under trial. However in RCTs, patient PLMI is rarely the primary outcome of interest when conducting sample size calculations, thus most RCTs are under-powered to see significant differences in PLMS [47]. This could be done ideally with un-medicated controls, however in our study the small number of patients on certain medications limited our ability to determine concise point estimates for the changes in PLMS.

The public health implications of this study are specific to clinical patient populations with RLS. RLS has been shown to be prevalent at a rate of 3-15% in European populations [3-9] and given that roughly 80% of RLS patients demonstrate PLMS [19], understanding the biological mechanisms of PLMS can direct more effective screening processes and treatments. It is clear from our findings that PLMS are more frequent in older patients and thus the field of geriatrics should closely monitor PLMS. Potentially, understanding PLMS in older populations, and providing proper treatments could help decrease the rate of cardiovascular events.

Our study further demonstrates that the measurements of iron metabolism are not robust predictors of the number of PLMS in RLS patients. TIBC was the only measurement shown to be significantly correlated with increased PLMS in one patient group, while controlling for potential confounders. However the predictive ability of TIBC was not robust when further considered a binary predictor, and appears to be a very limited finding. Future studies should consider the possibility of TIBC as a predictor, but other directions for PLM prediction should be taken. Also, dopaminergic medications and SSRI's appear to play a significant role in affecting the amount of observed PLMS in clinical patients with RLS.

Tables and Figures

Table 1: Population Characteristics (N=452)

Variable	N	Mean	Std. Dev.	Min.	Max.
Actigraphy 5-night mean PLMI	218	20	21	0	108
Actigraphy maxiumum PLMI	219	34	31	0	153
Actigraphy minimum PLMI	220	10	15	0	83
Polysomnigraph PLMI	232	35	37	0	223
Ferritin (ng/mL)	406	81	109	9	1364
Men	165	108	94	9	589
Women	237	63	117	9	1364
Iron (µg/dL)	406	79	34	9	211
Percent Saturation (%)	393	22	11	2	107
TIBC (µg/dL)	393	372	67	165	601

Table 2: Unadjusted population characteristics by categorical PLMI status.

Variable	0 to <5 PLMI N = 50	5 to <15 PLMI N = 70	15 to 30 PLMI N = 48	>30 PLMI N = 50	P-Value
Female (%)	26 (52%)	44 (62.86 %)	36 (75%)	25 (50%)	0.04
Num. of Pregnancies	1.76 (1.33)	2.14 (1.67)	2.19 (2.20)	2.42 (1.93)	0.64
Race (A.A.)	5 (12%)	5 (7%)	5 (11%)	2 (4%)	0.54
Age (yrs)	42 (15)	49 (18)	54 (16)	58 (13)	<.0001
Age of Onset (yrs)	32 (16)	33 (19)	33 (18)	39 (20)	0.26
Duration of RLS (yrs)	11 (13)	16 (16)	18 (16)	18 (17)	0.09
Alcohol (yes)	29 (58%)	37 (58%)	25 (52%)	19 (38%)	0.22
Ferritin (ng/mL)	88 (146)	72 (95)	83 (97)	83 (71)	0.88
Iron (µg/dL)	79 (35)	79 (31)	79 (31)	82 (29)	0.97
Percent Saturation (%)	22 (10)	22 (11)	23 (10)	22 (9)	0.98
TIBC (µg/dL)	355 (48)	382 (77)	366 (77)	378 (65)	0.18

Table 3: Pearson Correlations between measurements of iron metabolism.

Pearson Correlation Coefficients				
Variable	**Ferritin (ng/mL)**	**Iron (µg/dL)**	**Percent Sat. (%)**	**TIBC (µg/dL)**
Ferritin (ng/mL)	1	0.34066	0.51633	-0.35382
P-Value		<.0001	<.0001	<.0001
N	406	396	384	385
Iron (µg/dL)	0.34066	1	0.90756	-0.18132
P-Value	<.0001		<.0001	0.0003
N	396	406	393	393
Percent Saturation (%)	0.51633	0.90756	1	-0.48127
P-Value	<.0001	<.0001		<.0001
N	384	393	393	392
TIBC (µg/dL)	-0.35382	-0.18132	-0.48127	1
P-Value	<.0001	0.0003	<.0001	
N	385	393	392	393

Table 4: Pearson Correlations between Iron Panel measurement and the log transformed continuous PLMS variable, along with partial correlation coefficients determined by linear regressions of log-transformed PLMS, controlling for potential confounders.

Completely Un-Medicated Patients	**Type of PLMS Measurement**					
	Log avg. PLMS Actigraphy			***Partial Correlation Coef.**		
Variable	**Correlation Coef.**	**N**	**P-Value**	***Partial Correlation Coef.**	**N**	**P-Value**
Ferritin (ng/mL)	-0.010	67	0.94	-0.053	59	0.71
Iron (µg/dL)	-0.021	67	0.86	-0.137	59	0.32
Percent Saturation (%)	-0.067	66	0.60	-0.165	59	0.23
TIBC (µg/dL)	0.156	67	0.21	0.248	59	0.07

Patients not taking DAs, Opiates, Neurontins, or Iron	**Type of PLMS Measurement**					
	Log avg. PLMS Actigraphy			***Partial Correlation Coef.**		
Variable	**Correlation Coef.**	**N**	**P-Value**	***Partial Correlation Coef.**	**N**	**P-Value**
Ferritin (ng/mL)	-0.041	106	0.67	-0.091	97	0.39
Iron (µg/dL)	0.062	107	0.53	-0.034	98	0.74
Percent Saturation (%)	-0.002	106	0.99	-0.101	98	0.34
TIBC (µg/dL)	0.177	107	0.07	0.254	98	0.01

All Patients	**Type of PLMS Measurement**					
	Log avg. PLMS Actigraphy			***Partial Correlation Coef.**		
Variable	**Correlation Coef.**	**N**	**P-Value**	***Partial Correlation Coef.**	**N**	**P-Value**
Ferritin (ng/mL)	-0.012	200	0.87	-0.031	1?4	0.69
Iron (µg/dL)	-0.005	200	0.95	0.004	184	0.96
Percent Saturation (%)	-0.023	198	0.75	-0.028	183	0.71
TIBC (µg/dL)	0.120	198	0.09	0.144	182	0.06

* The partial correlation coefficient is determined from controlling for the potential confounders: age, race, sex, duration of RLS symptoms and alcohol use.

Table 5: Estimated Odds Ratios (OR), and 95% Confidence Intervals (CI) using the proportional odds model for increased categorical PLMS by age, sex, race, duration of RLS symptoms, and alcohol, in a clinical RLS cohort of 59 untreated patients from the Emory Sleep Lab.

	Un-Medicated Patients (n=59)				Patients on medication not suspected affect PLMs (n=98)				All patients (n=182)			
Variable	**OR**	**95% CI**		**P-value**	**OR**	**95% CI**		**P-value**	**OR**	**95% CI**		**P-value**
Age	1.05	1.02	1.09	<.0001	1.06	1.04	1.09	<.0001	1.04	1.02	1.06	<.0001
Sex	1.92	0.64	5.75	0.25	1.67	0.74	3.73	0.21	1.14	0.65	1.99	0.64
Race	2.39	0.40	14.26	0.34	0.96	0.23	3.98	0.95	0.88	0.31	2.46	0.80
Duration	1.08	1.03	1.14	0.001	1.05	1.02	1.08	0.004	1.00	0.98	1.02	0.78
Alcohol	0.33	0.11	0.98	0.05	0.46	0.21	1.01	0.05	0.93	0.54	1.59	0.78
TIBC	3.09	0.72	13.25	0.13	2.99	0.96	9.32	0.06	1.38	0.64	2.98	0.41

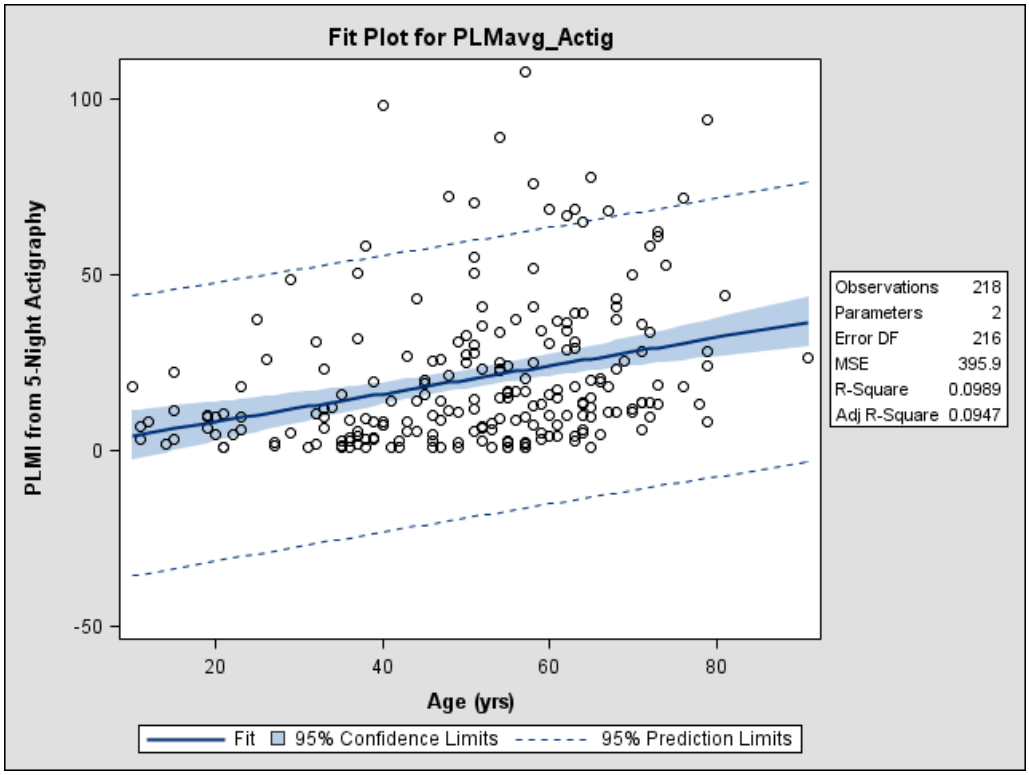

Figure 1: Mean PLMI from 5-night actigraphy vs age in years (n=218) (r=0.31). Slope = 0.40.

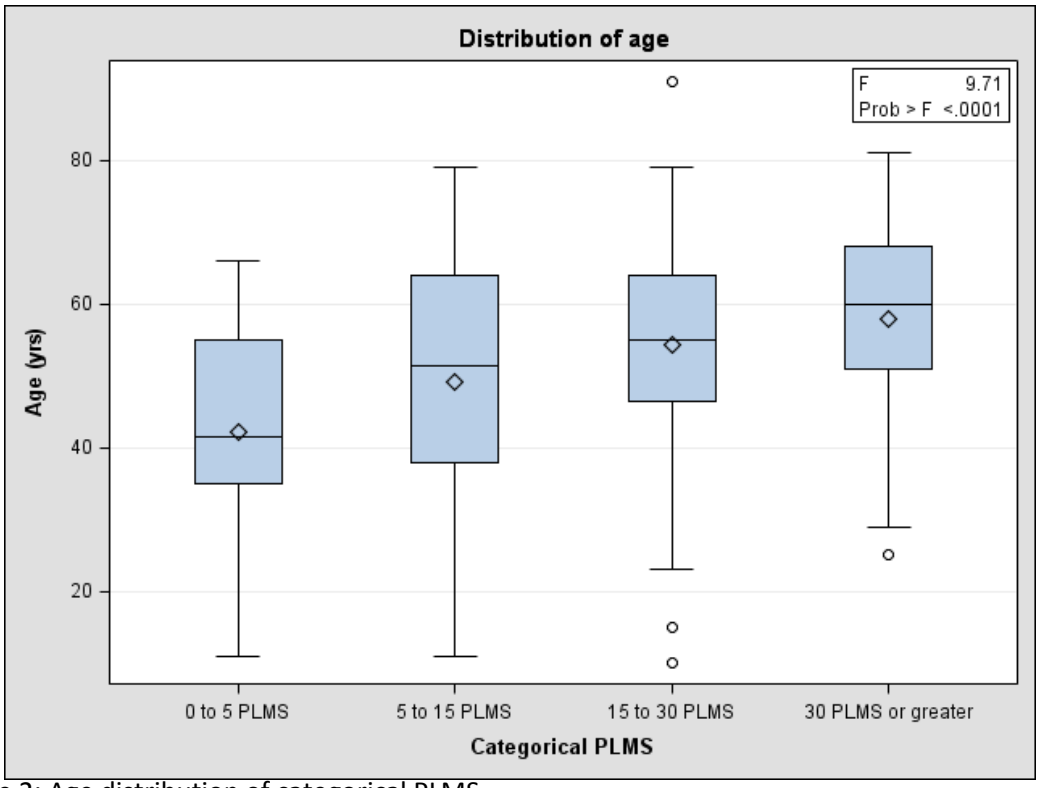

Figure 2: Age distribution of categorical PLMS.

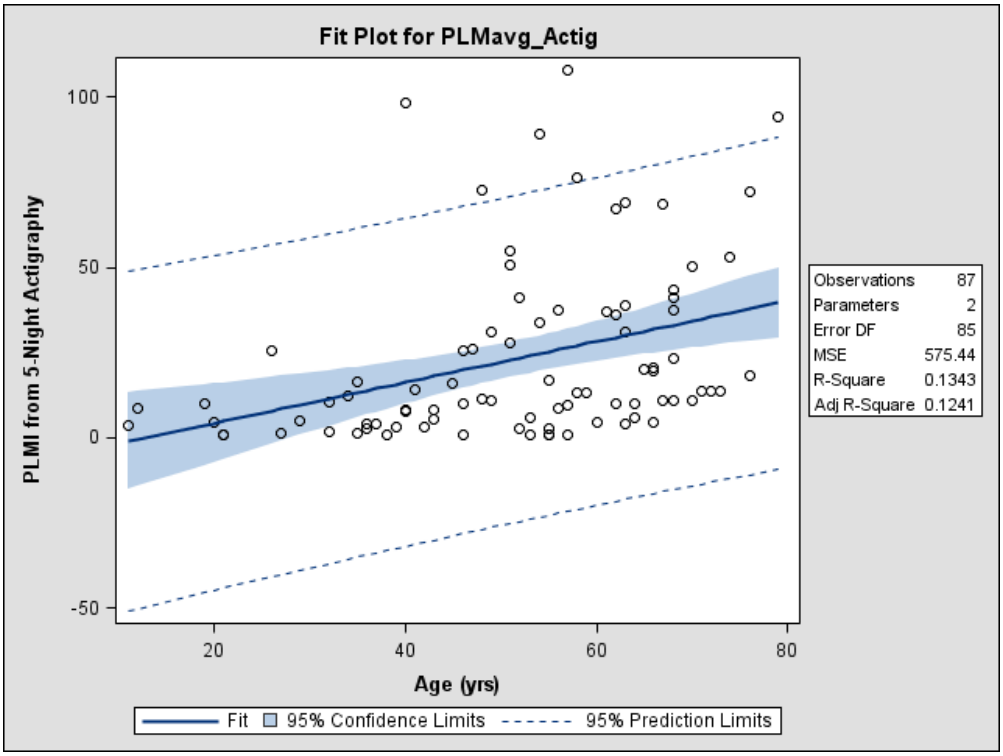

Figure 3: Mean PLMI from 5-night actigraphy vs age in years, for men (n=87) Slope=0.60.

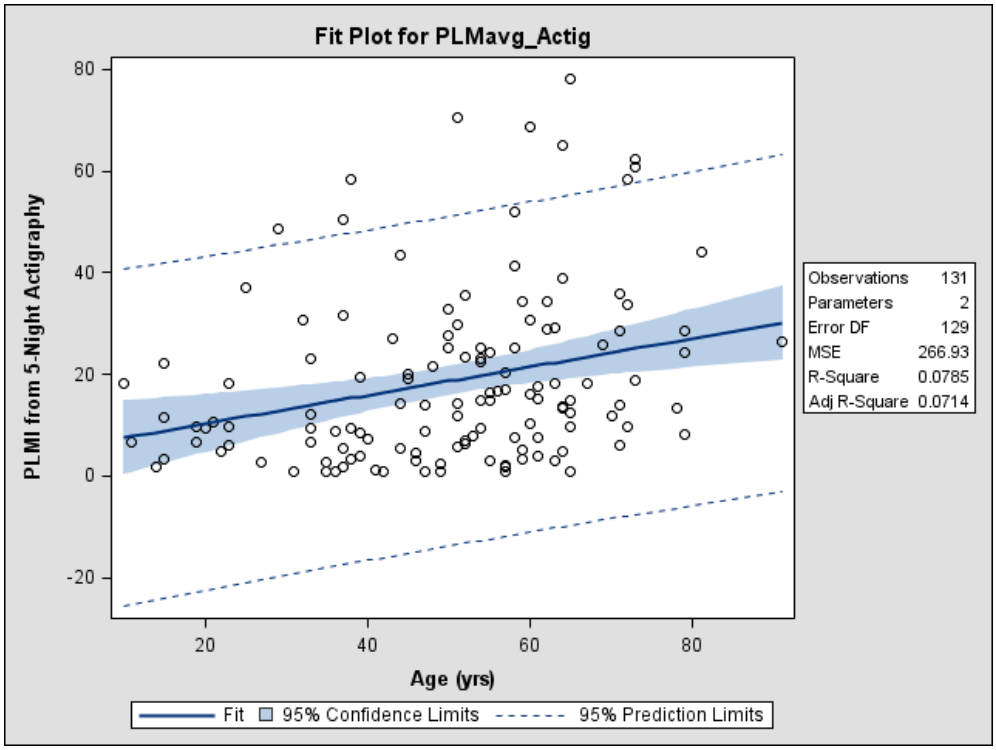

Figure 4: Mean PLMI from 5-night actigraphy vs age in years, for women (n=131) Slope=0.28.

Table 6: Estimated Odds Ratios (OR), and 95% Confidence Intervals (CI) for categorical PLMI by age, sex, race, duration of RLS symptoms, and alcohol, using <5 PLMS as the reference group, in a clinical RLS cohort of 202 patients from the Emory Sleep Lab.

Categorical PLMI	Age Years	(SD)	OR	95% Wald Confidence Interval	
< 5	42	15	1.00		
5 to < 15	48	17	1.02	1.00	1.05
15 to < 30	54	16	1.05	1.02	1.08
> 30	58	13	1.06	1.03	1.10

Test for trend using Proportional Odds model, Wald Chi-Squared (p<.0001, df=1)

Categorical PLMI	Sex Male	Female	OR	95% Wald Confidence Interval	
< 5	25	17	1.00		
5 to < 15	41	26	0.83	0.37	1.88
15 to < 30	35	12	0.43	0.17	1.10
> 30	23	23	1.23	0.49	3.07

Test for trend using Proportional Odds model, Wald Chi-Squared (p=0.96, df=1)

Categorical PLMI	Race White	A.A.	OR	95% Wald Confidence Interval	
< 5	37	5	1.00		
5 to < 15	62	5	0.61	0.16	2.34
15 to < 30	42	5	0.80	0.20	3.28
> 30	44	2	0.45	0.08	2.67

Test for trend using Proportional Odds model, Wald Chi-Squared (p=0.51, df=1)

Categorical PLMI	Duration of RLS Years	SD	OR	95% Wald Confidence Interval	
< 5	37	5	1.00		
5 to < 15	61	6	1.01	0.98	1.05
15 to < 30	43	4	1.01	0.98	1.05
> 30	44	2	1.01	0.98	1.05

Test for trend using Proportional Odds model, Wald Chi-Squared (p=0.45, df=1)

Categorical PLMI	Alcohol Yes	Alcohol No	OR	95% Wald Confidence Interval	
< 5	24	18	1.00		
5 to < 15	36	31	0.96	0.43	2.12
15 to < 30	25	22	1.14	0.47	2.80
> 30	18	28	0.66	0.27	1.66

Test for trend using Proportional Odds model, Wald Chi-Squared (p=0.36, df=1)

Table 7: Estimated Odds Ratios (OR), and 95% Confidence Intervals (CI) for categorical PLMI by medication group, using <5 PLMI as the reference group, controlling for age, sex, duration of RLS symptoms and alcohol consumption, in a clinical RLS cohort of 194 patients from the Emory Sleep Lab.

Categorical PLMS	Dopaminergics Yes	No	OR	95% Wald Confidence Limits	
< 5	13	23	1.00		
5 to < 15	22	39	0.66	0.25	1.77
15 to < 30	12	30	0.46	0.15	1.41
> 30	11	31	0.32	0.10	1.05

Test for trend using Proportional Odds model, Wald Chi-Squared (p=0.05, df=1)

Categorical PLMS	Opiates Yes	No	OR	95% Wald Confidence Limits	
< 5	4	32	1.00		
5 to < 15	9	52	1.07	0.22	5.06
15 to < 30	3	39	0.40	0.06	2.60
> 30	7	35	0.68	0.12	3.95

Test for trend using Proportional Odds model, Wald Chi-Squared (p=0.38, df=1)

Categorical PLMS	Low Level SSRI's Yes	No	OR	95% Wald Confidence Limits	
< 5	3	31	1.00		
5 to < 15	9	52	1.62	0.39	6.69
15 to < 30	16	26	5.35	1.30	22.04
> 30	15	26	6.02	1.38	26.24

Test for trend using Proportional Odds model, Wald Chi-Squared (p=0.001, df=1)

Categorical PLMS	High Level SSRI's Yes	No	OR	95% Wald Confidence Limits	
< 5	2	34	1.00		
5 to < 15	5	56	1.80	0.31	10.60
15 to < 30	3	39	2.36	0.32	17.11
> 30	8	34	12.11	1.85	79.44

Test for trend using Proportional Odds model, Wald Chi-Squared (p=0.004, df=1)

References

1. **Willis, T., *The London practice of physick*. 1685, Thomas Basset and William Crooke: London. p. 404.**
2. **Allen, R., et al., *Restless legs syndrome: Diagnostic criteria, special considerations, and epidemiology: a report from The RLS Diagnosis and Epidemiology Workshop at the National Instititutes of Health.* Sleep Med, 2003. 4: p. 101-119.**
3. **Hening, W., et al., *Impact, diagnosis and treatment of restless legs syndrome (RLS) in a primary care population: the RESt (RLS epidemiology, symptoms, and treatment) primary care study.* Sleep Med, 2004. 5: p. 237-246.**
4. **Allen, R.P., et al., *Restless legs syndrome prevalence and impact: REST general population study.* Arch Intern Med, 2005. 165(11): p. 1286-92.**
5. **Happe, S., et al., *Treatment wish of individuals with known and unknown restless legs syndrome in the community.* J Neurol, 2008. 255: p. 1365-1371.**
6. **Lavigne, G. and J. Montplaisir, *Restless legs syndrome and sleep bruxism: prevalence and association among Canadians.* Sleep, 1994. 17: p. 739-743.**
7. **Phillips, B., et al., *Epidemiology of restless legs symptoms in adults.* Arch Int Med, 2000. 160: p. 2137-2141.**
8. **Ulfberg, J., et al., *Comorbidity in restless legs syndrome among a sample of Swedish adults.* Sleep Med, 2007. 8: p. 768-772.**
9. **Ulfberg, J., et al., *Prevalence of restless legs syndrome among men aged 18 to 64 years: an association with somatic disease and neuropsychiatric symptoms.* Mov Disord, 2001. 16: p. 1159-1163.**
10. **O'Keeffe, S.T., et al., *The frequency and impact of restless legs syndrome in primary care.* Ir Med J, 2007. 100(7): p. 539-42.**
11. **Richard M. Coleman, C.P.P., Elliot D. Weitzman, *Periodic movements in sleep (nocturnal myoclonus): Relation to sleep disorders.* Annals of Neurology, 1980. 8(4): p. 416-421.**
12. **Ekbom, K., *Asthenia crurum paraesthetica ("irritable legs"): A new syndrome consisting of weakness, sensation of cold and nocturnal paresthesia in legs, responding to certain extent to treatment with priscol and doryl. Note on paresthesis in general.* Acta Medica Scandinavica, 1944. 118: p. 197-209.**
13. **Arthur S. Walters, W.A.H., Sudhansu Chokroverty,, *Review and videotape recognition of idiopathic restless legs syndrome.* Movement Disorders, 1991. 6(2): p. 105-110.**
14. **Lazzarini, A., et al., *Studies of penetrance and anticipation in five autosomal-dominant restless legs syndrome pedigrees [In Process Citation].* Mov Disord, 1999. 14(1): p. 111-6.**
15. **Ondo, W. and J. Jankovic, *Restless legs syndrome: Clinicoetiologic correlates.* Neurology, 1996. 47: p. 1435-1441.**
16. **Trenkwalder, C., et al., *Clinical symptoms and possible anticipation in a large kindred of familial restless legs syndrome.* Mov Disord, 1996. 11: p. 389-394.**
17. **Walters, A., et al., *Variable expressivity in familial restless legs syndrome.* Arch Neurol, 1990. 47: p. 1219-1220.**

18. **Trotti, L.M., et al., *Correlates of PLMs variability over multiple nights and impact upon RLS diagnosis.* Sleep Medicine, 2009. 10(6): p. 668-71.**
19. **Montplaisir, J., et al., *Clinical, polysomnographic, and genetic characteristics of restless legs syndrome: a study of 133 patients diagnosed with new standard criteria.* Movement Disorders, 1997. 12: p. 61-65.**
20. **Symonds, C.P., *Nocturnal myoclonus.* J Neurol Neurosurg Psychiatry, 1953. 16(3): p. 166-71.**
21. **ADSA, *Recording and scoring leg movements. A report from the Sleep Disorders Atlas Task Force of the American Sleep Disorders Association.* Sleep, 1993. 16: p. 748-759.**
22. **Winkelman, J.W., *Periodic Limb Movements in Sleep -- Endophenotype for Restless Legs Syndrome?* N Engl J Med, 2007: p. NEJMe078129.**
23. **Rye, D., et al., *A novel 2-step diagnostic approach for RLS disease classification.* Sleep, 2004. 27 (Suppl S): p. 306-307.**
24. **Aksu, M., S. Demirci, and W. Bara-Jimenez, *Correlation between putative indicators of primary restless legs syndrome severity.* Sleep Med, 2007. 8(1): p. 84-9.**
25. **Garcia-Borreguero, D., et al., *Correlation between rating scales and sleep laboratory measurements in restless legs syndrome.* Sleep Med, 2004. 5(6): p. 561-5.**
26. **Hornyak, M., et al., *Polysomnography findings in patients with restless legs syndrome and in healthy controls: a comparative observational study.* Sleep, 2007. 30(7): p. 861-5.**
27. **RM, C., *Periodic movements in sleep (nocturnal myoclonus) and restless legs syndrome*, in *Sleeping and waking disorders: indications and techniques*, G. C, Editor. 1982, Addison-Wesley: Menlo Park. p. 265-295.**
28. **Wetter T, P.T., *Restless Legs and Periodic Limb Movements in sleep syndromes.* Journal of Neurology, 1997. 244(Supplement 1): p. S37-S45.**
29. **Stefansson, H., et al., *A Genetic Risk Factor for Periodic Limb Movements in Sleep.* N Engl J Med, 2007: p. NEJMoa072743.**
30. **Medicine, A.A.o.S., *International classification of sleep disorders 2nd edition: diagnostic and coding manual (ICSD-2)*. 2005, Westchester: AASM.**
31. **Clemens, S., D. Rye, and S. Hochman, *Restless legs syndrome: revisiting the dopamine hypothesis from the spinal cord perspective.* Neurology, 2006. 67(1): p. 125-30.**
32. **Ferri, R., et al., *Heart rate and spectral EEG changes accompanying periodic and non-periodic leg movements during sleep.* Clinical Neurophysiology, 2007. 118(2): p. 438-48.**
33. **Sforza, E., et al., *Cardiac variability and heart-rate increment as a marker of sleep fragmentation in patients with a sleep disorder: a preliminary study.* Sleep, 2007. 30(1): p. 43-51.**
34. **Winkelman, J., *The evoked heart rate response to periodic leg movements of sleep.* Sleep, 1999. 22: p. 575-580.**
35. **Pennestri, M.H., et al., *Nocturnal blood pressure changes in patients with restless legs syndrome.* Neurology, 2007. 68(15): p. 1213-8.**
36. **Siddiqui, F., et al., *Rise of blood pressure with periodic limb movements in sleep and wakefulness.* Clin Neurophysiol, 2007. 118(9): p. 1923-30.**
37. **Morgan, B.J., et al., *Blood pressure perturbations caused by subclinical sleep-disordered breathing.* Sleep, 1998. 21(7): p. 737-46.**

38. Roman, M.J., et al., *Relation of blood pressure variability to carotid atherosclerosis and carotid artery and left ventricular hypertrophy.* Arteriosclerosis, Thrombosis & Vascular Biology, 2001. 21(9): p. 1507-11.
39. Zakopoulos, N.A., et al., *Time rate of blood pressure variation is associated with increased common carotid artery intima-media thickness.* Hypertension, 2005. 45(4): p. 505-12.
40. Frattola, A., et al., *Prognostic value of 24-hour blood pressure variability.* J Hypertens, 1993. 11(10): p. 1133-7.
41. Pringle, E., et al., *Systolic blood pressure variability as a risk factor for stroke and cardiovascular mortality in the elderly hypertensive population.* J Hypertens, 2003. 21(12): p. 2251-7.
42. Beard, J.L., *Iron status and periodic limb movements of sleep in children: a causal relationship?* Sleep Med, 2004. 5(1): p. 89-90.
43. Simakajornboon, N., et al., *Periodic limb movements in sleep and iron status in children.* Sleep, 2003. 26(6): p. 735-8.
44. Lee, J.H., et al., *A secondary analysis of racial differences in periodic leg movements in sleep and ferritin in hemodialysis patients.* Sleep Med, 2006. 7(8): p. 646-8.
45. Meir H. Kryger, K.O., John Foerster, *Low body stores of iron and restless legs syndrome: a correctable cause of insomnia in adolescents and teenagers.* Sleep Medicine, 2002. 3: p. 127-132.
46. Sun, E.R., et al., *Iron and the restless legs syndrome.* Sleep, 1998. 21(4): p. 371-7.
47. Earley, C.J., et al., *A randomized, double-blind, placebo-controlled trial of intravenous iron sucrose in restless legs syndrome.* Sleep Med, 2009. 10(2): p. 206-11.
48. O'Brien, L.M., et al., *Iron stores, periodic leg movements, and sleepiness in obstructive sleep apnea.* J Clin Sleep Med, 2009. 5(6): p. 525-31.
49. Brunner, C. and W.A. Wuillemin, *[Iron deficiency and iron deficiency anemia - symptoms and therapy].* Ther Umsch, 2010. 67(5): p. 219-23.
50. Beutler, E., *Iron storage disease: facts, fiction and progress.* Blood Cells Mol Dis, 2007. 39(2): p. 140-7.
51. Sforza, E., M. Johannes, and B. Claudio, *The PAM-RL ambulatory device for detection of periodic leg movements: a validation study.* Sleep Medicine, 2005. 6(5): p. 407-413.
52. Katinka Tuisku, et al., *Quantitative rest activity in ambulatory monitoring as a physiological marker of restless legs syndrome: A controlled study.* Movement Disorders, 2003. 18(4): p. 442-448.
53. Ananth, C.V. and D.G. Kleinbaum, *Regression models for ordinal responses: a review of methods and applications.* Int J Epidemiol, 1997. 26(6): p. 1323-33.
54. Kryger, M.H., T. Roth, and W.C. Dement, *Principles and practice of sleep medicine*. 3rd ed. 2000, Philadelphia: Saunders. xxxiii, 1336 p.
55. Cohrs, S., et al., *Nocturnal urinary dopamine excretion is reduced in otherwise healthy subjects with periodic leg movements in sleep.* Neurosci Lett, 2004. 360(3): p. 161-4.
56. Yang, C., D.P. White, and J.W. Winkelman, *Antidepressants and periodic leg movements of sleep.* Biol Psychiatry, 2005. 58(6): p. 510-4.

Emory IRB Approval Letter

FROM: Sam Roberts
Analyst Assistant

TO: Lynn Trotti
Principal Investigator

CC:

Rye	David	NeuroSleep
Schultz	Jonathan	NeuroSleep

DATE: July 8, 2010

RE: **Notification of Expedited Approval**
IRB00045221
Clinico-epidemiological study of periodic leg movements of sleep and restless legs syndrome

This is your notification that your above referenced study was reviewed and APPROVED under the Expedited review process per 45 CFR 46.110 and 21 CFR 56.110. The approval is valid from **7/8/2010 until 7/7/2011**. Thereafter, continued approval is contingent upon the submission of a continuing review request that must be reviewed and approved by the IRB prior to the expiration date of this study.

- Complete HIPAA waiver granted
- Waiver of informed consent granted

Any reportable events (serious adverse events, breaches of confidentiality, protocol deviation or protocol violations) or issues resulting from this study should be reported immediately to the IRB and to the sponsoring agency (if any). Any amendments (changes to any portion of this research study including but not limited to protocol or informed consent changes) must have IRB approval before being implemented.

All correspondence and inquiries concerning this research study must include the IRB ID, the name of the Principal Investigator and the Study Title.

Sincerely,

Sam Roberts
This letter has been digitally signed