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Prabhjyot Saini April 23, 2012

Screening for Iron Deficiency in Restless Leg Syndrome

By

Prabhjyot Saini
Masters of Science in Public Health

Kimberly Rask

Committee Chair

Laura M. Gaydos

Committee Member

Lynn Marie Trotti

Committee Member

David B. Rye

Committee Member

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Abstract

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By *Prabhjyot Saini*

Restless Legs Syndrome (RLS) is a sensorimotor neurological disorder primarily manifesting in the lower limbs in at least 5% of adult Americans that is associated with significant disability and increased risk for cardiovascular disease. Altered iron homeostatic mechanisms appear to play a key role in the underlying pathology of RLS. A high prevalence of iron deficiency has been found among patients with RLS, and treatment of the deficiency has been reported to improve or ameliorate symptoms- even resulting in complete remission of symptoms. A low serum ferritin level (less than 50 μg/L) has been associated with greater severity of RLS and with a reduction in sleep. Iron deficiency is a recognizable and reversible state that appears to modulate expression of RLS in a proportion of individuals with RLS. Despite the high prevalence of iron deficiency amongst subjects seeking treatment, there is still little data to go on to guide one's selection of patients deserving of iron testing, which test to rely upon, and who might benefit from iron treatment. Using data collected from a population based genetic study of RLS in Iceland; this analysis examines potential screens constructed from RLS signs and symptoms to develop a simple, observational screening algorithm that can aid clinicians in the management of RLS patients. A combination of sensitivity/specificity analyses and regression analyses were performed on the data set. A superior screen of testing all RLS subjects was dominant against other strategies identified. Test all strategies are highly sensitive to the derivation of estimated costs and are difficult to demonstrate medical utility. With that the second most dominant screen identified included test all with a history of anemia and test only women under 45 for those without a history of anemia.

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List of Tables, Figures, and Equations

List of Abbreviations

RLS	Restless Leg Syndrome
PLM	Periodic Limb Movements
PLMs	Periodic Limb Movements during sleep
IRLS	International Restless Legs Syndrome Study Group Severity Rating Scale
DPA	Data Protection Agency
CMS	Centers for Medicare and Medicaid Services
BMI	Body Mass Index
WHO	World Health Organization

List of Definitions

Medical Terms- Iron	
Ferritin	Is a blood cell protein that contains iron. A ferritin test helps your doctor understand how much iron your body is storing.
Anemia	is an insufficient mass of red blood cells circulating in the blood
Iron Deficiency	State in which there is insufficient iron to maintain the normal physiological function of tissues such as the blood, brain, and muscles. Iron deficiency can exist in the absence of anemia.
Medical Terms- Sleep	
Circadian	A biological processes recurring naturally on a twenty-four hour cycle
Polysomnography	A sleep study that monitors you as you sleep.
Medical Terms- Clinical Science	
Dopamine	In the brain, this functions as a neurotransmitter and activates the five types of dopamine receptors on a cell's surface
Dopaminergic Therapy	Involving dopamine as a neurotransmitter
Sensorimotor	Having or involving both sensory and motor functions and pathways.
Pathophysiology	The study of the disruption of normal bodily functions due to disease.
Genetic Terms	
Gene	The gene is the basic physical unit of inheritance. Genes are passed from parents to offspring and contain the information needed to specify traits. Humans have approximately 23,000 genes.
Allele	An allele is one of two or more versions of a gene. An individual inherits two alleles for each gene, one from each parent. If the two alleles are the same, the individual is homozygous for that gene. If the alleles are different, the individual is heterozygous.
Genetic variant	Genetic variation refers to gene frequencies. Genetic variation can refer to differences between individuals or to differences between populations.
Phenotype	The set of observable characteristics of an individual resulting from the interaction of its genotype with the environment.
Endophenotype	Quantitative phenotypes that are intermediate between genes and the disease outcome, that are directly influenced by a smaller number of genes than the disease phenotype, and that represent one of many facets of a disease

Introduction

Restless Legs Syndrome (RLS) is a sensorimotor disorder that is defined by a symptom complex that includes a) an intense urge to move the legs that is usually accompanied or caused by unpleasant sensation in the legs, b) worsening of symptoms at rest, c) relief of symptoms with movement or activity, and d) a circadian preference to emerge in the evening and at night. Variability in symptom expressivity is the rule rather than exception [1-5]. Eighty percent of RLS cases exhibit periodic limb movements (PLMs) during sleep, which are repetitive, non-volitional movements of the lower limbs evident during sleep [6]. Because symptoms are brought on by inactivity; distress intrudes upon everyday activities such as plane travel, car rides, attending school, meetings, theatre, and most importantly sleep.

In the first seminal description of RLS published in 1945, Ekbom noted iron deficiency in 25% of his patients with RLS and a favorable response to iron supplementation. Since his initial publication, the theory of iron deficiency as the underlying cause for RLS has been the subject of intense investigation. The most demonstrable association between iron and RLS comes in the form of serum iron storage levels, or ferritin. Ferritin is a blood cell protein that contains iron stored for use when the body is depleted of iron. When the body is depleted, ferritin is transported to where it is needed. Numerous studies have revealed that iron deficiency is common in RLS patients, reductions in serum ferritin correlate with RLS symptom severity [7], and iron therapy can lead to improvement in symptoms [7]. It has therefore been hypothesized that iron deficiency is principally involved in the pathophysiology of RLS.

Results from these studies were generalized by RLS experts into a recommendation that iron testing be performed on all RLS patients [8]. In 2006, this was followed by the American Academy of Sleep Medicine requesting Centers for Medicare and Medicaid Services (CMS) reimbursement for routine assessment of serum iron parameters, including ferritin in RLS patients. However, review by CMS determined that there was insufficient evidence for the use of ferritin levels for diagnosing or

monitoring therapy related to RLS. Further, they did not find that RLS in and of itself was sufficiently predictive of iron deficiency that a diagnostic or therapeutic presumption of reasonableness and necessity could be made, absent other indications for testing. Therefore, to date CMS does not reimburse for the cost of iron testing in RLS patients.

In 2007, Rye et al. [9] and Winkelmann et al [10] separately demonstrated that four unique genes account for nearly 80% of the population attributable risk for RLS and that one common allelic variant in one of these genes, *BTBD9,* alone accounts for 50% of the population attributable risk. This *BTBD9* gene confers risk for two clinically important features. That is, each copy of the risk allele predicts a 13% lower average serum ferritin and each copy of the gene increases the number of PLMs. Despite these advances in our knowledge of the molecular determinants underlying clinical features of RLS, a reliable algorithm for predicting iron deficiency in patients with RLS has not emerged. This study seeks to define a clinical algorithm for identifying RLS patients at high risk for iron deficiency.

Research Question

Can a clinical algorithm identify RLS patients at high risk for iron deficiency?

Literature Review

The majority of RLS patients report sensations in the legs ranging from creepy, crawling, or bubbling, to burning, tingling or pain, coincident with a compelling desire to move. Symptoms can affect both lower extremities simultaneously, can be unilateral, or can alternate one leg to the other. In half of the cases, the arms are also involved [11] and about one-in-four RLS patients have daily symptoms [12].

The symptoms of RLS can result in daytime dysfunction including: daytime sleepiness, decreased job performance, poor relationships, depression, anxiety, fatigue and poor quality of life [13-15]. Because symptoms are brought on by inactivity, distress intrudes upon everyday activities such as attending work and school, traveling by plane or car, engaging in social activities [14, 16], and most notably falling and staying asleep. Patients with RLS may suffer from insomnia and disturbed sleep which can lead to excessive daytime sleepiness, chronically reduced sleep times of 5 hours or less, 20% reduction in work productivity, higher cross-sectional rates of depression and anxiety, and diminished quality of life [17]. The quality of life is as low or lower than that reported for other chronic diseases such as arthritis, type 2 diabetes, and depression [17, 18].

Despite the significant negative impact of RLS on an individual, it remains under recognized by both patients and physicians. About 5% of adult Americans have some degree of RLS symptoms [19] with about 1.5-3% of adult Americans having moderate to severe RLS symptoms. Surveys of RLS patients have shown that between 32% and 81% report having consulted a physician about their symptoms, but only 7% report having received a diagnosis of RLS [20-22]. Even after receiving a diagnosis of RLS, as many as 13% of patients are treated with inappropriate medications [12].

Epidemiology

Symptoms of RLS exhibit a genuine circadian pattern and are also influenced by age, gender, pregnancy, ethnicity, genetic factors, and common medical conditions. The disorder can occur at any age, but symptoms often begin in middle-age with the mean age of onset being between 27 and 41 years of age [23, 24]. Approximately 40% of RLS patients first experience symptoms before the age of 20. RLS affects 2% of school aged children [25], 1 to 5% of young to middle-aged adults, and increases to 10 to 20% in those 60 years and older [26]. RLS affects women twice as frequently as men [27] and women exhibit greater night-to-night variability in PLMs [5, 28]. Nearly one-in-three pregnant women experience RLS in their third trimester and the risk of RLS increases linearly with the number of live births (OR=3.57 for >3 births) [5, 27]. RLS occurs more commonly in those with Northern European ancestry and approximately one-third of patients (especially those with early-onset disease, before 45 years of age) will have multiple affected family members. Prevalence in non-European ethnic groups are notably low: 0.1% in Singapore [29], 0.9% in South Korea [30], 2.0% in Ecuador [31], 3.2% in Turkey [32], and 3.3% in Japan [33], implicating genetic, environmental, or socio-cultural factors in expressivity [5, 9, 34-37]. The proportion of phenotypic variation attributable to genes is 54-83%. RLS is also seen as a co-morbidity with many common medical conditions, including iron deficiency [38], end stage renal disease [39], rheumatologic disorders [40], diabetes [41], pulmonary hypertension [42], chronic obstructive pulmonary disease [43, 44], liver disease [45], Crohn's [46] and celiac disease [46, 47], gastric surgery [48], medications [41], and neurological conditions such as Parkinson's disease [49-52] and multiple sclerosis [5, 53]. More significantly, RLS has been identified as a risk factor for cardiovascular disease [54-57].

RLS and Iron

In the first comprehensive description of RLS, published in 1945, Ekbom encountered iron deficiency in 25% of his patients and a favorable response to iron supplementation [58]. RLS was

more frequent in patients with co-morbid medical conditions such as uremia, anemia and pregnancy. Low iron blood level is common amongst these conditions and therefore supported a relationship between iron and RLS. Since Ekbom's initial publication, mixed results concerning the relationship between RLS and iron have been found through clinical experience, open label and clinical therapeutic trials, and population and genetic studies.

RLS and Iron – Supporting Evidence

Since Ekbom's observation, several reports of single cases, case series, clinical investigations, population studies, open label and clinical trials have demonstrated a significant relationship between RLS and iron.

Several clinical cases and case series have found decreased levels of serum iron and ferritin (iron stores) in patients with RLS [7, 59, 60], even in the absence of anemia [61]. Furthermore, clinical findings have demonstrated that patients with iron deficiency have a higher frequency of RLS (30%) than those without [38, 62] and iron deficiency has been reported to occur at a high frequency among RLS patients [63]. Moreover, the prevalence of RLS increases in other conditions that are associated with compromised iron status, including pregnancy [64] and end stage renal disease [65, 66]. Dr. Rye, in the evaluation of his own tertiary care clinic, found that approximately 40% of over 800 RLS patients were iron deficient [personal communication with Drs. Rye and Trotti], thus lending support to Ekbom's original observation.

Population based studies are necessary to further elucidate the association of iron deficiency in cases with RLS to determine if the observed frequencies of iron deficiencies found in case series and clinical investigations are reflective only of the subgroup of RLS patients who seek medical attention or a phenomenon genuine to the underlying disease itself. To this matter, in one study, the soluble transferrin receptor levels were significantly higher in RLS cases compared to controls without RLS, reflecting relative iron deficiency in the RLS group [34]. One recent multi-center study

in Iceland and Sweden [67] demonstrated significantly lower ferritin levels in RLS participants, but not after adjustment for geographic location, age, sex and smoking history. Additionally, in another study [27], among 53 men with low ferritin levels (less than 20μg/L), an increased prevalence of RLS was seen compared with men with higher ferritin levels [27, 67]. These findings, albeit small, suggest that the association between iron and RLS seen in clinic extends to the general population, but may be impacted by other factors.

Further supporting the role of iron in RLS, several reports have found an inverse relationship between the severity of RLS symptoms and levels of ferritin [7, 60, 68, 69]. That is, the sensory symptoms of RLS (i.e., the uncomfortable feelings in legs that are only relieved with movement) are worse in persons with low serum ferritin levels. Moreover, a low serum ferritin level (less than 50 μg/L) has been associated with a reduction in the quantity of sleep [7, 59, 60] as determined by standard overnight sleep study in RLS patients [60].

Open label reports suggest that various formulations of iron supplementation may improve symptoms of RLS in patients with iron deficiency. Treatment of RLS with iron was first reported in 1945 [70]. Nordlander pioneered this approach, reporting almost complete remission of RLS in 21 of 22 RLS subjects with repeated doses of IV iron *irrespective* of baseline iron status [71]. A retrospective analysis from 89 patients with RLS who had been treated using various iron formulations found between 67 and 86 percent of the patients had complete resolution of symptoms as determined by patients' subjective statement of improvements [72]. In an open label study, in which 10 RLS patients received a single 1000 mg intravenous iron infusion, 7 showed a substantial improvement in RLS symptoms 2 weeks later [73]. Two further studies investigated the effect of intravenous iron and found that 1000 mg iron dextran was superior to placebo for up to 4 weeks in 25 RLS patients with chronic renal failure [74].

To date six double blind, randomized placebo controlled trials have been conducted. In a recent meta-analysis of the four studies that reported average change in IRLS scores, the mean

improvement in IRLS was 3.8 points higher in those subjects taking iron than those receiving placebo (p=0.06) [75]. This estimate is lower than the 6-point difference that has been proposed as clinically meaningful improvement in IRLS scores [35] and the 5.7 point difference observed with first line dopamine agonist therapy [36]. In another, high-dose iron dextran infusion was associated with a significant, but transient, reduction in symptoms of RLS in patients with end-stage renal disease [74]. From these trials, there is insufficient evidence to conclude whether iron is beneficial for the treatment of restless legs syndrome.

RLS and Iron – Opposing Evidence

Because of these investigations demonstrating a significant relationship between iron and RLS, it was reasoned that iron deficiency is the mechanism underlying RLS. Despite these associations, however, the prevalence, pathophysiological mechanisms, and relationship of iron deficiency to RLS remain unclear. Several clinical investigations, population studies, open label and clinical trials have demonstrated contrasting conclusions that challenge these views of iron deficiency as causal to RLS.

Clinical experience with iron deficiency [personal communication with Drs. Rye and Trotti] and epidemiological findings [27, 76] are unambiguous in demonstrating that systemic iron-deficiency is neither sufficient or necessary to produce RLS. While RLS can develop or worsen in nearly one-third of pregnant women, iron parameters bear little to no relation to symptoms that otherwise resolve quickly after delivery despite the attendant loss of blood, and presumably iron [64, 77-79].

The population based studies that have examined this question suggest that the observed frequencies of iron deficiencies found in case series and clinical investigations are reflective of a subgroup of RLS patients [27, 76]. The findings for the association of iron metabolism and RLS on a population level have been generally consistent. In four population based studies that assessed iron,

only one study and one iron metric was significant [27]. Contrast this to one study in which five parameters of iron metabolism including serum ferritin, within or below clinical norms, were not associated with increased risks of RLS [76]. Moreover, two other population based studies [27, 34] found only equivocal associations between iron and RLS. In a study that found increased prevalence of RLS amongst men with abnormally low ferritin levels (less than 20μg/L) [27], no increase in prevalence was observed amongst women with ferritin levels of 20μg/L or lower (n=334, comprising 9.4% of the sample). The prevalence of RLS is higher in Iceland than in Sweden where it is comparable to other European populations, [67] yet the adult Icelandic population has unusually high ferritin levels [80].

These data, taken together with clinical experience, suggest that iron deficiency may only be a mechanism of RLS in a subgroup of people that has arisen from a selection bias for more severely affected or treatment resistant individuals more likely to present to specialized centers.

Several randomized controlled trials of iron have failed to show a benefit of iron therapy. In a randomized, double-blind, placebo-controlled trial that did not select patients according to their iron status as measured by serum ferritin levels, iron sulfate failed to improve RLS symptoms [81]. In a second study of intravenous iron sucrose in RLS with mild-to-moderate evidence of systemic iron deficiency, some positive symptom benefit was demonstrable, but not at all time points or in all subjects [82]. One open-label study [73], and two randomized, double blind, placebo-controlled studies [82, 83] of intravenous iron for RLS were unable to conclusively demonstrate reliable efficacy in alleviating RLS. One placebo controlled study of RLS subjects with normal iron status demonstrated no benefit in an interim analysis and was therefore halted prematurely [83]. In summary, these studies suggest the potential benefit of iron supplementation.

One of the difficulties in studying RLS that may account for discrepancies between various studies is the temporal variability of the phenotype. Symptoms such as PLM's are variable on a day–

to-day basis [28]. Normal everyday activities such as sleep restriction, tobacco, alcohol, and caffeine may exacerbate symptoms [84], while exercise can improve them [85].

When the underlying biology of the condition is complex and incompletely elucidated, it can be difficult to exclude sources of bias and confounding. Methodologically rigorous clinical trials are particularly important when considering conditions like RLS where the natural history includes waxing and waning of symptoms and a placebo effect is likely to be present [86]. Moreover, statistical methods to assess validity and accuracy are a necessity when evaluating health outcomes after intervention.

In conclusion, despite the high prevalence of iron deficiency amongst RLS subjects seeking treatment, there is still little data to go on to guide one's selection of patients who should be tested for iron deficiency. To this end, an alternative perspective and approach are warranted to accurately define the nature and extent of the relationship between low iron and RLS.

Genetic Studies

In 2007 Rye et al [9] and Winkelmann et al [10] separately demonstrated that alleles in 4 unique genes account for nearly 80% of the population attributable risk for RLS and in the *BTBD9* gene, alone carries a population attributable risk of 50%. This BTBD9 risk allele gene possesses 2 clinically important traits: 1) dose-dependent relationship to periodic limb movements of sleep and 2) a dose-dependent relationship to decreases in ferritin levels. That is, each copy of the risk allele increases the number of PLMs on average and each copy of the risk allele predicts a 13% lower average serum ferritin.

The results are a critical first step in understanding the pathophysiology of RLS and offer explanations for some of its epidemiologic features. The iron deficiency commonly seen in RLS, for example, appears to be an environmental and genetic interaction factor, or affected by the *BTBD9* gene product. In addition, these findings also shed light on ethnic differences in RLS prevalence,

suggesting that ethnic differences in RLS prevalence reflect the frequency of the RLS risk alleles in different populations. The Rye et al study also identified PLMs as a valuable endophenotype for future epidemiologic studies [38]. Endophenotypes, represent simpler clues to genetic underpinnings than the disease's symptom complex. This promotes the view that RLS diagnoses can be decomposed or deconstructed, for example, objective criteria involving PLMs and/or genetic testing rather than the current, subjective clinical assessment. These findings are a prime example of how genetic advances can inform disease classification, recognition, and measurement.

When the clinical, epidemiological and genetic data is carefully considered, iron deficiency emerges as recognizable and reversible state that may modulate expression of RLS in some individuals with RLS, but not all [5]. It may be more appropriate to view RLS as a complex disease within which potential sub-populations exist with specific attributes that are driven by underlying genetic factors [9].

Methods

This section presents the methods employed to achieve the research objective.

Study Population

This study used data that was obtained by deCODE Genetics and researchers at Emory University School of Medicine in a collaborative research project beginning in 2002 in Reykjavik, Iceland [9]. The primary population sample of 943 Icelanders was recruited from responses to a newspaper advertisement describing the clinical signs and symptoms of RLS. A questionnaire was administered asking about the presence and severity of RLS symptoms (according to the International Restless Legs Syndrome Study Group [IRLSSG]), clinical features, and coexisting conditions. Venous blood samples were obtained for serum iron measurements and genotyping. The Data Protection Authority (DPA) of Iceland encrypted all personal identifiers associated with information or blood samples with the use of deCODE genetics' third-party encryption system. Details on ascertainment have been previously published [9].

Sample Population- RLS Diagnosis

The sample population for this study included those subjects that met the 2003 revised RLS clinical diagnostic criteria. In line with the 2003 revised criteria, subjects were considered to be affected by RLS if they self reported an uncomfortable desire to move their legs with inactivity at least 2-4 times/month that was relieved by movement, and that predominated in the evening or at bedtime. The sample population consisted of 572 subjects positive for RLS.

Variables

Dependent Variable

Iron Deficiency

Bone marrow iron storage assessment is considered the gold standard for evaluating systemic iron storage, and it has been shown that a ferritin level <45 μg/L can detect 90% of patients with iron absent in bone marrow [37]. We therefore developed a binary variable for serum ferritin levels. Subjects with a ferritin below 45 μg/L were categorized as iron deficient.

Independent Variables

Demographics and Lifestyle Covariates

Demographic covariates include subject's age, gender, Body Mass Index (BMI), smoking history, caffeine and alcohol use, and physical activity participation. Demographic variables were binary for gender (**Male** and **Female**), age **(>45 Years of Age** and **≤ 45 Years of Age**), smoking history, caffeine use (Do you drink any caffeinated beverages?), alcohol use (Do you drink alcohol?) and participation in physical activity (Do you currently participate in sports or regular physical exercise?) (**Yes** and **No**) and continuous for BMI. BMI is a fairly reliable indicator of body fatness for most people. The standard weight status categories associated with BMI ranges for adults are displayed in Table 1. Previous studies at the population level have assessed these lifestyle factors and found statistically significant associations with RLS, for example positive associations between RLS and smoking and with alcohol as well [26].

Medications

Medication variables include first and second line treatments and iron supplementation. First line treatments for relieving RLS symptoms include dopamine agonists therapies, pramipexole and ropinirole. Dopaminergics have been known to be effective in treating RLS for at least two decades [87] , typically produce immediate and marked symptom relief in 70-90% of patients [88] and are first line therapy in clinical guidelines [5, 89-91].

Second line treatments demonstrating efficacy includes the anti-epileptic medication gabapentin and the dopaminergic therapy levodopa [37, 92-94]. The Movement Disorders Society (MDS) in a recent systematic review classified gabapentin as effective for RLS (in addition to dopaminergic agents) [95]. Levodopa is converted into dopamine in the body and therefore increases levels of this chemical. Iron treatment includes any iron supplement including over the counter and prescribed iron supplements. Three binary variables (**Yes** and **No**) were created to indicate whether a subject was currently taking a first line or second line treatment, or receiving iron supplementation.

RLS Severity

RLS has a wide variety of severities from rare, non disturbing episodes to severe, almost unremitting symptoms that last for most of the day and prevent sleep at night. RLS severity has been measured in clinical trials using the validated using International Restless Legs Syndrome Study Group Severity Rating Scale (IRLS). Advances in genetic discoveries have also established PLMs as a clinical and genetic phenotype that can be reliably and objectively measured.

RLS Rating Scale

Severity was assessed using the IRLS . The IRLS is a validated subjective rating scale that assesses RLS severity, reflecting both subjective primary features (diagnostic features 1 through 3 reflected in questions 1 through 3 and 6 of the scale, respectively), intensity and frequency of the disorder (questions 7 and 8 of the scale) and associated sleep problems (features 5 and 6 reflected in questions 4 and 5 of the scale). The scale also includes questions that probe for the presumed negative impact of symptoms on the patient's mood and daily functioning (questions 9 and 10 of the scale). The range of achievable total score is 0 – 40 and allows categorization of RLS as Mild (score 1-10), Moderate (score 11-20), Severe (score 21-30), or Very Severe (score 31-40) [96]. Validation of the scale supported that severity of the core symptoms is strongly related to their impact on the patient's life [96]. Therefore, a continuous variable was created to capture RLS severity as scored from the IRLS.

PLMs

While PLMs are not specific for RLS, they are linked to the biology of RLS, likely to portend some of the disorder's morbidity and mortality, and are typically sensitive to the same treatments that bring relief from RLS symptoms [9, 97, 98]. PLMs represent an important clinical and genetic phenotype of RLS [99]. Therefore, RLS severity was objectively measured using PLMs.

A PLMs index (PLMi; number of PLMs/hour) 5/hour is generally considered pathological [100], and 9.3% for European Americans meet a threshold of 15 PLMs/hr on a single night of a routine sleep study [101]. This threshold has been included in guidelines for the diagnosis and treatment of RLS. PLMs were measured using a well-validated [28, 102, 103] research device. Subjects wore a small (65-g), wristwatch-size accelerometer (PAM-RL detector, IM Systems) that

was affixed by a hook-and-loop strap to their more affected ankle (or, as a default position, the non-dominant ankle) for at least 1 night and up to 5 nights to derive a mean PLM index (PLMi; number of PLMs/hour) [9]. A continuous variable was created to capture the PLM index. Further details on the use of the PAM-RL and its validation with a standard overnight sleep study have been extensively detailed elsewhere [9, 102].

Phenotype- Sensory and Motor Symptoms

Phenotype of RLS was inquired in the questionnaire on two domains 1) Sensory and Motor symptoms and 2) Effects on sleep.

Sensory and motor symptoms that characterize subclinical features of RLS that would otherwise be unrecognized using standard clinical criteria were created from the RLS questionnaire. Previous population based studies are generally devoid of granular assessments with population samples; however several clinical samples have established notable associations with RLS [39, 104].

Affected Leg

A binary variable (**Either Leg** or **Both Legs**) was created from the question "In which leg do you get discomfort in?"

Leg Cramps

A second binary variable (**Infrequent** and **Frequent**) was created in response to the question "How often do you experience leg cramps?" Original responses of "Never /Almost Never" and "Sometimes" were re-coded as **Infrequent** and responses of "Often" and "Very Often" were re-coded as **Frequent.**

Arm Symptoms

Augmentation, exacerbation of symptoms following therapy often presented as earlier onset of symptoms and symptoms in upper extremities, has a significant positive-inverse association with iron deficiency. In addition, some unmedicated RLS patients present with symptoms in arms and feet [104]. To identify sensory symptoms not isolated to subject's legs, a third binary variable (**Yes** and **No**) was created in response to the question "Do you ever feel discomfort similar to RLS symptoms in your hands or arms?"

Growing Pains

The natural history of RLS has revealed that sensory symptoms often begin during childhood and manifest as "growing pains", estimated to affect 2% of school-aged children. To further elucidate the potential significance of the slow and gradual progress that culminates in clinical presentation during adulthood, a fourth binary variable (**Yes** and **No**) was created in response to the question "When you were a child, were you ever told that you had growing pains?"

Age of Onset

Patients with late onset of symptoms (≥45 Years of Age) rather than early-onset **(<**45 Years of Age) of RLS have serum ferritin levels correlate that with RLS severity and sleep efficiency [105-108]. Therefore, a fifth binary variable **(≥ 40 Years of Age** and **<40 Years of Age**) was created in response to the question ""How old were your when you first had discomfort in your legs?" Original responses included 8 categories of 10 year denominations starting with "Less than 10 years old" , "10-19 years old", etc… and ending with "70 years or older". The answer choices did not have a choice that corresponded to the clinical findings associated with age of onset and 45 years of age.

Therefore, four categories representing age of onset <40 years of age were re-coded as **<40 Years of Age** and four categories representing age of onset ≥40 were re-coded as **≥40 Years of Age.**

Effects on Sleep

Symptoms of RLS are worse in the evening or at night and are frequently associated with brief arousals from sleep that result in sleep fragmentation. To capture the effects on sleep, 2 binary variables were created from 2 questions inquiring about the frequency and severity of sleep disturbance because of RLS symptoms.

Sleep Disturbance Frequency

A binary variable (**Infrequent** and **Frequent**) was created in response to the question "How often does the restlessness in your legs delay you from falling asleep?" Original responses of "Never", "Less than once a week" and "Once to twice a week" were re-coded as **Infrequent** and responses of "three to five times a week" and "six to seven times a week" were re-coded as **Frequent.**

Sleep Disturbance Severity

A binary variable (**Low Severity** and **High Severity**) was created in response to the question "overall, how severe is your sleep disturbance from the leg discomfort?" Original responses of "None", "Mild", and "Moderate" were re-coded as **Low Severity** and responses of "Severe" and "Very Severe" were re-coded as **High Severity.**

History of Anemia

A history of anemia is suggestive of iron deficiency. A binary variable (**Yes** and **No**) was created for a history of anemia in response to the question "Have you ever been previously diagnosed with anemia?"

Table 1: Study Variables

Dependent Variable	Description
Iron Deficiency Status	Evaluated as a dichotomous variable 1. Iron deficiency: ferritin levels below 45µg/mL 2. No iron deficiency: ferritin levels equal and above 45µg/mL
Independent Variable	**Description**
Demographics	
Age	Evaluated as a binary variable: 1. > 45 years of age 2. ≤ 45 years of age
Gender	Evaluated as a binary variable: 1. Male 2. Female
BMI	Continuous variable The standard weight status categories associated with BMI ranges for adults are shown in the following table: **BMI** / **Weight Status** Below 18.5 / Underweight 18.5 – 24.9 / Normal 25.0 – 29.9 / Overweight 30.0 and Above / Obese
Lifestyle Factors	
Alcohol	"Do you drink alcohol?" Evaluated as a binary variable 1. Yes 2. No
Exercise	"Do you currently participate in sports or regular physical exercise?" Evaluated as a binary variable 1. Yes 2. No
Caffeine	"Do you drink any caffeinated beverages?" Evaluated as a binary variable 1. Yes 2. No
Smoking	History of smoking Evaluated as a binary variable 1. Yes 2. No
RLS Severity	
PLMi	Evaluated as a binary variable 1. PLMi < 15/hr

<table>
<tr><td></td><td>2. PLMI≥ 15/hr</td></tr>
<tr><td>RLS Rating Scale Score</td><td>Continuous variable
Ranging from 1-40
<table><tr><th>IRLS Score</th><th>Severity Scale</th></tr><tr><td>1 – 10</td><td>Mild</td></tr><tr><td>11 – 20</td><td>Moderate</td></tr><tr><td>21 – 30</td><td>Severe</td></tr><tr><td>31 – 40</td><td>Very Severe</td></tr></table></td></tr>
<tr><td colspan="2">Phenotypic Presentation – Sensory &Motor</td></tr>
<tr><td>Age of Onset</td><td>Evaluated as a binary variable:
1. <40 years of age
2. ≥ 40 years of age</td></tr>
<tr><td>Affected Leg</td><td>Evaluated as a binary variable
1. Either leg
2. Both legs</td></tr>
<tr><td>Growing Pains</td><td>Evaluated as a binary variable
1. Yes
2. No/ Do not know</td></tr>
<tr><td>Leg Cramps</td><td>Evaluated as a binary variable
1. Infrequent
2. Frequent</td></tr>
<tr><td colspan="2">Phenotypic Presentation – Sleep</td></tr>
<tr><td>Frequency of Sleep Disturbance</td><td>Evaluated as a binary variable
3. Infrequent
4. Frequent</td></tr>
<tr><td>Severity of Sleep Disturbance</td><td>Evaluated as a binary variable
1. Low Severity
2. High Severity</td></tr>
<tr><td colspan="2">History of Anemia</td></tr>
<tr><td>History of Anemia</td><td>Evaluated as a dichotomous variable
1. Yes
2. No</td></tr>
</table>

Data Analysis

SAS 9.3 (SAS Institute Inc. Cary, NC) was used for database construction, manipulation, and statistical analyses.

Missing Data

Multiple Imputations

A multiple imputation strategy was used to impute missing values so that the whole sample could be analyzed to avoid selection bias and biased regression estimates that can occur by deleting cases with missing covariates. Multiple imputation strategies based on maximum likelihood described by Rubin et al [109, 110] were used to impute missing values. Exploratory analysis confirmed data was missing at random. Four thousand datasets with 2000 iterations were imputed and estimates from these datasets were combined using methods described by Rubin et al [109, 110].

Logistic Regression Analysis

The PROC LOGISTIC procedure in SAS 9.3 was used with the binary outcome variable of iron deficiency. Covariates are seen in Table 1. These variables were included in models to assess how well the signs and symptoms of RLS predicted iron deficiency.

Calculating the probability of iron deficiency is possible by using the equation below:

Equation 1: Specified Logistic Regression Model

$$\Pr(IronDeficiency) = \frac{1}{1+e^{-z}}$$

$$z = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \ldots + \beta_i X_i + \varepsilon.$$

To determine the value of various predictors, we conducted a series of logistic regressions modeling iron deficiency (Table 2). Models 1-3 contained the same demographic and lifestyle variables while controlling for current RLS and iron medications. This was done to ensure treatment bias did not confound the selection of variables to include in the full models, which did not control for

any covariates. Model 1 was constructed with the *a priori* specification of RLS Severity as measured by RLS Rating Scale and PLMi. Model 2 was constructed with the *a priori* specification of RLS Phenotype, consisting of Sensory and Motor symptoms and sleep symptoms. Model 3 was constructed with *a priori* specification of History of Anemia. The models were constructed with these *a priori* specifications because each model represents a domain that captures particular features of the disease. Model 1 was constructed under the domain of disease severity. This was constructed with the presumption that the RLS Rating Scale and PLM Index will demonstrate significant differences between iron deficient patients and non-iron deficient patients as supported by previous studies and clinical experience [111]. Model 2 was constructed under the domain of RLS Phenotype with the focus on sensory and motor systems and sleep symptoms. Model 2 was constructed with the presumption that sensory and motor and sleep symptoms are associated with serum ferritin [108, 112-114]. Model 3 was construed under the domain of medical history, specifically history of anemia. This was constructed with the presumption that incidence of RLS is greater in co-morbid conditions with compromised iron [61, 114-116].

Results from this preliminary screening indicated that *Age* and *Gender* were significant predictors of iron deficiency in each of the three models regardless of other RLS features that were considered. A *History of Anemia* in Model 3 was statistically significant in predicting iron deficiency as well. Taken together, these results suggested that a history of anemia can be used as the first decision step in developing an algorithm. We were therefore interested in identifying predictors of iron deficiency in RLS subjects who did *not* have a history of anemia. Therefore, Models 1 and 2 (Table 2) were re-analyzed under the condition of no history of anemia (n=428). These models were labeled as Model 1A and 2A, respectively (not shown). Models 1A and 2A indicated that the metrics

for RLS severity and RLS symptoms are insignificant predictors of iron deficiency. Models 1A and 2A also demonstrated that *Age* and *Gender* are significant predictors of iron deficiency in subjects with no history of anemia. These results were consistent with Models 1, 2 and 3 in that *Age* and *Gender* are significant predictors of iron deficiency.

Table 2: Variables Included in Initial Screening (Models 1-3)

	Variable	Model 1 (n=572)	Model 2 (n=572)	Model 3 (n=572)
Demographics	Age (continuous)	X	X	X
	Age (binary)			
	Gender (Female)	X	X	X
	BMI	X	X	X
Lifestyle	Alcohol	X	X	X
	Exercise	X	X	X
	Smoking	X	X	X
	Caffeine	X	X	X
	History of Anemia			X
RLS Severity	PLMi	X		
	RLS Rating Scale	X		
RLS Phenotype	Age of Onset		X	
	Affected Leg		X	
	Growing Pains		X	
	Leg Cramps		X	
	Sleep disturbance frequency		X	
	Sleep disturbance severity		X	

Table 3: Variables Included in Final Models (Models 4-6)

Variable	Model 4 (n=572)	Model 5* (n=428)	Model 6 (n=572)
History of Anemia and Females Under 45 years of age without a History of Anemia			X
Females Under 45 years of age without a History of Anemia		X	
History of Anemia	X		

*indicates that the model was run in the sample that did not have a history of anemia.

The regression results from Models 1A and 2A were used to construct the 3 final models, Models 4-6 (Table 3), which were used to develop screening strategies. *History of Anemia* in Model 3 was significantly associated with iron deficiency and therefore included as the only independent variable in Model 4. Model 4 tests whether *History of Anemia* can be used as the first decision step in developing an algorithm and categorize subjects in clinically relevant subgroups. *Age* and *Gender* were significantly associated with iron deficiency with no history of anemia in Models 1A and 2A and therefore combined into a variable in Model 5, which tested whether *Age* and *Gender* are significant in the group of subjects with no history of anemia and therefore is only of 428 subjects.. Model 6 encompasses Models 4 and 5 and allows for comparison between Model 4 and Model 6 because it includes the whole sample population of 572 subjects.

Sensitivity, Specificity, PV+, PV-, FPR, FNR, Analysis with Cost Consideration

Measures of accuracy (sensitivity, specificity, positive predictive value PV+, negative predictive value PV-, False Positive Rate FPR, and False Negative Rate FNR) must be known if we

are to determine the ability of a test to correctly distinguish between diseased and well persons in a given population. Therefore, we included these measures in our analysis.

Sensitivity is defined as the proportion of true positive subjects that have a positive test result. Specificity is the proportion of true negative responders that have a negative test result. The false positive rate is the proportion of positive test results that are true negative responders. The false negative rate is the proportion of negative test results that are true positive responders. Logistic regression is often evaluated in terms of its predictive ability. In a logistic regression, a two by two table classification table can be created for any cut-off value of the fitted probability and hence the sensitivity and specificity are then available. The fraction calculated as a count of predicted positives divided by the actual total number of positives is the sensitivity (Equation 1) and the fraction calculated as the count of predicted negatives divided by the actual total negatives is the specificity (Equation 2).

The sensitivity and specificity of each screen was calculated using the following equations:

Equation 2: Sensitivity

$$\text{Sensitivity} = \Pr(\text{Positive test result} \mid \text{Disease})$$

$$= \frac{\text{Frequency of positive tests among diseased}}{\text{Frequency of diseased persons}}$$

Equation 3: Specificity

$$\text{Specificity} = \Pr(\text{Negative test result} \mid \text{No Disease})$$

$$= \frac{\text{Frequency of negative tests among disease-free}}{\text{Frequency of non-diseased persons}}$$

The sensitivity and specificity are descriptors of the accuracy of a test. Two measures concerning the estimation of the probability of the presence or absence of disease are the positive predictive value (PV+) and the negative predictive value (PV–). The PV+ is defined as the percentage of persons with positive test results who actually have the disease of interest. The PV+ therefore allows us to estimate how likely it is that the disease of interest is present if the test is positive. The PV– is defined as the percentage of persons with negative test results who do not have the disease of interest. PV+ and PV- are measures of the performance of a diagnostic test that depend on the prevalence of the disease in the screened population and on the sensitivity and specificity of the test. However, unlike sensitivity and specificity, they are not properties of the screening test itself, but of its application.

In addition to examining the sensitivity and specificity of the screens, screens were also analyzed in terms of direct medical costs. The direct medical costs were estimated under certain assumptions, primarily, that an inverse relationship exists between serum ferritin levels and RLS severity and subsequently a positive correlation between utilization of medical services and RLS severity. The cost of each screening strategy was estimated as the direct medical costs to test and treat each subject over one year. The estimated costs were derived from multiple sources as described in Table 4. Estimated costs for clinical visits were derived from clinical experience informed by RLS experts, Drs. Rye and Trotti who consulted on this study. Laboratory costs were derived from the median of the 2012 60% updated Base Fee Amounts from all Medicare Part B carriers made available from CMS. Therapy costs were based on pricing data available at Dynamed, a point of care information summary service, at the time of the analysis [117]. The cost of implementing each

screening strategy is negligible because the information necessary is routinely acquired during a clinical visit and therefore does not require extensive additional resources.

The medical costs associated with blood testing and iron treatments of iron deficient patients over one year are $160 and $360, respectively, totaling $520 per patient. Missed cases of iron deficiency in RLS patients are estimated to lead to 2 additional office visits within one year and increased dosage of first line therapy or possible introduction of a second or third line treatment. Estimated direct medical costs include 2 office visits of $300 each, and introduction of second and/or third line therapy estimated at $100/month, totaling $1,800 per patient.

Table 4: Estimated Costs

Item	Cost (per unit)	Direct Medical Cost Over One Year	Source
Iron Testing Assay of Iron Iron Binding Test Assay of ferritin Assay of transferrin	**$79.65** $12.40 $16.73 $26.08 $24.44	$160	2012 Clinical Diagnostic Laboratory Fee Schedule (CLAB) HCPCS Codes (83540, 83550, 82728,84466)
Iron Therapy Over the counter supplementation	$30/month	$360	Dynamed
RLS Therapy – Second and Third Line Therapy carbidopa/levodopa Gabapentin Opiods	 $40/month $100/month $60/month	$2,400	Dynamed
Follow up Office Visit	$300	$600	Study consultants (LMT, DBR)

Results

Descriptive statistics- Demographics, History of Anemia, Lifestyle Factors and Current Medications

The sample population for this study consisted of 572 subjects that met the clinical criteria for Restless Legs Syndrome. Out of the 572 subjects, 240 (41.92 %) have iron deficiency. The population consisted of 397 females and 175 males. The average age of all subjects was 53.076 (±14.612). Average BMI was 25.171 (±9.438). Of the sample population, 471 (82.34%) were alcohol users at the time of study, 545 (95.28%) consumed caffeinated beverages, 355 (62.06%) participated in regular physical exercise, and 376 (65.73%) endorsed a history of smoking. Of the sample population, only 4 (0.7%) were taking iron supplementation or were receiving iron therapy.

Table 1 details RLS severity, phenotype and symptoms found in the population. Sample data are provided prior to, and after, imputation to show changes in the variables.

Descriptive statistics- RLS Severity and Phenotype

The average PLMi of all subjects was 12.493 (±15.253) and the average RLS Rating Scale score was 20.122 (±7.606). The RLS scoring criteria are: Mild (score 1-10), Moderate (score 11-20), Severe (score 21-30), and Very Severe (score 31-40) [96]. The score of 20.122 is moderately severe. Of the sample population, 520 (90.91%) have symptoms in both legs while 52 (9.09%) have symptoms in only one leg. Four hundred eight (71.33%) subjects sometimes or often get leg cramps, compared to 164 (28.67%) that never or rarely get leg cramps. Three hundred thirteen (54.72%) had been told during childhood that they had "growing pains" compared to 259 (45.28%) that did not. One hundred forty seven (25.70%) of subjects experience RLS symptoms in their arms, compared to

425 (74.30%) that do not. One hundred twenty six (22.03%) first had symptoms after the age of 40, while 446 (73.43%) first had symptoms before 40 years of age. Of the sample population, 392 (68.53%) never or up to twice a week experience restlessness that delays them from falling asleep; and 180 (31.47%) experience delays in falling asleep due to restlessness 3-7 times a week. Four hundred twenty (73.43%) describe the severity of their sleep disturbance as Infrequent (None to Moderate); while 152 (26.57%) describe the severity of sleep disturbance as Frequent (Severe or Very Severe). Of the sample population, 144 (25.17%) endorsed a history of anemia compared to 428 (74.83%) that did not. In the sample population, 8 (1.40%) subject were on first line RLS treatment, 7 (1.22%) were on second line RLS treatment, and 4 (0.7%) were on iron treatment.

Table 5: Descriptive Statistics Pre and Post Imputation

Variable	Question	Categories	Before Imputation	After Imputation
Iron Deficiency				
			Frequency (Percent)	
Iron Deficiency		No Iron Deficiency	332 (58.04%)	
		Iron Deficiency	240 (41.96%)	
Demographics				
			Mean (Standard Deviation)	
Age (Continuous)			53.098 (±14.615)	53.076 (±14.612)
Age (Binary)		<45 years old ≥45 years or older		
BMI			25.168 (±9.446)	25.171 (±9.438)
			Frequency (Percent)	
Gender		Male	175 (30.59%)	175 (30.59%)
		Female	397 (69.41%)	397 (69.41%)
Lifestyle				
			Frequency (Percent)	

Alcohol	Do you drink alcohol?	No Yes	100 (18.32%) 446 (81.68%)	101 (17.66%) 471 (82.34%)
Caffeine	Do you drink any caffeinated beverages?	No Yes	27 (4.75%) 541 (95.25%)	27 (4.72%) 545 (95.28%)
Exercise	Do you participate in sports or regular physical exercise?	No Yes	206 (38.94%) 323 (61.06%)	217 (37.94%) 355 (62.06%)
Smoking	History of smoking	No Yes	193 (35.22%) 355 (64.78%)	196 (34.27%) 376 (65.73%)
RLS Severity				
			Mean (Standard Deviation)	
RLS Rating Scale			20.122 (±7.606)	20.122 (±7.606)
PLMi	Mean PLMi over 5 nights		12.494 (±15.388)	12.493 (±15.253)
RLS Phenotype – Sensory & Motor and Sleep Symptoms				
			Frequency (Percent)	
Leg	In which leg do you get discomfort?	Either Leg Both Legs	52 (9.76%) 481 (90.24%)	52 (9.09%) 520 (90.91%)
Leg Cramps	Do you get leg cramps?	Infrequent Frequent	164 (28.98%) 402 (71.02%)	164 (28.67%) 408 (71.33%)
Growing Pains	When you were a child, were you ever told that you had growing pains?	No/Do Not Know Yes	256 (45.71%) 304 (54.29%)	259 (45.28%) 313 (54.72%)
Arm Symptoms	Do you ever feel discomfort similar to RLS symptoms in your hands or arms?	No Yes	391 (72.95%) 145 (27.05%)	425 (74.30%) 147 (25.70%)
Age of Onset	How old were your when you first had discomfort in your legs?	≤ 40 years old >40 years or older	411 (77.11%) 122 (22.89%)	446 (77.97%) 126 (22.03%)
Frequency of Sleep Delay	How often does the restlessness in your legs delay you from falling asleep?	Infrequent Frequent	392 (68.53%) 180 (31.47%)	392 (68.53%) 180 (31.47%)

Severity of Sleep Delay	Overall, how severe is your sleep disturbance from the leg discomfort?	Low Severity High Severity	386 (71.75%) 152 (28.25%)	420 (73.43%) 152 (26.57%)
History of Anemia				
			Mean (Standard Deviation)	
History of Anemia	Have you been diagnosed with anemia?	No Yes	413 (74.41%) 142 (25.59%)	428 (74.83%) 144 (25.17%)
Medications				
			Mean (Standard Deviation)	
First Line RLS Treatment	Are you currently taking a first line RLS treatment?	No Yes	564 (98.60%) 8 (1.40%)	564 (98.60%) 8 (1.40%)
Second Line RLS Treatment	Are you currently taking a second line RLS treatment?	No Yes	565 (98.78%) 7 (1.22%)	565 (98.78%) 7 (1.22%)
Iron Treatment	Are you currently taking an iron supplement treatment?	No Yes	568 (99.30%) 4 (0.70%)	568 (99.30%) 4 (0.70%)

Predictive Value of Symptoms

To determine the value of various predictors, 3 models were developed to predict iron deficiency. Model 4 consisted of screening according to history of anemia; Model 5 consisted of screening according to age and gender with no history of anemia,; and Strategy 3 consisted of screening according to history of anemia and age and gender given no history of anemia. Because no interactions, either alone or in combination, added significantly to the equation, these parameters were not included.

Table 6: Logistic Regression Results for Models 4, 5 and 6

	Model 4	Model 5	Model 6
History of Anemia	2.7427 ***		
	(1.7751 - 4.2378)		
Age and Gender with No History of Anemia		8.6738 ***	
		(4.6239 - 16.2708)	
History of Anemia and Age and Gender for those with No History of Anemia			5.328 ***
			(3.5367 - 8.0267)
Constant	0.553 ***	0.359 ***	0.359 ***
	(0.4432 - 0.69)	(0.2738 - 0.4708)	(0.2738 - 0.4707)
Observations	**572**	**482**	**572**

Model 5 was run on the subset of patients that did not have a History of Anemia only
95% Confidence Intervals in parentheses
* *p < 0.05,* ** *p < 0.01,* *** *p < 0.001*

Model 4 (History of Anemia) tested whether a history of anemia can be used as the first decision step in an algorithm that categorizes subjects into clinically informative subgroups. In Model 4, the odds of being iron deficient are 2.74 times higher with a history of anemia than without a history of anemia. Model 5 (Age and Gender with no History of Anemia) tested whether age and gender are significantly associated with iron deficiency in a sub-sample of subjects with no history of anemia. In Model 5 the odds of a female under the age of 45 to be iron deficient are 8.67 times higher than any female over 45 and any male.Model 6 (History of Anemia and Age and gender for those with No History of Anemia) incorporates the variables in Models 4 and 5 and demonstrates the odds of being iron deficient are 5.33 times higher with a history of anemia and in women under 45 for those without a history of anemia than any other age and gender group. The regression results of Models 4, 5 and 6 informed the development of the following screening strategies respectively.:

Strategy 1- Test all with a History of Anemia, Strategy 2- Test All Women under 45 with No History of Anemia, and Strategy 3 – Test All with a History of Anemia and Test Only Women under 45 that do not have a History of Anemia. Strategy 2 only considers those subjects without a history of anemia and therefore is a smaller sample than Strategy 1 This strategy is informative for the further classification of subjects after the first step in the algorithm, history of anemia. Strategy 3 incorporates Strategy 1 and 2 which, now accounts for the entire population and is testing the entire algorithm and the classifications made at each step. The first step is a history of anemia, and the second step is age and gender, women under 45 specifically.

Diagnostic Accuracy of Screening Strategies

Strategy 1 – Test All with a History of Anemia

Table 7: 2 x 2 Table of Iron Deficiency vs. History of Anemia

Screen Results	True Disease Status		
	Iron Deficiency	**No Iron Deficiency**	**Total**
Iron Deficiency	88	58	**146**
No Iron Deficiency	152	274	**426**
Total	**240**	**332**	**572**

Strategy 2 – Test All Women under 45 with No History of Anemia

Table 8: 2 x 2 Table of Iron Deficiency vs. Women under 45 and No History of Anemia

Screen Results	True Disease Status		
	Iron Deficiency	**No Iron Deficiency**	**Total**
Iron Deficiency	60	19	**79**
No Iron Deficiency	92	255	**347**

Total	**152**	**274**	**428**

Strategy 3 – Test All with a History of Anemia and Test Only Women under 45 for those with No History of Anemia

Table 9: 2 x 2 Table of Iron Deficiency vs. Testing All with a History of Anemia and Testing Only Women under 45 for those with No History of Anemia

Screen Results	**True Disease Status**		
	Iron Deficiency	**No Iron Deficiency**	**Total**
Iron Deficiency	148	77	**225**
No Iron Deficiency	92	255	**347**
Total	**240**	**332**	**572**

Table 10: Accuracy of Strategies 1 and 3

Screening Strategy	**Sensitivity, %**	**Specificity, %**	**PV+, %**	**PV-,%**	**FPR,%**	**FNR,%**
Strategy 1- Test All with a History of Anemia	36.81	82.52	60.24	64.48	17.48	63.19
Strategy 3- Test All with a History of Anemia and Women Under 45 for those with No History of Anemia	61.84	76.69	65.67	73.59	23.31	38.16

Forty percent of the sample population was iron deficient and therefore the prevalence of iron deficiency was used as the optimal cut-off point for subsequent analyses. Based on the classification table derived from the logistic regression, two-by-two tables describing sensitivities and specificities of both strategies are displayed in Tables 7 – 10. Strategy 2 will not be discussed in much further detail because it was only a sub-sample population and does not allow for accurate comparison with Strategy 1. Strategy 1 is the first step in the algorithm and Strategy 2 is the second step which is

incorporated into Strategy 3, which is the complete model. Strategy 1 accurately identified approximately 37% of RLS patients with iron deficiency and 83% of RLS patients without iron deficiency. The probability that the patient has iron deficiency given that they have a history of anemia is approximately 60%, while the probability that the patient does not have iron deficiency given that they do not have a history of iron deficiency is approximately 64%.

Strategy 3 had a sensitivity of 61.84% and a specificity of 76.69%. Therefore, with this screen approximately 62% of RLS patients with iron deficiency were accurately detected, and approximately 77% of RLS patients without iron deficiency were accurately detected. The probability that the patient has iron deficiency given that they are a female under the age of 45 with no history of anemia is approximately 66%. The probability that the patient does not have iron deficiency given that they are not a female under the age of 45 without a history of anemia is approximately 74%. Using Strategy 3, 77 subjects were identified to be tested for iron deficiency when they truly were negative. Ninety-two subjects were screened to not have iron deficiency when they truly did.

Sensitivities and specificities are one approach to quantifying the discriminative power of a test. A test with a high sensitivity is useful for 'ruling out' a disease if a person tests negative. The sensitivities of Strategy 1 and 3 does not meet the clinically acceptable threshold of 0.70. If one of the strategies had a high sensitivity and a low specificity, that screen will detect a great majority of individuals that should be tested for iron deficiency and those who test negative are highly unlikely to have the deficiency. However, a relatively low specificity means it will be falsely positive for a number of people who do not have iron deficiency.

A test with a high specificity is useful for 'ruling in' a disease if a person tests positive. The sensitivity and specificity of Strategy 1 was 37% and 83% respectively. Strategy 3 also had a higher

	Strategy 1- Test All with a History of Anemia	Strategy 3- Test All with a History of Anemia and Women Under 45 for those with No History of Anemia	Test All	Test None

specificity and lower sensitivity, but not to the same absolute magnitude as Strategy 1. In Strategy 1 the sensitivity is pretty low, but the specificity is high. It is difficult to predict if an RLS patient without a history of anemia should be tested for iron deficiency because the screen's lack of sensitivity might lead to a false negative result. Since so many people without a history of iron deficiency test negative (82%), a person who returns a positive test is *likely* to have the disease. A highly specific test is, therefore, most helpful to the clinician when the test result is positive.

In clinical practice, however, the test result is all that is known and the intended use of a diagnostic test is to make a diagnosis. Therefore, we need to know the probability that the test will give the correct diagnosis. The sensitivity and specificity do not give us this information. Instead we must approach the data from the direction of the test results, using predictive values. The positive predictive values for Strategy 1 and Strategy 3 are similar at 60.24% and 65.67%. This means that a person who has a positive test has a 60-65% chance of being iron deficient. The advantage of Strategy 3 over Strategy 1 is due to the negative predictive value, 73.59% vs. 64.48%. This difference is significant in the ability of the screen to identify false positives and false negatives.

To determine if a screening strategy was dominant to testing all RLS patients and testing no one; assessment of each strategy in terms of costs and cases missed was carried out. Results are demonstrated in Table 11. In terms of costs, testing all 572 RLS patients once, with a blood test would cost $62,865.00. Subsequent treatment and repeat testing of those identified to have iron deficiency would cost $105,600.00. Alternatively, if none of the subjects were tested for iron deficiency direct patient costs from missed iron deficiency diagnoses would be $326,400.00.

Missed Cases (FN)	152	92	0	240
Detected Cases (FP)	58	78	332	0
Tested Cases	147	226	572	0
True Positive Cases	88	148	240	240
Missed Cases Costs	$272,700.00	$164,700.00	$ -	$431,550.00
False Positive Costs	$4,660.00	$6,200.00	$26,660.00	$ -
True Positive Costs	$45,890.00	$77,090.00	$124,670.00	$ -
Total Costs	$323,250.00	$247,990.00	$151,330.00	$431,550.00

Table 11: Costs vs. Missed Cases. FN= False Negative, FP= False Positive

Strategy 1's low sensitivity and higher specificity is evidenced by costs and missed cases. Using strategy 1, 152 subjects were identified as positive to test for iron deficiency. Of the 152, cost to test 88 subjects that are truly positive is $45,890.00; the cost to test false positives is $4,660.00. 147 subjects were identified to not test for iron deficiency although they truly are iron deficient, costing $272,700.00.

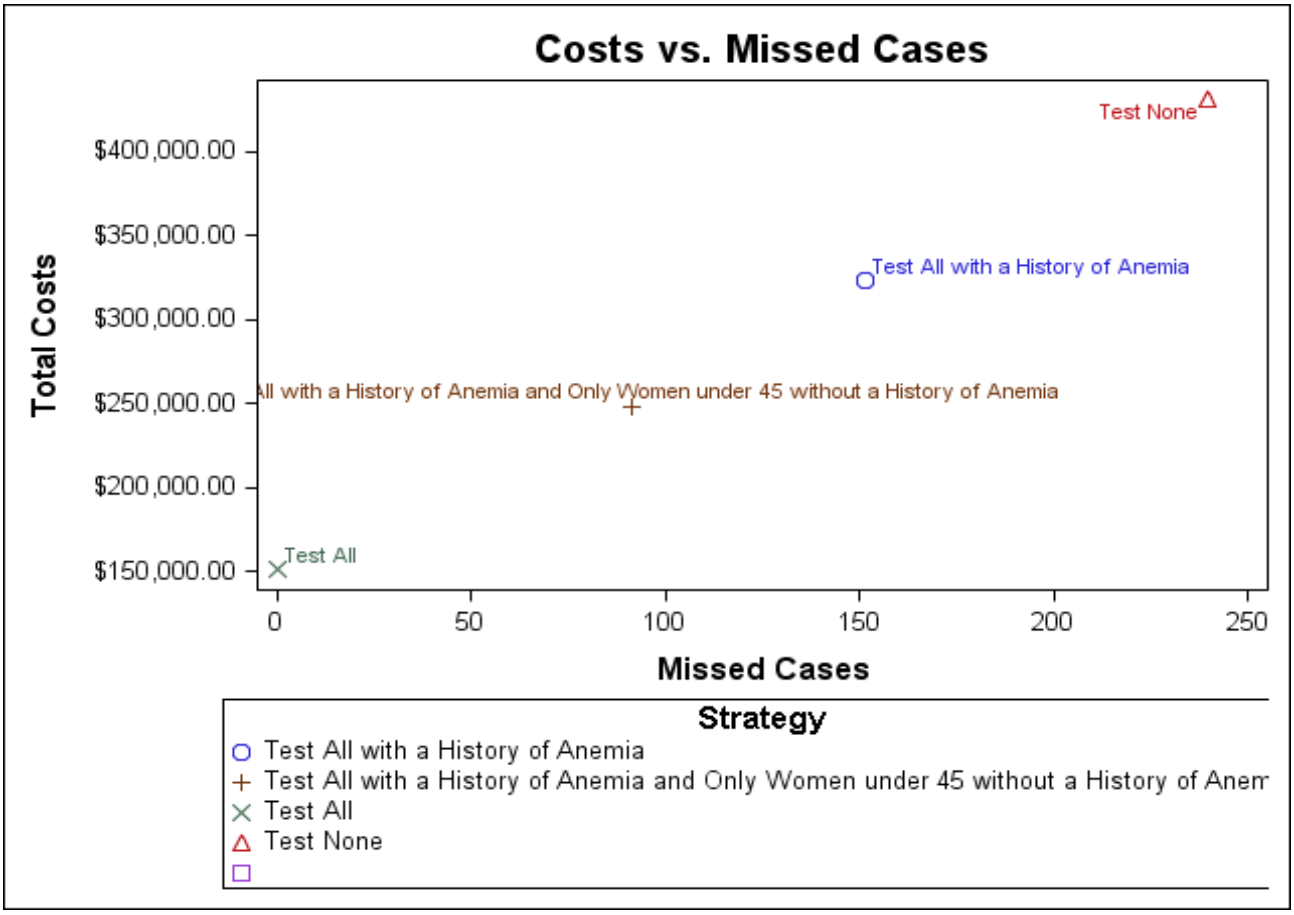
Strategy 3's high sensitivity and lower specificity is evidenced by costs and missed cases. Using strategy 3, 226 subjects were identified as positive to test for iron deficiency. Of the 226, cost to test 148 subjects that are truly positive is $77,090.00; the cost to test false positives is $6,200.00. 92 subjects were identified to not test for iron deficiency although they truly are iron deficient, costing $164,700.00.

Figure 2 illustrates exactly how the screens performed in terms of cost and cases missed when using the screens and taking two alternative approaches, testing all and testing no one. Figure 2 therefore illustrates the tradeoff between total costs and cases missed and highlights those tests that

are interesting with regard to both dimensions. Costs vs. Missed Cases plotted in Figure 2 demonstrate that the most dominant strategy is Test All. Test All is dominant over Strategy 3, Strategy 1 and the current status quo, testing no one. Testing all patients would only cost $151,330.00, which is nearly half the cost of Strategy 3 which had 92 missed cases that cost $164,700 and more than half the costs of Strategy 1 and Test None which had 152 and 240 missed cases with direct medical costs totaling $272,700 and $431,550, respectively.

In regards to clinical practice, current clinical practice guidelines indicate a history of anemia as a valid indication for iron testing, Strategy 1, however in an RLS population that approach would be very costly in terms of direct medical costs and the number of missed cases compared to Strategy 3 (Figure 2).

Figure 1: Costs vs. Missed Cases



Discussion

Sample Population

In this study population, RLS subjects were younger, largely unmedicated, and less affected by the disease's severity, as measured by the IRLS, compared to other population based studies that also assessed for iron parameters [27, 34, 67, 76, 118]. In this study, the average age was 53.076 years, less than 3% of all RLS subjects were taking first or second line treatments or iron supplementation, and average IRLS score was 20, evaluated as moderately severe RLS. Other population based studies had different age related exclusion criteria and none of them included subjects under the age of 40. RLS treatment data was collected but not published [67, 118] or briefly mentioned without great detail, even though there RLS cohort (n=74, 10.6%) have never taken any first line RLS treatment [34]. Only one study had assessed iron parameters and severity with the IRLS [34] and were evaluated to be moderately severe (n=33, 44.6%).

One of the strength's of this study is in the makeup of the population that allow it to be representative of a general population, while at the same time, representative of a disease or clinical population. Of 985 Icelanders included in the study, 572 met the 2003 clinical criteria for an RLS diagnosis [19]. Very few, less than 3% of all RLS subjects were taking first or second line treatments or iron supplementation. This removes potential bias of medication status on dependent variables of interest and, notably on independent variables, such as RLS signs and symptoms that are hypothesized to associate with iron deficiency. In RLS, 10-20% of subjects experience exacerbation of symptoms with chronic dopaminergic therapy known as augmentation [119]. Augmentation manifests as symptoms become more frequent and severe than before treatment. The spread of symptoms to previously unaffected body parts such as the arms, increase in symptom intensity, shorter duration of treatment effect, and the appearance of Periodic Limb Movements while awake (PLMw) [120] are all manifestations that potentially confound the magnitude and direction of

association between iron deficiency and RLS signs and symptoms. These RLS signs and symptoms were determined to be insignificant during the preliminary analysis. Moreover, the low prevalence of RLS subjects on treatment is more representative of populations presenting to primary or general care clinics where RLS recognition is low by both physician and patient and often goes untreated [12].

In this study population, RLS subjects we're less affected by the disease's severity, as measured by the IRLS, compared to RLS subjects that are included in clinical trials of first line dopamine agonists medications. Generally, inclusion into clinical trials for first line RLS therapies require an IRLS total score above 15, while the average baseline score is approximately 24 [36]. Moreover, these clinical trials often exclude iron deficient subjects, presumably, whose severity is worse as evidenced in the literature (See Literature Review section)

The study population is comparable to a clinical population in that subjects are eligible to participate in clinical drug trials for RLS. Of the 985 subjects in the population, 572 met the 2003 clinical criteria [19] for an RLS diagnosis. Had they walked into a clinic with a verbal complaint, they would in theory, all be given a diagnosis of RLS and with an average IRLS score of 20.122, many subjects would qualify to participate in clinical drug trials, which usually require an IRLS score >15. At the same time, this is an RLS population that is generally younger, largely unmedicated and less affected by the disease's severity. From what is known about the disease, diagnosis is usually made during the 4^{th} to 6^{th} decade of life and this population may not have reached the individual threshold to seek medical care. In addition, the sample is also largely unmedicated on iron treatment. The low prevalence of iron therapy amongst a large proportion of the sample that is iron deficient, irrespective of RLS, suggests that there can be systematic factors affecting low uptake of medical care for RLS and RLS related therapies in general. Iron assessment, either directly with iron parameters or indirectly with hemoglobin, is a general test often done during a routine annual check-up. The low frequency of iron therapy suggests caution before generalizing to other populations or developing further research on this population, depending on the research question.

Predictors of iron deficiency

In this study, even with the use of subjective (RLS Rating Scale) and objective (PLMi) metrics measuring severity, RLS sensory and motor symptoms were not associated with iron deficiency. Rye et al. demonstrated that subjects with a single copy of the RLS at risk allele had 13% lower serum ferritin values. In our study, there was no association found between PLMi and ferritin even though independently they share a common genetic association. These findings are consistent with previous population-based studies of RLS and iron [27, 34, 67, 76, 118], as well as the notion of a prevalent complex disease where the expression of the signs and symptoms of the disease are influenced by any multitude of biological, genetic and environmental factors.

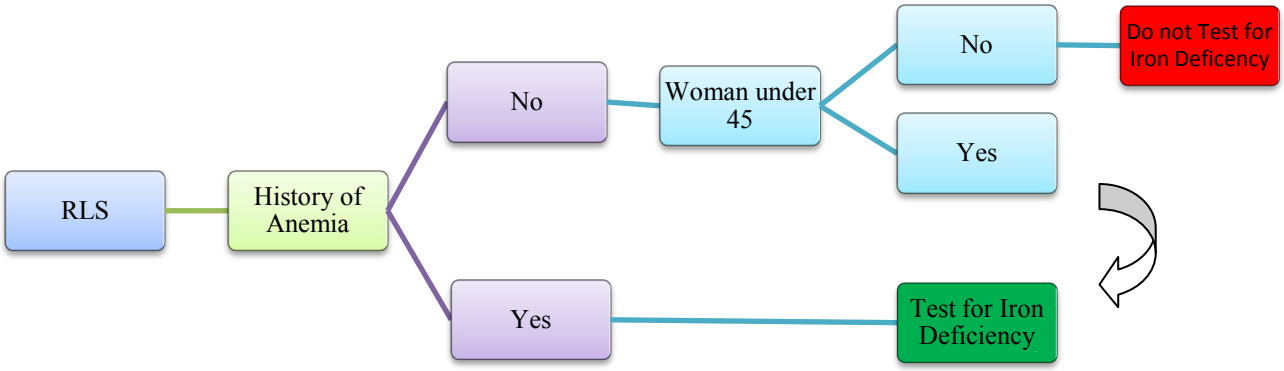


Figure 2: Screening algorithm for iron deficiency in RLS

Whereas RLS symptoms were not predictive, the findings did demonstrate that female gender, age under 45, and a previous history of anemia were independent predictors of iron deficiency. Therefore, we suggest that these variables are the components of a simple, effective clinical screen.

Screening strategies

A decision regarding acceptable levels of sensitivity and specificity involves weighing the consequences of leaving cases undetected (false negatives) and classifying healthy individuals as abnormal (false positives). Highly specific screening tests minimize the number of false-positive results but increase the number of false-negative results. They are preferable if the failure to make an early diagnosis and initiate treatment does not have dire health consequences and/or excessive costs. At the moment, iron testing of RLS patients, absent of any other indication, is not reimbursed by Medicare. A few private health insurance companies and continue to reimburse iron testing of RLS patients with less restrictive criteria on who can be tested or what medical indications are necessary. In instances when positive cases of iron deficiency arise, some health insurance companies require a trial of iron supplementation *before* starting first line RLS treatments. Likewise, when RLS patients with worsening symptoms are prescribed an alternative or additional treatment, some insurance companies would request iron studies to be completed before they approve and cover the costs of the additional treatment option if they believe exacerbating factors like iron deficiency have not been ruled out [personal communication with Dr. Rye].

The four screening strategies that were examined in terms of costs and cases missed include the 2 strategies indentified in this study and 2 extremes, Test All and Test None. The Test All strategy resembles AASM's request for reimbursement for iron testing of RLS patients and the Test None strategy resembles the status quo of not reimbursing for iron studies. The Test All strategy which in this sample costs $151,330 was the most dominant strategy (Figure 2) based on direct medical costs vs. missed cases given the assumptions that an inverse relationship exists between serum ferritin levels and RLS severity and subsequently a positive correlation between utilization of medical services and RLS severity. It follows that if a patient's iron deficiency was missed, their lower ferritin values would increase the severity of their RLS, which over the course of one year would require increased utilization of medical services than otherwise had their iron deficiency was not missed. In

addition, the estimates of direct medical costs used in this study are not comprehensive or extensive and were only classified by missed cases and identified cases. This implies that the direct medical costs that were estimated only resemble a proportion of the clinical care and its associated costs with the disease state of iron deficiency. In Figure 2, the Test None strategy missed 240 cases of iron deficiency at the highest cost of $432,000. Strategy 3 is dominant against Strategy1 and Test None, but not against Test All (Figure 2) and Strategy 1 is dominant against Test None.

Screening strategies generally are developed to maximize efficiency of resources at the lowest cost. Generally, a test all strategy is rarely advocated and implemented without some further disease classification that is reflective of the natural history of a disease or risk of disease, for example, the different frequencies of having a mammogram for breast cancer screening is classified by age ranges 40-49 and 50-74. In this study, the Test All strategy was dominant and most likely a consequence of the methods employed for the estimation of the direct medical costs. In this study, 2 screening strategies were identified that classified iron deficiency in an RLS population. Strategy 3 is dominant against Strategy 1 because the negative predictive values and false negative rates of the two strategies are remarkably different.

Iron and Genetic Considerations

The high frequency of *BTBD9* amongst the population and its risk in reducing serum ferritin means a large proportion of the population is genetically predisposed to low iron storage values, *irrespective* of a complaint of RLS, as evidenced by the low frequency of RLS subjects (n=15) on treatment and disease severity (IRLS≈20). Subjects with a single copy of the RLS at risk allele, had 13% lower serum ferritin values and 50% of subjects had a 26% reduction in ferritin. RLS is *not* considered a disease of exclusion when medical institutions, health agencies, non-profit organizations, and the World Health Organization (WHO) determine “normal” distributions of iron parameters (e.g. iron, serum ferritin, serum transferring receptor etc.) for iron-related diseases, such as

anemia, and iron deficiency with and without anemia. The population that is predisposed to lower ferritin because of *BTBD9* would be included when these institutions are determining "normal" distributions, which serve as reference ranges as to what is considered "normal" in "healthy" individuals. The inclusion of individuals with the *BTBD9* variant positively skews the distribution towards lower values of iron storage, which the WHO has recognized as a global health concern. The Centers for Disease Control and Prevention (CDC) and WHO rely on these distributions to be accurate so as to make recommendations and plan effective interventions for health ministries and agencies worldwide.

A skewed distribution of normal because of an underlying condition NOT recognized as exclusion for determining "normal" affects the interpretation of the health status of an individual and entire population. Given the iron and genetic considerations, one could advocate for improving the situation by defining new "normal" ranges with a re-defined or more granular method. This can include further stratification by race or genetics in addition to the current classifications of men, women, pre/post menopausal, pregnancy status etc [121]. This would allow new ranges, and presumably more accurate, for particular sub-populations. In addition, it would be essential to investigate and analyze RLS and these iron considerations using other iron metrics such as soluble transferrin receptor (sTfr).

Clinical Implications

The role of iron in RLS is much disputed, but it would be reasonable clinical practice to investigate for iron deficiency and correct it, if present [122]. Our findings suggest that screening RLS females under the age of 45 even if no history of anemia would be cost effective regarding their RLS treatment plan over the course of one year (a conservative estimate since most patients will require much longer pharmacotherapy). The findings can aid clinicians in the treatment and management of RLS patients in a simple and practical manner.

These findings are especially important for primary care physicians (i.e. not neurologists/sleep specialists who regularly treat RLS) who serve as gatekeepers to specialist care referrals. The ability for primary care clinicians to identify and treat the underlying disease, or exacerbating factor is crucial for devising a proper treatment and management plan. Most often, patients are referred to specialists for augmentation, treatment resistant symptoms or other management complications, sometimes which turn out to simply be iron deficiency. The lack of guidelines on iron deficient RLS patients affects the management strategies and treatment options available and usually perpetuates a cycle of frequent treatment changes and clinical visits; add further the distress to the patient, and the whole clinical picture begins to be disruptive, burdensome and expensive. Therefore, the algorithm described in this study may address some of these issues.

New Innovations

This is the first study which investigated an exhaustive list of RLS signs and symptoms and their association with iron deficiency in a population-based sample that had few exclusion criteria that would negatively affect the distribution and variance of disease expressivity, for example, there was no age –related exclusion criteria. Previous population based studies have examined iron parameters have often been limited to specific demographic features, most notably, age. population Previous investigations have occurred in convenience samples of clinical populations with RLS and are, therefore, far more homogenous than what extensive literature and genetics have shown to be a more complex, variable and heterogeneous disease. Additionally, much of the inconsistency between the iron deficiency hypotheses of RLS likely reflects a selection bias for more severely affected or refractory individuals who are more likely to present to specialized centers, which has not been corrected for in previous studies. When the underlying pathophysiology of the condition is complex and incompletely elucidated, it can be difficult to exclude sources of bias and confounding that may not be readily apparent. This study took a population based genetic approach, which implicitly

requires few inclusive limitations to answer the study question and therefore avoids some of these concerns. This study contained adequate sample population selection, sample sizes with sufficient statistical power and included measures of accuracy.

Although other population based studies mentioned in this analysis have examined iron and RLS, the breadth of coverage of the phenotype in this analysis was much larger. In this analysis, sensory and motor symptoms that characterize subclinical features of RLS that would otherwise be unrecognized using standard clinical criteria were examined. Previous population based studies often excluded these granular assessments, such as affected leg, frequency of sleep disturbance, growing pains etc. However, several clinical samples have established notable associations and were included in the original RLS questionnaire distributed in Iceland. Moreover, the advantage of having subjective and objective severity measurements, PLMs and the IRLS Rating Scale allowed for a broader and more objective assessment of severity.

Limitations

Whereas this study corrects many of the previous methodological concerns regarding accuracy, it also faces limitations. The predictive models were constructed on a small population size relative to disease prevalence and statistical power. This limitation may have contributed to less precise logistic regression coefficients or caused some disease phenotypes to not seem indicative of an important contributor to iron levels where they would otherwise have been considered important.

In addition, the Icelandic population, from which the sample was drawn, exhibits a greater prevalence of RLS compared to the US, 5%-15% in the US vs. 18% in Iceland [67]. Finally, we use a cross-sectional study design, which is observational in nature and, therefore, cannot establish a causal relationship. The incidence of RLS increases in normal and pathological conditions in which iron deficiency is common and includes pregnancy, gastrointestinal conditions that interfere with iron absorption, and end-state renal disease (ESRD). Our study did not have data on pregnancy or end

stage renal disease, and therefore is limited in its true representation of RLS. If these data were available, it seems plausible that the direction of the current results would remain, but the magnitude might be altered

This study estimated the costs associated with iron testing, treatment and management on an assumption that iron deficiency remarkably increases severity of symptoms. Up to the point where patients are engaged in seeking additional medical care to relieve the distress. While there is some data to suggest this may be true, on the contrary, this analysis demonstrated that iron deficiency is not associated with RLS signs, symptoms, and severity. The accuracy of these costs along with the subsequent cost analysis should be taken cautiously.

Future Research

It is striking to note that 25-30% of pre-menopausal (≤45 years of age) Icelandic women have low serum ferritin [67] while also a high prevalence of RLS amongst women in Iceland [67]. Taken together with the four at-risk gene variants being common and conferring large risks (odds ratios = 1.5–2.3) begs the question if it would be possible to develop a screening strategy that can meet higher statistical thresholds of sensitivity, specificity, and predictive values. With 2 common features, prevalent amongst the population warrants further assessment into other discriminative factors to identify these individuals, such as the use of other iron parameters alone or in combination with the findings from this study.

Future research would include data on pregnancy and ESRD patients because their compromised iron status might lend to further characteristics that are important for screening for iron deficiency in RLS patients. In addition, comprehensive and an exhaustive estimation of costs derived from clinical data would further improve the study's objectives.

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