1. Introduction

Several studies have linked restless legs syndrome (RLS) to problems with insufficient iron both systemically [1] and in the brain [2–4]. Moreover, systemic iron deficiency has been found to be common among RLS patients. Both Ekbom and Nordlander noted a high frequency of iron deficiency among RLS patients [5,6]. The obverse also occurs with reports of a high frequency of RLS among patients with iron deficiency. One study reported 40% of patients with iron deficiency had RLS [1], a percentage substantially higher than the usual population prevalence of 5–10% [7–9]. Reduced peripheral iron stores not only increase the risk of RLS but are also associated with increased severity of RLS symptoms [10,11]. Conditions that compromise iron stores should, therefore, be associated with both increased risk of RLS and increased severity of RLS symptoms. The standard blood donation of one pint of blood will at least temporarily reduce iron stores, potentially increasing the risk of RLS. Repeated blood donations have been reported to produce significant iron deficiency in 10–15% of donors [12,13]. Results from two recent studies appear to indicate that iron loss from frequent blood donation depletes iron stores enough to cause RLS. In one clinical series, frequent blood donors were found to have RLS that could be relieved by reducing the frequency of donations [14]. A Scandinavian epidemiological study found that the frequency of those reporting RLS symptoms was high in blood donors, including as many as one quarter of female donors [15]. However, neither study used a control population, an adequate sample of the blood donor population or a well-standardized method for making the RLS diagnosis. An unpublished Polish study comparing blood donors to controls did find evidence of higher RLS amongst the blood donors, but in this context the average number of blood donations was 3.8 times per year over the past 7.9 years [16]. Thus these studies fail to clearly demonstrate that blood donation itself significantly increases the risk of RLS, provided that the maximum permitted rate of donating is limited to three times per year as in the UK. Moreover, the studies fail to clearly relate RLS occurrence to the amount of blood donation, a critical test for discriminating a causal relation from a selection bias.

To more carefully explore the relation of blood donation to RLS, we developed a donor-completed questionnaire to diagnose RLS using a previously validated question set[17], validated a modified version of the questionnaire on the blood donor population [18] and then sought to obtain a consecutive sample of blood donors...
at the time of the visit to donate blood. We hypothesized that indices of greater levels of blood donation—including both the total number of donations over a lifetime and frequency of donation—should be directly related to the frequency of RLS symptoms. To test this hypothesis, we compared both donor self-report of the number of blood donations and official records of donation histories with likelihood of donors reporting RLS symptoms. We also examined whether specific groups of donors who were more likely to have decreased iron stores would show a higher frequency of RLS.

2. Methods

2.1. Subjects

Subjects were all drawn from three blood donation units in the Cambridge England area. One was a permanent unit at a large hospital in Cambridge City, and two others were mobile units that collected blood from the city as well as its surrounding towns and villages. Posters and questionnaires were left for the blood donors in the recuperation areas where refreshments were consumed after blood donation. Information was available explaining the study, and donors were invited to complete a questionnaire before leaving the blood donation unit. Nurses and Donor Carers were asked to mention the study to blood donors and to encourage them to take part.

Computation of a response rate is not possible; on some occasions the mobile units forgot to take the research materials with them, on other days they were not displayed due to high workloads or staff shortages. But the basic proposed within-sample population analyses protect against bias introduced by self-selection to complete the questionnaire.

2.2. Data sources

The Cambridge-Hopkins RLS diagnostic questionnaire (CH-RLSq) used in this study contained a number of questions to enable diagnosis of RLS. In addition, demographic information was collected on age, gender, diet (vegetarian or vegan), height and weight, and donors were asked on how many occasions in the past they had donated blood. Because of the way in which donors are rewarded with bronze, silver and gold medals, donors can usually provide this information accurately.

Records on the national database were linked to the patient’s questionnaire data. The database provided the dates of past blood donations, as well as other information such as whether donors had failed the anaemia test administered on each visit before blood donation. Records were available for 1933 of the respondents. However, this database was incomplete and imperfect. For instance, habitual donors who moved location might have been entered as a new case in the dataset. Coverage before April 1996, whilst card index records were in use, is incomplete. For this reason, statistical tests reported here have been carried out using records and self-report blood donation obtained after April 1996. There were no systematic differences in the correlations with RLS frequency between these three different sets of blood donation variables (i.e., post-1996 National Blood Service [NBS] data, all NBS data and self-report data), increasing confidence that our results are not an artefact due to measurement error of blood donation histories.

2.3. Measures of frequency of blood donations

Little evidence is available from previous studies concerning the patterns of blood donations that might increase the risk of RLS. Therefore, a number of variables were created to measure the frequency of giving blood:

- The total number of blood donations (self-report).
- The overall frequency of blood donations since the first donation (derived from self-report).
- The total number of blood donations (based on NBS records).
- The overall frequency of blood donations since the first donation (NBS records).
- The maximum number of blood donations in any 1-, 2-, 3-, 5- or 8-year period (NBS records).
- The minimum period of time that would cover any 2, 3, 5 or 8 donations (NBS records).

In one type of blood donation, leukapheresis, only white blood cells are extracted from the donor’s blood. The remainder flows directly back into the donor’s body. A small quantity of blood is outside the body at any time to enable the white blood cells to be extracted. NBS records of donations of this type have not contributed to any of the measures of blood donation used in this study. Some of the respondents’ self-reports include donations of this type. In the questionnaire, no reference was made to this apheresis type of donation. Thus, inclusion of these sessions in self-reports was at the option of the respondents. The leukapheresis donations are, in any case, only a small fraction of blood donations in this sample (6.8%). Furthermore, this group had the same frequency of RLS as the whole-blood donors, so our results have not been biased by this small sub-group of leukapheresis donors.

2.4. Diagnosis of RLS

The diagnosis of RLS for each respondent was based on their responses (“positive” or “negative”) to seven of the questionnaire items. These items covered the 4 basic diagnostic criteria for RLS [19] and also excluded mimics of RLS related to leg cramps or positional discomfort. This questionnaire was validated comparing its results to that of the Hopkins Telephone Diagnostic interview [20] conducted by 2 RLS experts. This validation was made for a random sample of 185 of these donors stratified for balance by the number of RLS positive questions answered. The questionnaire had a positive predictive value of 87.2%, sensitivity of 87.2% and specificity of 94.4% for identification of subjects with a clinical diagnosis of RLS [18].

In this study we attempted to separate out those RLS patients more typical of the clinical population from those generally not seen in a clinical setting. The clinical type of RLS was defined as presenting with sensory symptoms as well as an urge to move that occurred while lying down or sleeping. For this study we refer to this as “definite RLS.” Those with symptoms almost exclusively while sitting or with no symptoms aside from an urge to move when lying down were seen as differing from our experience with clinical populations. They may represent either a milder form of RLS or a somewhat different phenotype. This distinction was driven by the importance of relating our findings both to clinical significance and also to the more theoretical issue of the relation of iron to any RLS symptoms. Thus for this study we spent the effort to separate these two populations. To be very thorough, we report here the results from all diagnoses of RLS and also from these two sub-populations: clinically typical RLS which we refer to as definite RLS and this possibly milder or subclinical RLS which we refer to here as probable RLS.

2.5. Statistical analyses

The demographics of rate of RLS occurrence in relation to gender and age (over and under the age of 40) were evaluated using a $\chi^2$-statistic. The occurrence of RLS in relation to overall age,
height, weight, and BMI were evaluated both using standard regressions and multiple regression and ANOVA models as appropriate for the distributions of data. The relation between the measures of frequency of blood donations and occurrence of RLS were evaluated using logistic regression with correction for subject variables found to be related to RLS occurrence. This was evaluated both for the entire population and for special populations considered to possibly be more vulnerable to RLS. These analyses were obtained for both the combination of definite and probable RLS vs. not-RLS and for definite RLS vs. not-RLS.

3. Results

3.1. Demographic incidence of RLS

In total, 2005 questionnaires were completed and returned between 28 October 2003 and 14 Feb 2004. Of the 2005 respondents, 1988 could be diagnosed satisfactorily on the basis of their responses. Of these, 27% were classified as RLS.

Rates of RLS in different demographic groups have been compared. Table 1 shows the proportion of RLS by several of the demographic variables.

As expected, a clear effect for sex is evident, with 22.9% of women and 9.9% of men diagnosed as definite RLS ($\chi^2 = 59$, df = 1, $p < .0005$). Within genders neither height nor weight are significantly correlated with RLS ($\chi^2 < 1.6$). However, among female respondents (though not among males), higher body mass index (BMI) correlated significantly with more RLS. The interaction of sex and BMI was statistically significant on the 0.05 level in a log-linear model of definite RLS.

An age effect is also evident. Of the sample aged 40 or more years 20.7% were diagnosed as definite RLS, compared with 11.6% of younger persons ($\chi^2 = 1$, df = 1, $p < .0005$). RLS diagnosis is weakly correlated with age among men ($\chi^2 = 5.0$, df = 1, $p = .033$). The correlation of age and RLS diagnosis among women is relatively strong ($\chi^2 = 24$, df = 1, $p < .0005$). With sex and age in a log-linear model, the interaction of age and gender is statistically significant.

The 6% of the sample who are vegetarian had a lower rate of definite RLS diagnosis from the rest of the population, but this effect was not statistically significant.

3.2. RLS and blood donation

A logistic regression was used to model the effect of donating blood on RLS diagnosis. A strong correlation exists between older age and more times that blood was donated; so, given the correlation of age and RLS (as described in Demographic incidence of RLS), the analyses controlled for age to avoid a spurious relation between blood donation and RLS. Sex is strongly correlated with RLS so the analyses also controlled for this factor. These analyses were analysed by grouping the definite and probable diagnostic categories and contrasting them with the not-RLS category. Secondly, the probable RLS group was omitted, and the definite RLS category was compared with the not-RLS group.

These logistic regression analyses were repeated using all of the different measures of the number of times and frequency of giving blood. In no case was there any evidence of increased (or decreased) risk of RLS being associated with giving blood.

Additional material provided online shows a sample SPSS output of a logistic regression model for one of the measures of blood donation based on NBS records (the number of blood donations since the first donation). The results are typical of models involving measures of blood donation based on NBS records. Table 2 summarises the contribution of each blood donation variable within the logistic regression models.

The use of the more accurate data from NBS records since 1996 had only a slight and inconsistent effect on correlations.

None of the interactions of blood donation variables and age and sex were significant in a model already containing their main effects. Consequently, no significant differential effect between men and women or between different age groups is to be expected. The rate of definite RLS diagnosis is almost 20% greater for women than men aged over 37. None of the six groups defined by the age groups (17–37, 38–49, 50–70) and sex and age and frequency of blood donation is 159. The groups with fewest cases are the youngest age group with more than 8 donations and the oldest age group with 8 or fewer donations. These groups have between 82 and 85 cases for both sexes.

Therefore, there is no evidence of an effect of the frequency or number of blood donations on RLS for the whole sample. But one might expect to find a relationship among certain sub-groups of the population. Accordingly, statistical tests have been carried out on data from the following “vulnerable” groups:

- Vegetarians (126 cases).
- People of low weight (60 kg or less; 297 cases).
- People who have at least once failed the test for anaemia carried out on prospective blood donors prior to each session (292 cases).
- Young adults (aged 25 or under; 208 cases).

Again a logistic regression model was used, controlling for sex and age, with each form of RLS diagnosis and with each measure of blood donation. Again, no significant indications of increased risk of RLS as a result of either frequent or sustained blood donation were found. In fact, among respondents who had failed the test for anaemia, the blood donation was significantly associated with a reduced risk of RLS for 16 out of the 28 measures of blood donation (one for each combination of the various blood donation variables and the two types of RLS diagnosis: Wald >4, 0.01 <sig <0.05). For the other groups, blood donation was not a statistically significant factor.

A large number of additional exploratory analyses were performed to examine whether there were any other more complex relationships between the number and frequency of donations or if donations were in any way related to the risk of RLS. These included comparison of the 109 first-time blood donors with the rest of the sample. None of these analyses gave any suggestion of any significant relationship, positive or negative, between blood donation and RLS.

4. Discussion

Contrary to expectations raised by prior studies of RLS in blood donors, we not only failed to confirm our primary hypothesis of an association between risk of RLS and frequency of blood donations but actually found a slight indication for decreased risk of RLS with increased blood donations. This tendency occurred even when comparing first-time donors to those who had donated more and occurred for both males and females separately. Thus, we conclude from this study there is no evidence of any relationship between frequency and number of blood donations and risk of RLS in this UK sample. As this conclusion is at odds with the two previous published papers on this topic, it is instructive to examine in more detail these two previous studies.
Silber and Richardson reported on a very small sample of eight RLS patients who were frequent blood donors [14]. Self-report severity of RLS decreased when they were instructed to cease donating blood. There was no control group, and although the clinicians involved were RLS experts they did not report using a standardized or validated measurement of diagnosis or severity, and with such a small sample, no statistical analysis. Furthermore, for some of their patients, they had been giving blood up to six times per year, whereas the maximum in the UK is three times a year. Moreover, using subjective reports of RLS symptoms to evaluate treatment benefits is known to produce a large placebo effect [21–24], and the Silber & Richardson study would have been prone to these effects.

Ulfberg and Nystrom [15] found prevalence of RLS in their sample of Swedish blood donors that was much higher than what had been found in general population surveys of RLS in Sweden, but different diagnostic instruments were used in each of these studies. More importantly, they did not show any relationship between the number of blood donations and occurrence of RLS in this UK population compared those with RLS to ones without. The results showed that a history of more than five lifetime blood donations was significantly more likely for men [25] with RLS than men without RLS. This was not a significant factor for women. This study was, however, a retrospective recall of family members of patients diagnosed with RLS; given the iron issues for RLS patients it is likely that some discussion about iron may contribute to remembering blood donations. The recall of blood donation was not verified and there was no information about the frequency of the blood donation. The blood donations in the United States are encouraged to be as much as every other month and are not limited to 3 per year. It seems likely that this American sample contained several cases sn Wald Sig

<table>
<thead>
<tr>
<th>Variable(s)</th>
<th>Demographic group</th>
<th>RLS diagnosis</th>
<th>Total non-missing, diagnosable cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Not-RLS (%)</td>
<td>Probable (%)</td>
</tr>
<tr>
<td>All cases</td>
<td></td>
<td>73</td>
<td>9.9</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>66</td>
<td>11.4</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>82</td>
<td>8.0</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 40 years</td>
<td></td>
<td>79</td>
<td>9.3</td>
</tr>
<tr>
<td>40 years or more</td>
<td></td>
<td>69</td>
<td>10.7</td>
</tr>
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<td>Sex and age</td>
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<td></td>
<td></td>
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<td>Female, less than 40 years</td>
<td>75</td>
<td>10.0</td>
<td>15.2</td>
</tr>
<tr>
<td>Female, 40 years or more</td>
<td>60</td>
<td>12.3</td>
<td>28.0</td>
</tr>
<tr>
<td>Male, less than 40 years</td>
<td>85</td>
<td>8.4</td>
<td>6.9</td>
</tr>
<tr>
<td>Male, 40 years or more</td>
<td>81</td>
<td>7.6</td>
<td>11.5</td>
</tr>
<tr>
<td>Sex and body mass index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female, less than 27 kg/m²</td>
<td>68</td>
<td>10.8</td>
<td>20.9</td>
</tr>
<tr>
<td>Female, 27 kg/m² or more</td>
<td>59</td>
<td>11.8</td>
<td>28.8</td>
</tr>
<tr>
<td>Male, less than 27 kg/m²</td>
<td>82</td>
<td>8.2</td>
<td>9.4</td>
</tr>
<tr>
<td>Male, 27 kg/m² or more</td>
<td>81</td>
<td>8.1</td>
<td>10.8</td>
</tr>
<tr>
<td>Diet</td>
<td></td>
<td>72</td>
<td>10.1</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td>80</td>
<td>7.1</td>
</tr>
</tbody>
</table>

Table 2 Contributions of blood donation variables to binary logistic regression models with sex and age included. All variables are derived from NBS records unless otherwise stated. The sign (sn) indicates the tendency of high values of the variable to produce negative RLS diagnosis (−) or positive RLS diagnosis (+).

<table>
<thead>
<tr>
<th>Blood donation variable</th>
<th>Cases</th>
<th>sn</th>
<th>Wald</th>
<th>Sig</th>
<th>Definite vs. not-RLS</th>
<th>Cases</th>
<th>sn</th>
<th>Wald</th>
<th>Sig</th>
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</thead>
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<tr>
<td>Number of donations</td>
<td>1907</td>
<td>1</td>
<td>0.0</td>
<td>.97</td>
<td>1721</td>
<td>1.0</td>
<td>.33</td>
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<td></td>
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<tr>
<td>Frequency of donations</td>
<td>1815</td>
<td>0.9</td>
<td>0.33</td>
<td>.19</td>
<td>1640</td>
<td>0.8</td>
<td>.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of donations</td>
<td>1896</td>
<td>1.6</td>
<td>0.20</td>
<td>.25</td>
<td>1705</td>
<td>1.3</td>
<td>.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max. no. of donations</td>
<td>1759</td>
<td>2.4</td>
<td>0.12</td>
<td>.26</td>
<td>1588</td>
<td>1.3</td>
<td>.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max. no. in 1-year period</td>
<td>1961</td>
<td>0.1</td>
<td>0.81</td>
<td>.98</td>
<td>1767</td>
<td>0.0</td>
<td>.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max. no. in 2-year period</td>
<td>1896</td>
<td>0.7</td>
<td>0.93</td>
<td>.93</td>
<td>1705</td>
<td>0.9</td>
<td>.93</td>
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<td></td>
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<tr>
<td>Max. no. in 5-year period</td>
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<td>0.32</td>
<td>.25</td>
<td>1705</td>
<td>0.3</td>
<td>.25</td>
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<tr>
<td>Max. no. in 8-year period</td>
<td>1896</td>
<td>2.1</td>
<td>0.14</td>
<td>.19</td>
<td>1705</td>
<td>1.7</td>
<td>.19</td>
<td></td>
<td></td>
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<tr>
<td>Max. no. in 12-year period</td>
<td>1896</td>
<td>2.0</td>
<td>0.16</td>
<td>.20</td>
<td>1705</td>
<td>1.6</td>
<td>.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum time for any 2 donations</td>
<td>1759</td>
<td>0.1</td>
<td>0.73</td>
<td>.37</td>
<td>1588</td>
<td>0.0</td>
<td>.85</td>
<td></td>
<td></td>
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<tr>
<td>Maximum time for any 3 donations</td>
<td>1629</td>
<td>0.5</td>
<td>0.46</td>
<td>.47</td>
<td>1470</td>
<td>0.5</td>
<td>.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum time for any 5 donations</td>
<td>1380</td>
<td>3.7</td>
<td>0.054</td>
<td>.054</td>
<td>1257</td>
<td>3.7</td>
<td>.054</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum time for any 8 donations</td>
<td>1063</td>
<td>0.0</td>
<td>0.98</td>
<td>.77</td>
<td>955</td>
<td>0.1</td>
<td>.77</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
days (17 months), so the average time between donations is just under 6 months. The prior studies involved more frequent donations, up to six per year. It has been reported that full recovery of iron stores takes 2 to 4 months after donating a pint of blood [12]. Limiting donations to three a year may, therefore, not produce the significant challenge to the iron stores required for the level or type of iron deficiency that engenders RLS, while more frequent donations could. This raises both an interesting theoretical issue regarding RLS and iron deficiency and an important public safety issue. The theoretical issue concerns the question of persistence and nature of the iron challenge that would be related to RLS. The impact of both degree and also intermittent vs. persistence of iron deficiency and the interaction with subject variables need to be better explored. The important public safety issue would be to establish the frequency of blood donation, if any, within the currently accepted limits that would increase the risk of RLS. This requires evaluating the risk of RLS in a population permitting more frequent donations using validated measures similar to those in this study.

This study was designed to evaluate our hypothesized relation between frequency of donation and RLS and not to determine RLS prevalence in the blood donor population. Such a prevalence study would require close attention to obtaining random sampling of the population of blood donors. In contrast, the frequency relation to RLS can be answered by any convenience sample, provided there is no differential participation bias involving the interaction of donation frequency and RLS occurrence, i.e., more frequent donors with RLS were more likely to volunteer than less frequent donors with RLS. It is hard to imagine how such a complicated bias could occur.

Still, one puzzle in this study was the higher-than-expected prevalence of RLS in our blood donor population than in other recent surveys of the general population [9,26]. There are several possible explanations for this. For instance, it may be that there is a higher-than-average prevalence of RLS in the Cambridge area, or, alternatively, those who volunteer to donate blood even once have a higher-than-average prevalence of RLS. This study was specifically designed for within-sample analyses for effects of frequency of donation that would not require a control group. We, therefore, did not establish a control group and cannot compare the RLS prevalence here with the local general population. A more plausible explanation for the observed high prevalence of RLS might be that those blood donors who experienced symptoms of RLS were more likely to complete the questionnaire. To comply with the ethical standards required by the NBS, blood donors were told in advance that the questionnaire was being administered to determine whether unpleasant leg sensations were linked to donating blood. This may have caused those with RLS to have had a higher response rate than those who had not experienced RLS symptoms. We also did not evaluate RLS severity or duration, so subjects with RLS starting at any time of life could have responded to the request to complete the questionnaire. This could account for the unexpectedly high rate of RLS in this study.

This raises an important question as to whether or not our method of sampling and the possible associated response bias towards those with RLS could have produced a further interactive association such that novice donors with RLS were even more likely to complete the questionnaire than experienced donors with RLS. If this were the case, it might have masked a causal link between blood donating and RLS. We could not find a possible explanation for how our methods would lead a person with RLS symptoms to be more likely to answer the questionnaire if they were new vs. a more frequent blood donor. Without any possible explanation of how such a specific confounding relationship might have come about, we conclude that the only explanation for our findings is that there is no relationship between the frequency and number of blood donations in an environment where the donations are limited to no more than 3 per year.

Also, while our questionnaire was similar to others that have been used to ascertain RLS, there are certain critical differences, including the requirement of some studies that subjects complain not only of an urge to move, but also of discrete sensations [9,26]. That may have depressed the frequency observed in population studies. However, the Instant Study [26] evaluated this effect and found it at most produced a 10% decrease in the prevalence, producing prevalence rates somewhat less than 1% lower.

There is one other incidental finding that is worthy of brief note: for women, there was a correlation of BMI with frequency of RLS diagnosis. But unfortunately this study cannot inform us of the direction of causation—for instance, it could be the case that those with higher BMI are more vulnerable to RLS, or alternatively that RLS encourages “comfort eating” as a way of reducing or coping with the unpleasant limb sensations, or this could be a consequence of sleep loss occurring with RLS.

The conclusions reached in this study could have been strengthened with modifications to the method. For instance, a longitudinal prospective study could have followed new donors through their first, say, 10 donations and monitored their RLS along with their serum ferritin and other iron levels before and after each bleed. The frequency of donations could be manipulated in a randomized design, and a control group of non-donors could also be incorporated. Such a study would take several years and would be costly. Until then, this paper is the best evidence we have for the relationship between RLS and the frequency of blood donations.

Because this study was unique in using a questionnaire validated in the same population that was studied, we believe that there can be greater confidence in our results than there would be in other studies, almost none of which have used a validated means of ascertaining those with RLS. We also had excellent data on donating frequency. We therefore feel we can be confident in the primary finding of this study that no relation exists between blood donation frequency at rates of no greater than 3 times per year and the occurrence of RLS.

Acknowledgements

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Appendix A. Supplementary data


References


