

PEER REVIEW HISTORY

This paper was submitted to the BMJ but declined for publication following peer review. The authors addressed the reviewers' comments and submitted the revised paper to BMJ Open. The paper was subsequently accepted for publication at BMJ Open. The comments of the BMJ reviewers are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Restless legs syndrome and risk of incident cardiovascular disease in women and men: prospective cohort study
AUTHORS	Anke C. Winter, Markus Schürks, Robert J. Glynn, Julie Buring, J Michael Gaziano, Klaus Berger and Tobias Kurth

VERSION 1 - REVIEW

REVIEWER	Giancarlo Logroscino, Harvard School of Public Health
REVIEW RETURNED	10/10/2011

GENERAL COMMENTS	<p>1. Introduction: it is not clear how the hypotheses of sympathetic hyperactivity may rise from a clinical study. The statement is not clear (ref 15).</p> <p>2. Was the diagnosis of RLS validated within these cohorts? The MEMO study (ref 22 for validation) is a cross-sectional study in southern Germany where all subjects were examined neurologically and interviewed in person using standardized questions addressing the four minimal criteria for RLS. The WHS and PHS are based on mailed questionnaire. Is this relevant for the assessment? May the presence of neurological comorbidity be relevant?</p> <p>3. The lack of information on duration of the RLS may undermine some of the results of the study.</p> <p>4. The presentation as the results of sensitivity analyses with the statement "the association remained insignificant " with CI 0.99 to 2.05 is not convincing.</p> <p>5. No discussion on the results on stroke is present.</p>
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REVIEWER	John Gallacher, University of Wales College of Medicine
REVIEW RETURNED	28/10/2011

GENERAL COMMENTS	<p>This analysis is of observational data from two trials. The paper is of significance to clinicians as it clarifies the ambiguity surrounding the clinical importance of RLS, a frequently reported symptom. It makes a significant contribution to the debate on the association between restless legs syndrome (RLS) and vascular disease; adding to the single other longitudinal study reported to date.</p> <p>The design of the studies is critical in providing longitudinal data. The studies are sufficiently large, have been rigorously conducted and appropriately analysed. It would be helpful for the authors to clarify in the PHS analysis, for the 7641 PHS II participants who also participated in PHS I, (presumably completing RLS questions in</p>
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	<p>both) which were used in the analysis. That the follow-up period is slightly shorter and the population more healthy than the previous study are not significant objections to the author's interpretation of their findings.</p> <p>The authors' interpretation of the findings is inconsistent. The findings are interpreted to show that RLS is not implicated in the causal pathway to vascular disease, but may be an indicator of poor health, including vascular health. This is entirely reasonable. However, their application is less clear. If RLS is a marker for vascular co-morbidities, then it is a marker for increased risk of vascular disease, contrary to the last sentence of the abstract. The solution is to make clear the distinction between a marker for disease risk and a determinant of disease risk. The double negative in the first sentence of the clinical implications section compounds this difficulty. This section should be re-written to state more clearly the authors' view.</p> <p>This is an important paper as it clarifies the ambiguity surrounding the clinical significance of RLS.</p> <p>I recommend its publication once clarifications have been made.</p>
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VERSION 1 – AUTHOR RESPONSE

We appreciate the detailed and thorough comments of the associate editor and reviewers. We respond point-by-point and to the concerns raised by the reviewers. We have repeated the comments of the reviewers in italics before our response and indicate where we have made changes in the text of the paper, where appropriate.

Detailed comments from the BMJ meeting:

We thought this a well written and carefully analyzed study.

However, our main concern was that RLS is not sufficiently well known and the association with CVD not widely known about. As your paper reports a negative finding for a rare association that most readers will not know existed, overall we think it better suited to a specialty journal.

Response: Numerous cross-sectional studies have evaluated the association between RLS and CVD and reported positive association. Although the conclusions that can be drawn from cross-sectional studies are limited and a causal link between RLS and CVD cannot be established with this study design, these results have caused a lot of attention. Given the high prevalences of both RLS and CVD in the general population, a potential association would be relevant and have major public health consequences.

We think that the populations in these cohorts are probably not representative of patients who consult a doctor with the problem of RLS.

Response: While RLS may not appear to be an important issue in a general medicine practice, numerous population-based studies have shown that RLS is a very prevalent condition. Many patients

with minor RLS symptoms do not seek medical care and only severe cases are treated by physicians. However, RLS is an important disease in the general population and evaluating risk factors and comorbidities of RLS on a more general population level have implications for the RLS populations seen by primary care physicians as well.

Our statistician had no major concerns about your paper except the handling of the missing data. You have used a missing value indicator method that has been shown in numerous studies to almost always result in biased estimates, so wonders why you did not opt for the multiple imputation method.

Response: While we understand the comment of the statistical editors, we believe that there are advantages and disadvantages to all methods dealing with missing data. We have carefully outweighed advantages and disadvantages of every single approach and decided to use the missing value indicator method for the following reasons. We have excluded missing information for our exposure and outcome of interest and have only used the missing value indicator approach for the covariate information. We have decided to choose this approach since a) with this approach we are only using real data and do not make any assumptions about the distributions of the covariates and the mechanisms led to the missing data (as in the multiple imputation method) b) the sample size/power/generalizability for the covariate with missing data and for the other covariates is at the maximum for the actually collected 'real' data. The effect estimate for the covariate could be biased if the reason that data are missing is related to the outcome and to the predictor. In addition, the effect estimates of other covariates may be biased. However, since we have chosen to include confounders based on a priori knowledge and not chosen any data driven selection algorithms, we consider this disadvantage of our missing data approach as negligible. Furthermore, the total number of missing covariate information in our cohort is fairly small (e.g. maximum of 4%). Thus, we believe that our approach is not biasing our analyses.

Reviewer Comments:

Reviewer: 1

1. Introduction: it is not clear how the hypotheses of sympathetic hyperactivity may rise from a clinical study. The statement is not clear (ref 15).

Response: Small clinical based studies have evaluated patients with periodic limb movement disorder (PLMS) and observed PLMS-related changes in heart rate and blood pressure. These changes were associated with EEG microarousals. Based on these observations it has been hypothesized that PLMS may be associated with sympathetic nerve hyperactivity. Since PLMS can occur in patients with RLS, it has been discussed that these transient elevations in heart rate and blood pressure occurring during the night may increase the risk for developing CVD events in patients with RLS. However, we believe that this information is too specific as introduction for this paper and we have simplified the section in the introduction.

2. Was the diagnosis of RLS validated within these cohorts? The MEMO study (ref 22 for validation) is a cross-sectional study in southern Germany where all subjects were examined neurologically and interviewed in person using standardized questions addressing the four minimal criteria for RLS. The

WHS and PHS are based on mailed questionnaire. Is this relevant for the assessment? May the presence of neurological comorbidity be relevant?

Response: The issue on validation of RLS is not easy to answer. Based on the criteria established by the International Restless Legs Study Group (IRLSSG), RLS is defined by self reports and there is no diagnostic test confirming this disease. In two studies, self reported information has been validated against a physician's diagnosis (who is also referring to the criteria established by the IRLSSG). We have applied the identical 3-item RLS questionnaire which has been used and validated in the cited cohorts from Germany and Italy. In our methods paragraph outlining the RLS assessment (page 7), we cite two different RLS studies using data from the MEMO cohort. As mentioned by the reviewer, two trained physicians assessed RLS by using the 3-item standardized questionnaire in the study by Rothdach et al (reference 22). Along with references 20-21, we cite reference 22 in the context of studies that have used and established the 3-item questionnaire we have assessed our RLS cases with. In the second study using data of the MEMO cohort (reference 23), Berger et al. performed a validation of this questionnaire comparing the RLS classification based on the questionnaire against a RLS diagnosis of an examining neurologist, yielding a high degree of concordance ($\kappa=0.67$). The statistical properties of the 3-item questionnaire were also very good with a sensitivity of 87.5% and a specificity of 95.6%. As our studies are very large, we obviously cannot validate the RLS self-reports against a physician diagnosis. However, the main aspect of a physician's diagnosis of RLS is to rule out other conditions that can mimic RLS symptoms, in particular diabetes and peripheral neuropathy. We have information available about a long list of potential co-morbid conditions that could result in RLS mimics and we have performed careful sensitivity analysis and reported the results (which are similar to our main findings). As both of our cohorts are composed of health professionals that have been shown to very accurately provide health related information, we believe that our sensitivity analyses sufficiently address potential misdiagnosis/categorization of RLS.

3. The lack of information on duration of the RLS may undermine some of the results of the study.

Response: Results of a previous study evaluating the association between RLS and CVD do not indicate an association between duration of RLS symptoms and CVD.¹ Thus, we believe that this is unlikely a major issue in our data.

4. The presentation as the results of sensitivity analyses with the statement "the association remained insignificant" with CI 0.99 to 2.05 is not convincing.

Response: When comparing the main effects, the results are not very different. Based on the a priori chosen alpha cutoff, the results of the sensitivity analysis are not significant. However, we have removed that statement that the association remained insignificant.

5. No discussion on the results on stroke is present.

Our study shows no association between RLS and major CVD as well as the individual endpoints. There is a suggestion from our sensitivity analysis that the risk of total stroke may be increased ($RR=1.42$ (0.99 to 2.05)). We have now included brief statements about this fact when discussing the

positive result of a Swedish study (on page 12). However, we believe that this finding is only a suggestion and we do not want to put a main focus on results of secondary analyses that do technically not reach the significance level.

Reviewer: 2

This analysis is of observational data from two trials.

The paper is of significance to clinicians as it clarifies the ambiguity surrounding the clinical importance of RLS, a frequently reported symptom. It makes a significant contribution to the debate on the association between restless legs syndrome (RLS) and vascular disease; adding to the single other longitudinal study reported to date.

The design of the studies is critical in providing longitudinal data. The studies are sufficiently large, have been rigorously conducted and appropriately analyzed.

It would be helpful for the authors to clarify in the PHS analysis, for the 7641 PHS II participants who also participated in PHS I, (presumably completing RLS questions in both) which were used in the analysis. That the follow-up period is slightly shorter and the population more healthy than the previous study are not significant objections to the author's interpretation of their findings.

Response: While participants in both PHS cohorts received the questionnaire containing the RLS questions approximately at the same time, the two cohorts differ somewhat in age and prevalent comorbidities. As we, however, have tested for effect modification by age and other variables and we have found no significant results, we do not believe that these differences have influenced our results.

The authors' interpretation of the findings is inconsistent. The findings are interpreted to show that RLS is not implicated in the causal pathway to vascular disease, but may be an indicator of poor health, including vascular health. This is entirely reasonable. However, their application is less clear. If RLS is a marker for vascular co-morbidities, then it is a marker for increased risk of vascular disease, contrary to the last sentence of the abstract. The solution is to make clear the distinction between a marker for disease risk and a determinant of disease risk. The double negative in the first sentence of the clinical implications section compounds this difficulty. This section should be re-written to state more clearly the authors' view.

Response: Based on the lack of clear biological concept why RLS should lead to CVD, we do not want to argue that RLS is a causal factor (i.e., risk factor). We thus have opted to call RLS a marker of risk similar to other concepts (such as C - reactive protein being a marker of increased risk for CVD). We have edited the text to avoid confusion about this point.

This is an important paper as it clarifies the ambiguity surrounding the clinical significance of RLS.

I recommend its publication once clarifications have been made.

Note: There is a typo in page 7, paragraph 2 line 1 word 1.

Response: We have edited the text.

References

1. Winkelman JW, Shahar E, Sharief I, et al. Association of restless legs syndrome and cardiovascular disease in the Sleep Heart Health Study. *Neurology*. Jan 1 2008;70(1):35-42.