PEER REVIEW HISTORY

This paper was submitted to a another journal from 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.

(This paper received two reviews from its previous journal but only one reviewers agreed to published their review.)

ARTICLE DETAILS

TITLE (PROVISIONAL)	Longitudinal Effect of Nocturnal R-R Intervals Changes on Cardiovascular Outcome in a Community-Based Cohort
AUTHORS	Sankari, Abdulghani; Ravelo, Laurel; Maresh, Scott; Aljundi, Nawar; Alsabri, Bander; Fawaz, Serene; Hamdon, Mulham; Al-kubaisi, Ghazwan; Hagen, Erika; Badr, Safwan; Peppard, Paul

VERSION 1 - REVIEW

REVIEWER	Roche, Frédéric CHU Nord, Physiologie Clinique
REVIEW RETURNED	15-Jan-2019

GENERAL COMMENTS	The paper submitted here tests for the first time the cardiovascular prognostic role of autonomic fragmentation during sleep. The authors relying on the follow-up of a long-standing cohort (and quality with regard to the diagnostic tools used) finds a deleterious effect of the density of strong changes in nocturnal heart rate irrespective of the Hypoxemic burden on the cardiovascular risk of this population. The strengths of the study are: the mode of selection of the cohort (general population of Middle age), the length of the follow-up and the completeness of this follow-up, the search for threshold determination of fragmenting cardiac autonomic events density, the new methodology of quantification of this fragmentation. This is a convincing study but the authors still have to answer some methodological questions and amend the manuscript. The first critical element is the lack of explanation regarding in the end the (relative) small percentage of subjects that may have been included in this study compared to the initial population of the Wisconsin Sleep cohort study. The quality of the recording of the trace ECG was also problematic on the polysomnographies to the inclusion? A second limitation on the inclusion criteria relates to the few cardiovascular events that have occurred in this population: authors must insist on this limit in the application of such results in

	other populations at higher cardiovascular risk (the hypoxemic load can in this case have a more important independent impact). A high proportion of cardiac autonomous awakenings are not related to a nocturnal respiratory event (spontaneous fragmentation, altered awakening threshold, and above all periodic motor pathology during sleep). It can be estimated here that this represents nearly 40% of these autonomic microarousals. Authors should be asked to classify these autonomous events according to the synchronous presence of a EEG arousal. This is all the more important since this fragmentation EEG appears to have little impact on mortality (including cardiovascular) in other cohorts. The choice of the method of detection of autonomous awakenings is poorly justified: it is perfectly understandable that these sympathetic heart burts at the ventilatory recovery are deleterious on the cardiovascular level. However, the method used by the authors is lacking an experimental pharmacological background. Why did the authors not use a classical method of spectral analysis or time-frequency of RR (HRV) variability which is a well validated method of quantifying sympathetic (cardiac) activation? This fragmentation of sleep (whatsoever is its cause) has been associated with other authors at the risk of developing high blood pressure. The authors need to better assess the associated role of this risk factor in this context (stratification) and also associated drug therapies in this cohort (not only beta-blockers medication). It must be possible to dissociate the events autonomous contemporary from the Desaturating events and the others. A separate analysis of each of these indices is indispensable in the survival study presented in my opinion. It is also important to mention that this type of analysis is not possible during a complete arrhythmia, if one has frequent extrasystoles and in case of cardiac pacemaker (limits). The authors should tell us whether this new marker has an equivalent prognostic impact in both men and women: the autonomic response to the same hypoxic stress can be different depending on the gender. Finally in the perspectives they must mention the recent work (Eur Heart J) demonstrating the interest of calculating a hypoxic charge index taking into account the integration of the severity of the desaturation and its length. In this context I am convinced that the addition of an associated autonomic fragmentation would strengthen this parameter. The bibliography on methods of quantification of autonomic fragmentation and its cardiovascular impact must be strengthened (and discussion also). I also noted that in the first chapter of the results the correlative analysis of the 3236 RRI had already been presented in another paper (to be removed I think by keeping the reference)

VERSION 1 – AUTHOR RESPONSE

Reviewer #1:

- The first critical element is the lack of explanation regarding in the end the (relative) small percentage of subjects that may have been included in this study compared to the initial population of the Wisconsin Sleep cohort study.

Response: The incidence of CVD in this population is relatively smaller than what was observed in other high cardiovascular risk population. This is might be due to the inclusion of only those who have no prior history of CVD and not on beta blockers. This issue is clarified in the revised manuscript.

- The quality of the recording of the trace ECG was also problematic on the polysomnographies?

Response: The ECG tracing was not clear in Fig.1 and a clearer representative polygraph is added with magnification to the ECG segment during period of heart rate acceleration corresponding to RRI dips.

- A second limitation on the inclusion criteria relates to the few cardiovascular events that have occurred in this population: authors must insist on this limit in the application of such results in other populations at higher cardiovascular risk (the hypoxemic load can in this case have a more important independent impact).

Response: This issue of low incidence of CVD is clarified now in the limitation.

-A high proportion of cardiac autonomous awakenings are not related to a nocturnal respiratory event (spontaneous fragmentation, altered awakening threshold, and above all periodic motor pathology during sleep)….Authors should be asked to classify these autonomous events according to the synchronous presence of a EEG arousal. ... I also noted that in the first chapter of the results the correlative analysis of the 3236 RRI had already been presented in another paper (to be removed I think by keeping the reference)

Response: The current study did not correlate RRI dips to specific arousals for the whole sample. As mentioned by the reviewer later it was done on a sub sample of studies which is now removed per the reviewer suggestion.

-“This fragmentation of sleep (whatsoever is its cause) has been associated with other authors at the risk of developing high blood pressure. The authors need to better assess the associated role of this risk factor in this context (stratification) and also associated drug therapies in this cohort (not only beta-blockers medication).. “

Response: We agree with the reviewer that sleep fragmentation may play important role in the increased incidence of CVD and development of blood pressure. The model of regression was adjusted for hypertension and other comorbidities. Despite further adjustments the relationship between total RRDI and CVD events remained significant. In this study we excluded not only beta blockers but also other medications that can affect heart rate (see supplement). Adding more restriction to this sample will decrease the size of the study further.

It is also important to mention that this type of analysis is not possible during a complete arrhythmia, if one has frequent extrasystoles and in case of cardiac pacemaker (limits).

Response: In this cohort sample only 2 individuals had pacemaker after the PSG which was counted as an outcome but did not affect the ECG analysis.

The authors should tell us whether this new marker has an equivalent prognostic impact in both men and women.

Response: We added a stratified analysis for men and women to assess the gender effect on RRDI and CVD outcome. We found that there were significant difference between men and women and that RRDI predicted CVD in men but not in women. This data is included in the revised manuscript.

-Finally in the perspectives they must mention the recent work (Eur Heart J) demonstrating the interest of calculating a hypoxic charge index taking into account the integration of the severity of the

desaturation and its length…The bibliography on methods of quantification of autonomic fragmentation and its cardiovascular impact must be strengthened (and discussion also).

Response: We added suggested references and strengthened the bibliography and discussion as suggested.

VERSION 2 - REVIEW

REVIEWER	Frederic Roche Clinical Physiology Dpt, University Hospital, Saint Etienne, France EA 4607 SNA EPIS, Lyon University, Jean Monnet University, Saint Etienne, France
REVIEW RETURNED	01-May-2019

GENERAL COMMENTS	The authors have significantly improved the paper and brought important complementary analyses. The results are interesting and the work merit to be published I believe.

REVIEWER	Luciano Drager Associate Professor of Medicine, University of Sao Paulo Medical School.
REVIEW RETURNED	02-May-2019

GENERAL COMMENTS	This is a revised version of a sub-analysis of the Wisconsin cohort addressing the role of nocturnal R-R intervals dips on CV events. The manuscript is much improved. I have a couple of comments: 1) Please update some references; 2) The authors performed adjustments for hypertension, diabetes, stroke, etc. (page 11). Please review tables 3 to 6. The authors only reported adjustments for age, sex, body mass index, and apnea-hypopnea index.

VERSION 2 – AUTHOR RESPONSE

Specific responses to the reviewers:

Reviewer #1:

- The authors have significantly improved the paper and brought important complementary analyses. The results are interesting and the work merit to be published I believe.

Response: Thank you for your comments and feedback.

-Reviewer 2

1) Please update some references;

Response: We thank the reviewer for insightful feedback. The references are verified and updated.

2) The authors performed adjustments for hypertension, diabetes, stroke, etc. (page 11). Please review tables 3 to 6. The authors only reported adjustments for age, sex, body mass index, and apnea-hypopnea index.

Response: We agree with the reviewer suggestions and have revised the manuscript for more clarified adjustments in table 3-4. For table 5-6 we did not do additional adjustment for hypoxia and other comorbidities in the sub categories during REM and NREM sleep.