Original Research Article

Association between nocturnal heart rate variability and incident cardiovascular diseases: The HypnoLaus population-based study

Short title: Heart rate variability and cardiovascular disease

Mathieu Berger, PhD[1,2], Vincent Pichot, PhD[2], Geoffroy Solelhac, MD[1], Pedro Marques-Vidal, MD, PhD[3], José Haba-Rubio, MD[1], Peter Vollenweider, MD[3], Gérard Waeber, MD[3], Martin Preisig, MD, Jean-Claude Barthélémy, MD, PhD[2], Frédéric Roche, MD, PhD[2], Raphael Heinzer, MD[1]

[1]Center for Investigation and Research in Sleep (CIRS), Lausanne University Hospital (CHUV) and University of Lausanne, Lausanne, Switzerland

[2]SNA-EPIS Laboratory, EA 4607, University Hospital of Saint-Etienne, Saint-Etienne, France

[3]Department of Medicine, Internal Medicine, Lausanne University Hospital (CHUV) and University of Lausanne, Lausanne, Switzerland

[4]Department of Psychiatry, Lausanne University Hospital (CHUV) and University of Lausanne, Lausanne, Switzerland

Corresponding Author: Mathieu Berger, PhD, Center for Investigation and Research in Sleep (CIRS), Lausanne University Hospital (CHUV), Rue du Bugnon 46, 1011 Lausanne, Switzerland. E-mail: mathieuberger@outlook.com

Total manuscript word count: 5286

Abstract word count: 217

Conflict of interest: The authors have no conflicts to disclose.

ABSTRACT

Background: Although heart rate variability (HRV) is widely used to assess cardiac autonomic function, few studies have specifically investigated nocturnal HRV.

Objective: We aimed to assess the association between nocturnal HRV and cardiovascular disease (CVD) incidence over 4 years in a population-based sample.

Methods: 1784 participants (48.2% male, 58±11 years) from the HypnoLaus population-based cohort free of CVD at baseline were included. Polysomnography-based electrocardiograms were exported to analyse time and frequency-domain HRV, Poincaré plots indices, detrended fluctuation analysis, acceleration (AC) and deceleration capacities (DC), entropy, heart rate fragmentation (HRF), and heart rate turbulence. Multivariable-adjusted cox regression analysis was used to assess the association between HRV indices and incident CVD.

Results: 67 participants (3.8%) developed a CVD over a mean follow-up time of 4.1±1.1 years. In a fully adjusted model, AC (hazard ratio per one SD increase [95% confidence interval]: 1.59 [1.17-2.16]; p=0.004), DC (0.63 [0.47-0.84]; p=0.002) and HRF (1.41 [1.11-1.78]; p=0.005) were the only HRV metrics significantly associated with incident CVD events after controlling for false discovery rate.

Conclusion: Nocturnal novel HRV parameters such as AC, DC and HRF are better predictors of CVD events than time and frequency traditional HRV parameters. These findings suggest a form of dysautonomia and fragmented rhythms but further experimental studies are needed to delineate the underlying physiological mechanisms of these novel HRV parameters.

Key Words: heart rate variability; heart rate fragmentation; cardiovascular disease; electrocardiogram; sleep; prospective study.

INTRODUCTION

Sleep is a complex homeostatic and circadian function during which autonomic nervous system (ANS) regulation is largely independent of external stimuli and therefore provides a reasonable representation of basal autonomic regulatory equilibrium.[1] For over three decades, analysis of heart rate variability (HRV) has been proposed as a non-invasive and accessible tool to evaluate cardiac autonomic function (CAF) in health and disease.[2] In practice, normal beat-to-beat intervals are extracted from the electrocardiogram (ECG) and numerous HRV indices can be calculated using time-domain, frequency-domain and non-linear analysis.[3-5] These indices provide information about sympathetic and parasympathetic modulations as well as randomness of heart rate.[3, 4] Several epidemiological studies have shown that altered HRV is an independent predictor of cardiovascular outcomes and all-cause mortality.[6-8]

Despite this, HRV is sometimes criticized due to its lack of reproducibility and reliability if standardized procedures are not utilized. To improve reproducibility, HRV indices are usually calculated from 24-h ECG Holter monitoring while participants perform their usual daily activities[3] but Holter monitoring is mostly used by cardiologists. Moreover, unrestricted activities for the same participant may vary within and between days, which may have unpredictable effects on 24-h HRV parameters and thus decrease reproducibility.[9] HRV can also be acquired during daytime using short-term recordings, but methods of measurement need to be highly standardized (position, breathing pace, recording time, etc.) and short-term measurements have lower predictive power than 24-h recordings.[4, 10, 11]

Recently, sleep has been proposed as a highly standardized condition to time-efficiently measure HRV in a setting that is less influenced by environmental factors compared with daytime measures.[12] However, although the association between 24-h HRV and cardiovascular events is well established, it is still largely unknown whether nocturnal HRV metrics can predict the occurrence of cardiovascular diseases (CVD) events.

Thus, the primary objective of this study was to determine whether nocturnal HRV indices can predict the incidence of fatal and non-fatal CVD events over a 4-year follow-up period.

METHODS

Study Population

The HypnoLaus study[13] is a nested study of CoLaus|PsyCoLaus,[14, 15] a prospective cohort study including 2,162 participants randomly selected according to the civil register of the city of Lausanne (Switzerland) who completed an ambulatory full polysomnography (PSG) in addition to a full clinical work-up between 2009 and 2013 to assess the prevalence and correlates of sleep characteristics and sleep disorders (more details in Supplemental Appendix).

Ethical Statement

Both the CoLaus|PsyCoLaus and HypnoLaus studies were approved by the Ethics Committee of the Vaud Canton (approval numbers 16/03 and 33/09), and written informed consent was obtained from all participants.

Heart Rate Variability Measurements

Single-lead ECG data were extracted from the polysomnography recordings in European Data Format (EDF) using Somnologica Studio (version 5.1.1, Embla® Flaga). Each QRS complex was validated and raw RR series were imported to the *HRVanalysis* software version 1.2.[16] An accurate preprocessing was performed as suggested in the HRV Task Force.[3] HRV was first analyzed for the whole sleep period using sleep onset and wake-up timestamps from the PSG (including wake after sleep onset periods). In a secondary analysis, HRV was analyzed from the average of 5-minute stable epochs during non-rapid eye movement (NREM, including sleep stages 1, 2 and 3) and rapid eye movement (REM) sleep using the sleep scoring of the PSG. Moreover, HRV was analyzed from the average of 5-minute stable epochs without events (arousals, apnea-hypopnea or periodic leg movements) during NREM and REM sleep. HRV analysis included standard time-domain, frequency-domain, non-linear and novel indices according to standard criteria (more details in Supplemental Appendix).[3, 5]

Linear HRV Analysis

In the time-domain, the following indices were reported: mean peak-to-peak R intervals (RR), the standard deviation of normal-to-normal (N-N) intervals (SDNN) and the root mean square of successive N-N differences (rMSSD). In the frequency-domain, a Fast Fourier Transform algorithm was applied based on 5-min epochs and the following spectral power parameters were calculated: very low frequency (VLF), low frequency (LF), high frequency (HF) and the ratio LF/HF.[3]

Nonlinear HRV Analysis

Non-linear measurements provide information on the complexity of autonomic regulations, and included Poincaré plot analysis (short-term [SD1] and long-term variability indices [SD2]),[17] detrended fluctuation analysis (short- and long-term fluctuations indices DFA α1 and α2)[18] and entropy (approximative entropy [ApEn] and sample entropy [SampEn]).[19]

Novel HRV analysis

Novel HRV analysis included deceleration capacity (DC) and acceleration capacity (AC),[20] heart rate fragmentation (HRF; measured as the percentage of inflection point [PIP])[21] and heart rate turbulence (HRT; quantify by turbulence onset [TO] and turbulence slope [TS]).[22, 23]

Clinical Assessment

Information on sociodemographic characteristics, medical and treatment history was obtained by trained interviewers using standardized questionnaires during the first follow-up of CoLaus|PsyCoLaus (corresponding to the baseline of HypnoLaus).[14, 15] Detailed anthropometric measures and definition of comorbidities (diabetes, hypertension, dyslipidemia, depression) are available in Supplementary Appendix.

Outcomes

The incidence of a composite cardiovascular disease variable was established based on all the fatal and non-fatal cardiovascular disease events adjudicated by a local expert committee including a panel of cardiologists and neurologists according to international recommendations.[24] Fatal events were defined as death from myocardial infarction or stroke. Non-fatal outcomes included the occurrence of non-fatal myocardial infarction, stroke, acute coronary insufficiency (>50% stenosis) needing percutaneous coronary intervention or coronary artery bypass grafting, and peripheral arterial disease defined by angiological examination (US-doppler) and/or revascularization (by peripheral bypass surgery or stenting).

Exclusion criteria

Participants with a prior CVD and participants loss to follow-up were excluded (n=198) (Figure 1). Exclusion criteria also included recordings with insufficient ECG quality or technical failure as well as participants with frequent ectopic beats (percentage of supraventricular and ventricular extrasystoles >20% of the recording), atrial fibrillation or flutter, pacemaker, bundle branch block and ≥10% RR corrected due to their known influence on HRV (n=149) (Figure 1).

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics version 26.0 for Macintosh (IMB Corp, Armonk, NY, USA). Baseline characteristics are presented as mean ± SD, median (interquartile range) or as n (percentage) unless otherwise state. Baseline characteristics and HRV indices between “incident CVD events” and “no incident CVD events” groups were compared using Student’s t-test, Mann Whitney U test or Chi-square test as appropriate. Normality was checked with Q-Q plots. Non-normal HRV parameters were log transformed (log10) prior to analysis. A two-tailed *p*-value <0.05 was considered statistically significant. To determine the associations between HRV indices and CVD events

incidence, univariate and multivariable-adjusted COX regressions were used with each HRV parameter tested separately. Model 1 was adjusted for age, sex and body mass index (BMI). Model 2 was additionally adjusted for smoking status, education, depression, hypertension, dyslipidemia, diabetes, sleep drugs, beta-blockers, apnea-hypopnea index (AHI) and periodic leg movement during sleep (PLMSI). Model 3 was additionally adjusted for self-reported treatment for sleep-disordered breathing during the follow-up. Results are expressed as hazard ratio (HR) with 95% CI per one SD increase for each HRV parameter tested. To avoid type 1 error, we corrected for multiple testing using the false discovery rate (FDR) approach.[25] To facilitate interpretation, continuous HRV parameters that were statistically significant in the fully adjusted model were also dichotomized according to their median. Predictive power of these metrics was compared to a base model including age, BMI, diabetes, hypertension, smoking status and dyslipidemia. Discrimination performance of these latter models was assessed with area under the receiver operating characteristic curve (AUROC) while concordance was assessed using Harrell's C index and goodness of fit with Akaike information criterion (AIC) and Bayesian information criterion (BIC).

Additional analyses were done to determine: (1) the association of HRV during NREM and REM sleep with incident CVD events; (2) the association of HRV during sleep epochs in NREM and REM without sleep events (arousals, apnea-hypopnea or periodic leg movements) with incident CVD events; (3) the association of HRV with both incidence and recurrence of CVD events; (4) the association between the inability to measure HRV due to frequent ectopic beats, atrial arrhythmia, pacemakers, bundle branch block or lots of corrected RR intervals (≥10 %) and incident CVD events.

RESULTS

Population Characteristics

Of the 2162 participants from HypnoLaus, 1784 without any CVD at baseline were included in the present study. Detailed exclusion criteria are shown in Figure 1. The mean age was 58 years (range 40-84), 46.4% were men and the mean BMI was 26.0±4.3 $kg.m^{-2}$.

Sixty-seven (3.8%) participants developed a CVD event during follow-up (mean 4.1±1.1 years). Of these, 26 (1.5%) had a stroke, 30 (1.7%) developed coronary heart disease (including 1 fatal event), and 11 (0.6%) developed PAD. The mean time to incident CVD event was 2.6±1.4 years. Clinical and sleep characteristics of the participants with and without incident CVD events are shown in Table 1. Participants who developed a CVD event were older, had a higher BMI, were more often smokers, and had a higher prevalence rate of hypertension, dyslipidemia and diabetes. They were also more frequent users of sleep drugs and beta-blockers, and had higher AHI and PLMSI at baseline.

Association between HRV and incident CVD

Bivariate analysis for heart rate variability parameters according to cardiovascular status are shown in Table A.1. Numerous HRV indices were significantly associated with incident CVD events in unadjusted analysis and most of them remained significant after adjustment for age, sex and BMI (Model 1) (Table 2). In the Model 2 (additionally adjusted for smoking status, education, depression, hypertension, dyslipidemia, diabetes, sleep drugs, beta-blockers, AHI and PLMSI), AC, DC and PIP were the only significant predictors of incident CVD events after FDR correction. After additional adjustment for self-reported sleep-disordered treatment during follow-up (Model 3), none remains significantly associated with incident CVD events after FDR correction.

When AC, DC and PIP were dichotomized according to their median, we found that participants with low AC, low DC and high PIP were at higher risk of incident CVD events compared to those with high AC, high DC and low PIP respectively (Figure 2).

Predictive value of HRV metrics

Performance metrics of the risk prediction models are summarized in Table 3. Results showed a minimal improvement in discrimination (AUROC), concordance (Harrell's C), and goodness of fit (AIC and BIC) when AC, DC or PIP were added to the base model.

Secondary analysis

When considering HRV during NREM and REM sleep separately, results remained consistent although associations between incident CVD events and AC, DC as well as PIP were only significant in NREM sleep after FDR correction (Table.A.2.). Moreover, DFA α2 was significantly associated with incident CVD events during NREM sleep.

When considering only stable epochs without sleep events (arousals, apnea-hypopnea or periodic leg movements), only PIP during NREM remained significantly associated with CVD events after FDR adjustment (Table.A.3.).

When considering both incidence and recurrence of CVD (n=86 events), results remained consistent for AC and DC but PIP was no longer associated with the occurrence of CVD events during the follow-up (Table A.4).

Lastly, patients excluded from the primary analysis due to the inability to measure HRV because of frequent ectopic beats, atrial arrhythmia, pacemakers, bundle branch block or lots of corrected RR intervals were at higher risk of incident CVD events after adjustment for age, sex and BMI (HR: 1.86 [1.01–3.42]; p=0.046). However, this was no longer significant in the fully adjusted model (1.85 [0.98–3.48]; p=0.059).

DISCUSSION

To our knowledge, this study is the first to assess the predictive value of a comprehensive panel of linear, non-linear and novel nocturnal HRV indices based on a large population-based sample of middle-to-older age participants. Our findings showed that only novel nocturnal HRV metrics were independently associated with incident fatal and non-fatal CVD events over 4 years of follow-up. By contrast, traditional HRV parameters during sleep were not associated with incident CVD event. Although widely used in both research and clinical practice, nocturnal SDNN, rMSSD, HF and oldest non-linear indices (DFA, entropy) did not provide consistent results in the fully adjusted model after FDR-correction. However, our findings corroborate one recent study performed in the community-based MESA cohort[26] and

provide further evidence that nocturnal traditional HRV indices are not as accurate in participants without CVD as they can be in the cardiac populations in which they were originally developed.[3, 6] The discrepancy may be due to the paradoxical increase in short-term variability (rMSSD, HF) for some participants at high risk of CVD, which is actually not attributable to vagal tone modulation but rather to erratic sinus rhythms,[27, 28] and by the fact that they had been developed on 24-h measurement. Moreover, some nocturnal metrics such as VLF may be influenced by sleep disordered breathing. Even though the analysis was adjusted for the AHI, we cannot exclude that this interference could influence its predictive value.[29]

In contrast, some more novel metrics such as nocturnal PIP, AC and DC appear to be better predictors of incident CVD events. Nocturnal HRF already showed promising results as a predictor of CVD events at 3 years in the MESA cohort[26] with an adjusted hazard ratio for PIP in that community-based sample similar to the one obtained in our population-based cohort (HR values of 1.43 and 1.42, respectively), providing additional support for this novel approach. Surprisingly, our results showed that, in addition to DC, AC was strongly associated with incident CVD events whereas previous studies investigating these metrics only found DC to have prognostic value after myocardial infarction.[30] These HRV indices likely reflect cardiac dysautonomia not clearly captured by other HRV parameters during sleep but further studies are needed to elucidate the exact underlying physiological mechanisms. Furthermore, unlike previous studies in CVD populations,[22] we found no association between HRT indices and the incidence of CVD events in our sample, suggesting that HRT is a better predictor in cardiac populations than in general population. Lastly, secondary analysis showed that HRV during NREM sleep did not improve the prediction of cardiovascular risk compared to HRV during the whole night. This suggests that respiratory polygraphy recordings without sleep stage scoring and with a single-lead ECG may be used in clinical practice to analyze HRV and predict cardiovascular risk.

Strengths and Limitations

This study has several strengths including its prospective design in a large population-based cohort with CVD events adjudicated by an expert panel. Moreover, sleep was analyzed using gold-standard in-home PSG and the most commonly used HRV metrics were analyzed and adjusted according to comorbidities, CVD risk factors and treatments.

Nonetheless several limitations should be mentioned. First, we used a composite CVD events endpoint to evaluate CVD risk and therefore could not determine whether HRV parameters were better able to predict the incidence of cardiovascular or cerebrovascular diseases due to a relatively low number of events at four years. Further studies with more events are needed to shed light on CVD subgroup analysis. Second, this study might not be applicable to participants with arrhythmia, frequent ectopic beats and/or an implanted cardiac pacemaker because participants with these characteristics were excluded due to the alteration of the cardiac autonomic response. However, these subjects are known to be at risk of CVD and our findings confirmed that the inability to measure HRV may be a predictor of CVD events *per se*. Third, although we found significant prospective associations between some HRV metrics and incident CVD events, the predictive capacity of HRV appears to be relatively low when added to the base risk prediction model, but further studies are needed to confirm these results.

Conclusions

Our study showed that “novel” HRV indices are probably better predictors of incident CVD events than traditional HRV indices. In particular, low acceleration and deceleration capacity as well as high heart rate fragmentation showed the strongest associations with incident CVD events in our population-based cohort. Such features can be easily captured by HRV analysis of the PSG ECG channel. Further experimental studies are needed to shed light on the putative underlying physiological mechanisms of these novel HRV parameters.

Acknowledgments

The authors thank Prof Mehdi Tafti, Prof Vincent Mooser, Daniela Andries and Nadia Tobback for their important contribution to the HypnoLaus and CoLaus Cohorts, the Lausanne population who volunteered to participate in the CoLaus and HypnoLaus studies, as well as the whole team of CoLaus. English language editing assistance was provided by Nicola Ryan, independent medical writer, funded by the University of Lausanne.

Sources of Funding

The CoLaus study was and is supported by research grants from GlaxoSmithKline, the Faculty of Biology and Medicine of the University of Lausanne, and the Swiss National Science Foundation (grants 33CSCO-122661, 33CS30-139468, 33CS30-148401, 32003B_173092 and 32473B-182210). The HypnoLaus study received additional support from the Ligue Pulmonaire Vaudoise and the Leenaards Foundation.

Author's contributions

MB, VP, FR and RH designed the study. VP performed HRV analysis. MB and PMV made the statistical analysis. MB wrote the initial draft. All authors interpreted the data and critically reviewed the manuscript.

Data access

Due to the sensitivity of the data and the lack of consent for online posting, individual data cannot be made accessible. Only metadata will be made available in digital repositories. Metadata requests can also be made via the study website: www.colaus-psycolaus.ch.

References

1. Miglis MG. Sleep and the Autonomic Nervous System. Sleep and Neurologic Disease2017:227-244.
2. Malik M, Camm AJ. Heart rate variability. Clin Cardiol Aug 1990;13:570-576.
3. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Eur Heart J Mar 1996;17:354-381.
4. Shaffer F, Ginsberg JP. An Overview of Heart Rate Variability Metrics and Norms. Front Public Health 2017;5:258.
5. Sassi R, Cerutti S, Lombardi F, Malik M, Huikuri HV, Peng CK, Schmidt G, Yamamoto Y. Advances in heart rate variability signal analysis: joint position statement by the e-Cardiology ESC Working Group and the European Heart Rhythm Association co-endorsed by the Asia Pacific Heart Rhythm Society. Europace : European pacing, arrhythmias, and cardiac electrophysiology : journal of the working groups on cardiac pacing, arrhythmias, and cardiac cellular electrophysiology of the European Society of Cardiology Sep 2015;17:1341-1353.
6. Kleiger RE, Miller JP, Bigger JT, Jr., Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. The American journal of cardiology Feb 1 1987;59:256-262.
7. Tsuji H, Venditti FJ, Jr., Manders ES, Evans JC, Larson MG, Feldman CL, Levy D. Reduced heart rate variability and mortality risk in an elderly cohort. The Framingham Heart Study. Circulation Aug 1994;90:878-883.
8. Dekker JM, Schouten EG, Klootwijk P, Pool J, Swenne CA, Kromhout D. Heart rate variability from short electrocardiographic recordings predicts mortality from all causes in middle-aged and elderly men. The Zutphen Study. American journal of epidemiology May 15 1997;145:899-908.

9. Bernardi L, Valle F, Coco M, Calciati A, Sleight P. Physical activity influences heart rate variability and very-low-frequency components in Holter electrocardiograms. Cardiovascular research Aug 1996;32:234-237.

10. Fei L, Copie X, Malik M, Camm AJ. Short- and long-term assessment of heart rate variability for risk stratification after acute myocardial infarction. Am J Cardiol Apr 1 1996;77:681-684.

11. Kleiger RE, Stein PK, Bigger JT, Jr. Heart rate variability: measurement and clinical utility. Annals of noninvasive electrocardiology : the official journal of the International Society for Holter and Noninvasive Electrocardiology, Inc Jan 2005;10:88-101.

12. Herzig D, Eser P, Omlin X, Riener R, Wilhelm M, Achermann P. Reproducibility of Heart Rate Variability Is Parameter and Sleep Stage Dependent. Front Physiol 2017;8:1100.

13. Heinzer R, Vat S, Marques-Vidal P, et al. Prevalence of sleep-disordered breathing in the general population: the HypnoLaus study. The Lancet Respiratory medicine Apr 2015;3:310-318.

14. Firmann M, Mayor V, Vidal PM, et al. The CoLaus study: a population-based study to investigate the epidemiology and genetic determinants of cardiovascular risk factors and metabolic syndrome. BMC Cardiovasc Disord Mar 17 2008;8:6.

15. Preisig M, Waeber G, Vollenweider P, Bovet P, Rothen S, Vandeleur C, Guex P, Middleton L, Waterworth D, Mooser V, Tozzi F, Muglia P. The PsyCoLaus study: methodology and characteristics of the sample of a population-based survey on psychiatric disorders and their association with genetic and cardiovascular risk factors. BMC Psychiatry Mar 17 2009;9:9.

16. Pichot V, Roche F, Celle S, Barthelemy JC, Chouchou F. HRVanalysis: A Free Software for Analyzing Cardiac Autonomic Activity. Front Physiol 2016;7:557.

17. Tulppo MP, Makikallio TH, Takala TE, Seppanen T, Huikuri HV. Quantitative beat-to-beat analysis of heart rate dynamics during exercise. The American journal of physiology Jul 1996;271:H244-252.

18. Peng CK, Havlin S, Stanley HE, Goldberger AL. Quantification of scaling exponents and crossover phenomena in nonstationary heartbeat time series. Chaos 1995;5:82-87.

19. Pincus SM. Approximate entropy as a measure of system complexity. Proceedings of the National Academy of Sciences of the United States of America Mar 15 1991;88:2297-2301.

20. Bauer A, Kantelhardt JW, Barthel P, Schneider R, Mäkikallio T, Ulm K, Hnatkova K, Schömig A, Huikuri H, Bunde A, Malik M, Schmidt G. Deceleration capacity of heart rate as a predictor of mortality after myocardial infarction: cohort study. Lancet 2006;367:1674-1681.

21. Costa MD, Davis RB, Goldberger AL. Heart Rate Fragmentation: A New Approach to the Analysis of Cardiac Interbeat Interval Dynamics. Front Physiol 2017;8:255.

22. Schmidt G, Malik M, Barthel P, Schneider R, Ulm K, Rolnitzky L, Camm AJ, Bigger JT, Jr., Schomig A. Heart-rate turbulence after ventricular premature beats as a predictor of mortality after acute myocardial infarction. Lancet Apr 24 1999;353:1390-1396.

23. Bauer A, Malik M, Schmidt G, et al. Heart rate turbulence: standards of measurement, physiological interpretation, and clinical use: International Society for Holter and Noninvasive Electrophysiology Consensus. Journal of the American College of Cardiology Oct 21 2008;52:1353-1365.

24. Cutlip DE, Windecker S, Mehran R, et al. Clinical end points in coronary stent trials: a case for standardized definitions. Circulation May 1 2007;115:2344-2351.

25. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approch to multiple testing. J R Stat Soc Series B Stat Methodol 1995;57:289-300.

26. Costa MD, Redline S, Davis RB, Heckbert SR, Soliman EZ, Goldberger AL. Heart Rate Fragmentation as a Novel Biomarker of Adverse Cardiovascular Events: The Multi-Ethnic Study of Atherosclerosis. Front Physiol 2018;9:1117.

27. Stein PK. Heart rate variability is confounded by the presence of erratic sinus rhythm. Comput Cardiol 2002;26:669-672.

28. Nicolini P, Ciulla MM, De Asmundis C, Magrini F, Brugada P. The prognostic value of heart rate variability in the elderly, changing the perspective: from sympathovagal balance to chaos theory. Pacing Clin Electrophysiol May 2012;35:622-638.

29. Roche F, Court-Fortune I, Pichot V, Duverney D, Costes F, Emonot A, Vergnon JM, Geyssant A, Lacour JR, Barthelemy JC. Reduced cardiac sympathetic autonomic tone after long-term nasal continuous positive airway pressure in obstructive sleep apnoea syndrome. Clin Physiol Mar 1999;19:127-134.

30. Bauer A, Kantelhardt JW, Bunde A, Barthel P, Schneider R, Malik M, Schmidt G. Phase-rectified signal averaging detects quasi-periodicities in non-stationary data. Physica A 2006;364:423-434.

Figure legends

Figure 1. Study flow chart. Afib indicates atrial fibrillation; CV, cardiovascular; CVD, cardiovascular disease; ECG, electrocardiogram; HRV, heart rate variability; SVES, supraventricular extrasystoles; VES, ventricular extrasystoles. *Frequent VES and SVES were defined by a rate >20% of the recording.

Figure 2. Adjusted risk curves for the incidence of cardiovascular disease (CVD) according to high and low: A) acceleration capacity (AC); B) deceleration capacity (DC); and C) percentage of inflection points (PIP).
Curves were obtained after adjustment for age, sex, body mass index, education, smoking, depression, hypertension, dyslipidemia, diabetes, sleep drugs, beta-blockers, apnea-hypopnea index and periodic leg movement index during sleep. High and low values for each parameter was determined according to the median in the whole population.
HR: hazard ratio; Ref, reference.

Table 1. Population characteristics according to cardiovascular status at 4 years

	No incident CVD events (N=1717)	Incident CVD events (N=67)	p-value
Age (years)	56.1 (40.5-84.1)	67.6 (55.4-72.9)	**<0.001**
BMI ($kg.m^{-2}$)	25.5 (23.0-28.3)	26.6 (24.3-29.2)	**0.022**
Male (%)	790 (46.0%)	38 (56.7%)	0.085
Education (years)	13.8 ± 4.0	12.8 ± 4.0	0.054
Alcohol, units/week	4 (1-9)	4 (0-10)	**0.128**
Coffee (mL/day)	156 ± 100	148 ± 95	0.502
Smoking status (%)			**<0.001**
Current smoker	301 (17.7%)	24 (35.8%)	
Former smoker	669 (39.4%)	27 (40.3%)	
Depression (%)	327 (19.0%)	18 (26.9%)	0.112
Hypertension (%)	625 (36.4%)	45 (67.2%)	**<0.001**
Dyslipidemia (%)	448 (26.1%)	25 (37.3%)	**0.042**
Diabetes (%)	133 (7.8%)	14 (20.9%)	**<0.001**
Medication (%)			
Sleep drugs (%)	133 (7.7%)	13 (19.4%)	**0.001**
Beta-blockers (%)	101 (5.9%)	10 (14.9%)	**0.003**
ACE inhibitors	83 (4.8%)	11 (16.4%)	**<0.001**
Angiotensin receptor blockers	190 (11.1%)	12 (17.9%)	0.083
Calcium channel blockers	76 (4.4%)	7 (10.4%)	**0.022**
Diuretics	96 (5.6%)	13 (19.4%)	**<0.001**
Antiarrhythmic†	4 (0.2%)	0 (0%)	**1.000**
Sleep characteristics			
Total sleep time (min)	403 ± 71	402 ± 83	0.882
Sleep efficiency (%)	88.5 (81.0-93.0)	83.4 (77.2-89.1)	**0.002**
N1 (%)	10.1 (7.2-14.4)	12.2 (8.5-16.5)	**0.020**
N2 (%)	46.2 ± 10.2	50.1 ± 11.1	**0.002**
N3 (%)	20.1 ± 8.4	16.2 ± 8.5	**<0.001**
REM (%)	22.1 ± 6.0	20.3 ± 6.9	**0.018**
AHI (events/h)	9.4 (3.9-19.0)	16.3 (6.5-26.8)	**<0.001**
ODI (events/h)	9.4 (4.1-18.2)	15.1 (7.1-27.1)	**0.001**
Mean SpO_2 (%)	94.2 ± 2.9	93.4 ± 1.6	**0.021**
PLMSI (events/h)	1.8 (0-17.2)	13.5 (0.9-37.6)	**<0.001**
OSA treatment (%)††	116 (7.2%)	15 (27.3%)	**<0.001**

Values are presented as mean ± standard deviation or median (interquartile range) for normal and non-normal continuous data, respectively. Categorical data are presented as number of patients (%). P-values <0.05 are in bold.

ACE indicates angiotensin-converting enzyme; AHI, apnea-hypopnea index; ODI, oxygen desaturation index; OSA: obstructive sleep apnea; PLMSI, periodic leg movement during sleep; SpO_2, oxygen saturation. Detailed definitions of anthropometrics and comorbidities are presented in the supplementary method.

†Antiarrhythmic drugs include amiodarone (C01BD01) and flecainide (C01BC04). ††Self-reported OSA treatment was available in only 1645 participants.

Table 2. Nocturnal heart rate variability predictors of incident fatal and non-fatal adjudicated cardiovascular disease events

	Crude (n=1717/67)		Model 1 (n=1711/64)		Model 2 (n=1686/64)		Model 3 (n=1593/52)	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Mean RR (ms)	0.83 (0.65-1.07)	0.154	0.78 (0.60-1.02)	0.068	0.90 (0.69-1.18)	0.448	0.91 (0.67-1.23)	0.529
SDNN (ms)	0.69 (0.52-0.91)	0.008	0.76 (0.56-1.02)	0.064	0.85 (0.63-1.14)	0.280	0.84 (0.60-1.16)	0.286
RMSSD (ms)	0.46 (0.32-0.67)	**<0.001**	0.57 (0.39-0.84)	**0.005**	0.66 (0.45-0.98)	0.037	0.69 (0.45-1.05)	0.082
VLF (ms2/Hz)	0.64 (0.46-0.91)	0.011	0.69 (0.48-1.01)	0.055	0.77 (0.54-1.10)	0.154	0.77 (0.51-1.15)	0.195
LF (ms2/Hz)	0.51 (0.34-0.77)	**0.001**	0.67 (0.44-1.01)	0.056	0.75 (0.50-1.13)	0.169	0.80 (0.52-1.22)	0.296
HF (ms2/Hz)	0.29 (0.14-0.57)	**<0.001**	0.43 (0.22-0.84)	0.013	0.53 (0.28-1.00)	0.049	0.61 (0.32-1.16)	0.135
LF/HF ratio	1.17 (0.95-1.44)	0.129	1.11 (0.88-1.39)	0.396	1.09 (0.84-1.40)	0.526	1.09 (0.82-1.45)	0.573
SD1 (ms)	0.47 (0.32-0.68)	**<0.001**	0.57 (0.39-0.84)	**0.005**	0.67 (0.46-0.98)	0.039	0.69 (0.46-1.06)	0.087
SD2 (ms)	0.71 (0.54-0.94)	0.015	0.78 (0.58-1.04)	0.041	0.87 (0.65-1.16)	0.337	0.85 (0.61-1.17)	0.312
SD1/SD2 ratio	0.50 (0.35-0.70)	**<0.001**	0.63 (0.45-0.89)	0.008	0.69 (0.49-0.98)	0.036	0.73 (0.50-1.06)	0.100
DFA α1	1.37 (1.05-1.79)	0.020	1.30 (0.98-1.71)	0.067	1.25 (0.94-1.67)	0.128	1.20 (0.87-1.66)	0.269
DFA α2	1.58 (1.23-2.04)	**<0.001**	1.30 (1.01-1.67)	0.044	1.26 (0.98-1.63)	0.074	1.25 (0.93-1.67)	0.140
AC (ms)	2.31 (1.75-3.04)	**<0.001**	1.89 (1.39-2.57)	**<0.001**	1.58 (1.16-2.16)	**0.004**	1.53 (1.09-2.15)	0.015
DC (ms)	0.44 (0.33-0.57)	**<0.001**	0.53 (0.39-0.72)	**<0.001**	0.63 (0.47-0.85)	**0.002**	0.64 (0.46-0.88)	0.006
ApEn	0.76 (0.60-0.96)	0.022	0.90 (0.70-1.16)	0.396	0.93 (0.71-1.21)	0.589	0.92 (0.68-1.25)	0.610
SampEn	0.81 (0.63-1.04)	0.099	0.95 (0.74-1.22)	0.707	0.99 (0.76-1.29)	0.950	0.97 (0.72-1.31)	0.839

PIP (%)	1.64 (1.31-2.05)	**0.001**	1.46 (1.15-1.85)	**0.002**	1.42 (1.11-1.82)	**0.005**	1.39 (1.06-1.81)	0.016
TO (%)*	0.89 (0.70-1.14)	0.350	0.74 (0.56-0.98)	0.037	0.75 (0.55-1.01)	0.057	0.72 (0.52-0.99)	0.040
TS (ms/RR)*	0.50 (0.33-0.75)	**0.001**	0.68 (0.45-1.04)	0.076	0.82 (0.54-1.25)	0.353	0.94 (0.61-1.44)	0.785
HRT severity						0.896		0.992
Normal	Ref	-	Ref	-	Ref	-	Ref	-
TO or TS abnormal	1.66 (0.97-2.84)	0.065	1.13 (0.64-2.00)	0.683	1.15 (0.64-2.08)	0.642	0.98 (0.51-1.90)	0.954
TO & TS abnormal	2.69 (1.32-5.46)	**0.006**	1.25 (0.59-2.65)	0.565	1.09 (0.51-2.32)	0.827	0.94 (0.37-2.38)	0.903

Values presented are standardized hazard ratios (HR) per one-standard deviation (SD) increase in the independent variable, with 95% confidence intervals (CI). n indicates number of participants free of events / number of participants with incident cardiovascular disease in each model.

Model 1 was adjusted for age, sex and body mass index. Model 2: Model 1 + adjustment for educational level, smoking, depression, hypertension, dyslipidemia, diabetes, sleep drugs, beta-blockers, apnea-hypopnea index and periodic leg movement index during sleep. Model 3: Model 1 + adjustment for self-reported sleep-disordered treatment at follow-up. Detailed definitions of confounding factors are presented in the supplementary method.

False discovery rate (FDR) corrected significant results are in bold.

AC indicates acceleration capacity; ApEn, approximative entropy; DC, deceleration capacity; DFA α1, detrended fluctuation analysis describing short term fluctuations (4 to 11 beats); DFA α2, detrended fluctuation analysis describing long-term fluctuations (>11 beats); HF, high frequency power; LF, low frequency power; PIP, percentage of inflection points; rMSSD, square root of mean squared differences between successive NN intervals; SampEn, Sample entropy; SD1, Poincaré plot standard deviation perpendicular the line of identity; SD2, Poincaré plot standard deviation along the line of identity; SDNN, standard deviation in normal-to-normal (NN) RR intervals; TO, turbulence onset; TS, turbulence slope; VLF, very-low frequency power.

For TO and TS, n=1069/57 for crude; n=1067/55 for model 1, n=1056/55 for model 2 and n=993/45 for model 3.

Table 3. Predictive risk metrics for the model with cardiovascular risk factors (base model) and the model with cardiovascular risk factors plus HRV markers.

	AUROC	Harrell's C	AIC	BIC
Base model†	0.752 (0.690 - 0.814)	0.772 (0.709 - 0.835)	848.0	891.9
+ PIP	0.764 (0.705 - 0.823)	0.779 (0.719 - 0.840)	843.4	892.8
+ AC	0.763 (0.698 - 0.828)	0.780 (0.713 - 0.846)	840.8	890.2
+ DC	0.766 (0.701 - 0.831)	0.783 (0.718 - 0.849)	839.9	889.3

Results are presented as value and (95% confidence interval). AUROC, area under the receiver operating curve; AIC, Akaike's information criterion; BIC, Bayesian information criterion. PIP: percentage of inflection point; AC: acceleration capacity; DC: deceleration capacity.

†Base model included age, body mass index, diabetes, hypertension, smoking status and dyslipidemia.