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The Effect of Head-up Tilt upon Markers of Heart Rate Variability in Patients with Atrial Fibrillation

Running head: HRV in AF, is it plausible?

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Abstract

Background: Heart rate variability (HRV) analysis is uncommonly undertaken in patients with atrial fibrillation (AF) due to an assumption that ventricular response is random. We sought to determine the effects of head up tilt (HUT), a stimulus known to elicit an autonomic response, on HRV in patients with AF; we contrasted the findings with those of patients in sinus rhythm (SR).

Methods: Consecutive, clinically indicated tilt tests were examined for 207 patients: 176 in SR, 31 in AF. Patients in AF were compared to an age-matched SR cohort (n=69). Five minute windows immediately before and after tilting were analysed using time-domain, frequency-domain and non-linear HRV parameters. Continuous, non-invasive assessment of blood pressure, heart rate and stroke volume were available in the majority of patients.

Results: There were significant differences at baseline in all HRV parameters between AF and age matched SR. HUT produced significant haemodynamic changes, regardless of cardiac rhythm. Co-incident with these haemodynamic changes, patients in AF had a significant increase in median [quartile 1, 2] DFA-α2 (+0.14 [-0.03, 0.32], $p<0.005$) and a decrease in sample entropy (-0.17 [-0.50, -0.01], $p<0.005$).

Conclusion: In the SR cohort, increasing age was associated with fewer HRV changes on tilting. Patients with AF had blunted HRV responses to tilting, mirroring those seen in an age matched SR group. It is feasible to measure HRV in patients with AF and the changes observed on HUT are comparable to those seen in patients in sinus rhythm.

Keywords: Atrial Fibrillation; Heart Rate Variability; ECG Signal Processing; Head-up Tilt

Heart rate variability (HRV) is a surrogate marker for the function of the autonomic nervous system (ANS) and the technique is widely available (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). There are a variety of methods for the derivation of HRV, through the application of different mathematical functions to consecutive RR-intervals. These mathematical functions fall broadly into three groups: time domain, frequency domain and non-linear analysis (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

A relationship between reduced HRV and prognosis has been shown in health (Hillebrand et al., 2013) and in numerous conditions, including after myocardial infarction and in patients with heart failure (Bilchick et al., 2002).

HRV techniques are generally not applied to patients in atrial fibrillation (AF) (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). This is an important limitation, as AF is not only very prevalent but it is present in 30-50% of the heart failure population, a condition in which HRV has been shown to be useful in predicting outcomes (Bilchick, et al., 2002). Recently, bridging this gap in knowledge has become even more pertinent due to the introduction of ablative interventions or device implantation (e.g. renal denervation, baroreceptor stimulators, vagal nerve stimulators, spinal cord stimulators) which modulate the ANS as a potential treatment strategy in heart failure and other diseases (Ardell et al., 2014; Patel et al., 2013).

The argument against the use of HRV techniques in patients with AF is based upon the assertion that the RR intervals in AF are truly less dependent on physiological mechanisms measureable with HRV. Though in clinical examination AF is characterised crudely by an irregularly irregular pulse, generally considered random, there is a growing body of evidence that supports a different view (Carrara et al., 2015; Cygankiewicz et al., 2015; Hayano, Sakata, Okada, Mukai, & Fujinami, 1998; Hayano et al., 1997; Rawles & Rowland, 1986). Rawles and Rowland demonstrated in 74 patients in AF, using an auto-correlation technique, that at rest approximately a third of patients had a non-random ventricular rhythm (Rawles & Rowland, 1986). While the effect of the ANS on the sinus node is a major determinant of HRV in sinus rhythm (SR), the ANS is equally important in AF, through its effects on the refractory period and conductivity of the AV node, the frequency and irregularity of atrial impulses and the degree of concealed conduction (Bollmann et al., 2006; Hayano, et al., 1998; Lim et al., 2011).

The purpose of this study was to determine the validity of measuring HRV in patients with AF. To achieve this we used head-up tilt testing (HUT) as an intervention that predictably activates the sympathetic nervous system (SNS) and leads to withdrawal of the parasympathetic nervous system (PNS) (Mehlsen, Kaijer, & Mehlsen, 2008). We contrasted the effects of HUT on HRV in a cohort of individuals with AF and a group in SR.

The ageing process is an important consideration in studies of autonomic physiology (Petersen, Williams, Gordon, Chamberlain-Webber, & Sutton, 2000). Not only is increasing age a risk factor for AF but it has also been shown to reduce HRV in cross sectional studies (Laitinen, Niskanen, Geelen, Lansimies, & Hartikainen, 2004; Sosnowski, Macfarlane, & Tendera, 2011; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). It is vital that we match for age and interpret our findings in the context of a more elderly population. To aid this interpretation we carried out additional analyses on a cohort in SR to establish the effect of aging on HRV responses to tilt in our patients.

Methods

Study Patients

We obtained data retrospectively on consecutive patients with permanent AF who underwent clinically indicated tilt testing at two hospitals (over a cumulative 9 years). All patients in SR from one of the hospitals also had their data analysed to provide the control population. Patients were excluded from this analysis if they experienced syncope or pre-syncope in the tilt phase or had a paced rhythm. Data was available for 176 patients in SR and 31 in AF. National Health Service (UK) management permission for use of anonymised patient data for ethical research was obtained.

Tilt-test protocol

The tilt table examination was performed in a dedicated room. Patients were fasted for two hours prior to the HUT and did not have medications stopped. A motorised bed with footplate support was used to achieve tilt angles of 60-80°. Each patient had a 10 minute supine baseline period after which they were subjected to 20 minutes of tilt.

Data acquisition and pre-processing

Continuous, non-invasive, high resolution, beat-to-beat heart rate (1000 Hz sampling frequency) and blood pressure monitoring (500 Hz sampling frequency) was performed at both sites using either the Task Force® Monitor (CNS SystemsMedizintechnik AG, Graz, Austria) or Nexfin® (BMEYE B.V, Amsterdam, Holland). The Task Force® Monitor also estimates cardiac output and total peripheral resistance using impedance cardiography.

Time series for heart rate (beat to beat NN intervals) were extracted and automatically filtered to exclude artefacts and ectopics using a validated and freely available programme Kubios HRV (http://kubios.uef.fi).

Heart Rate Variability

We standardized our analysis windows to five minutes to minimize bias as it is known that the total variance of HRV increases in proportion to the length of recording, in line with international recommendations (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Windows immediately before and during the first five minutes of HUT were analysed. Time domain, frequency domain and non-linear methods for determining HRV were applied to the data (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

Time domain analysis involves application of simple statistical techniques straight to the successive RR intervals. We elected to study only SDRR (standard deviation of successive RR intervals) and RMSSD (root of the mean squared differences of successive RR intervals) (Tarvainen, Niskanen, Lipponen, Ranta-Aho, & Karjalainen, 2014) as both of these parameters can be used in 5 minute recordings of RR intervals and the other time domain parameters are either derived from them or are highly correlated to them (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

Frequency domain analysis required the RR interval time series to be converted to an equidistantly sampled series and this was performed using the cubic spline interpolation method (Tarvainen, et al., 2014). The power spectral density was estimated using parametric autoregressive modelling (order number 16 without factorisation) and absolute power in the low frequency (LF: 0.04-0.15 Hz) and high frequency (HF: 0.15-0.4 Hz) bands calculated (Tarvainen, et al., 2014). These powers can be normalised to minimise the effects of changes in total power on this parameters, e.g. normalised LF (LFnu) is calculated as: LF/(Total power- very low frequency power). Due to the algebraic relationship with normalised frequency domain parameters, whereby the sum of LFnu and HFnu is always equal to one, we have opted to present only unique data and arbitrarily chose to present LFnu.

Finally we also applied the following non-linear methods of HRV analysis: Poincaré plots, detrended fluctuation analysis and entropy. Poincaré plots are a graphical representation of the correlation between successive RR intervals. It can be assessed qualitatively by looking at the shape of the plot and quantitatively by fitting an ellipse to the plot and calculating the standard deviation of the points perpendicular to the line of identity (SD1) and along the line of identity (SD2) (Tarvainen, et al., 2014).

Detrended fluctuation analysis measures correlation with the signal for different time scales. A series of RR intervals are integrated and are divided into a series of regular intervals. For each interval the fluctuation of the data from a straight line of linear interpolation is calculated. DFA-$\alpha 1$ corresponds to short-term fluctuations within an interval range of 4-16 whereas DFA-$\alpha 2$ characterises longer-term fluctuations within the interval range of 16-64 (Tarvainen, et al., 2014).

Sample entropy, which refers to the degree of irregularity or randomness with a series and are estimates for the negative natural logarithm of the condition probability that a length of data having repeated itself within a tolerance *r* for *m* points, will also repeat itself for *m+1* points. We used the default value of *m*=2 and *r*= 0.2SDRR (Tarvainen, et al., 2014).

Statistics

Some of the HRV parameters were not normally distributed and so we adopted non-parametric statistical analysis throughout for consistency. Continuous variables are summarised as median (quartile 1, quartile 3) and compared using the Mann-Whitney U test (independent samples), the Kruskal-Wallis test (more than two independent samples) and the Wilcoxon signed rank test (dependent samples, i.e. comparing parameters before and after HUT in the same cohort). Categorical variables are presented as counts or proportions (%) and analysed using Fisher's exact text. Strength of correlation between variables are presented using the Pearson's product-moment correlation (r). A $P \leq 0.05$ was considered statistically significant for analysis of baseline clinical features and haemodynamics of patients. This level of significance was made more stringent to $P \leq 0.005$ when analysing the HRV parameters using the Bonferroni method to correct for multiple testing. A concern with the Bonferroni method is that it can elevate the type II error rate (accepting the null hypothesis when the alternative is correct) and for that reason we have also provided complete P values or at least made a summative distinction between a parameter that changed at $P<0.05$ and one at $P<0.005$. All analyses were performed using SPSS (Version 22, IBM).

Results

Data were available in total for 176 patients in SR and 31 patients in AF. Of these, all SR patients and 19 AF patients were from the same institution and had a full data set including non-invasive beat to beat heart rate, blood pressure, stroke volume and peripheral resistance. Data for the remaining 12 patients in AF was obtained from another institution for whom non-invasive stroke volume or peripheral resistance measurements were not available. Correlations between HRV variables at rest

The effect of HUT in AF and SR

The demographics of the 31 patients in AF and 69 age-matched patients in SR are summarised in Table 1. Patients with AF were significantly more likely to have hypertension and be on more medications (angiotensin converting enzyme inhibitor, angiotensin receptor blocker, beta-blocker, calcium channel blocker and digoxin). Only 7 (22.6%) patients with AF were not on any of the six classes of medication detailed in Table 1, compared with 54 (78.3%) in the SR cohort.

HUT causes a decrease in stroke volume, which is coupled with an increase in blood pressure (diastolic), heart rate and total peripheral resistance (Table 2). The magnitude and direction of change, though similar for both cohorts, were statistically more convincing in patients with SR.

All HRV parameters at rest were significantly different between the AF and SR cohorts. On HUT only 2 parameters (DFA-α2 and sample entropy) changed significantly ($P<0.005$) in both groups (Table 2). SDRR, LFnu and SD2 increased in patients in SR on HUT, whereas HF decreased in patients with AF at the uncorrected significance level of $p<0.05$. There was no overall difference in the direction of change between either group.

The effect of aging on cardiovascular autonomic reflexes

The cohort of 176 patients in SR was divided into tertiles of age (with median ages 22, 47 and 73 years). Their demographic data are detailed in Table 3 and suggests that the three groups were balanced apart from there being proportionally more females in the youngest cohort. In particular there were no differences with respect to prescribed medications.

There were significant differences at rest between the 3 tertiles of age with respect to haemodynamic function and HRV (Table 4). Stroke volume index decreased with age, whereas resting heart rate did not change. With advancing age all of the HRV parameters except for DFA-α1 and DFA-α2 were significantly attenuated (Table 4). Throughout the tertiles of age, upon HUT, blood pressure, heart rate and total peripheral resistance index increased whilst stroke volume index decreased. However, the augmentation in heart rate was attenuated as was the decline in stroke volume index with increasing age. Furthermore, the HRV response to HUT became blunted with age, with 11/12 HRV parameters changing at a significance level of $p<0.005$ in the youngest tertile, 9/12 in the middle cohort and only 2/12 in the oldest tertile.

Correlations between each of the HRV parameters in the SR (N=176) and AF (N=31) cohorts are shown in Table 5. There were strong correlations some of the non-linear parameters (SD1, SD2 and DFA-α1) and linear parameters.

Discussion

The main findings of this study are: 1) For patients in sinus rhythm, HRV at rest and in response to HUT attenuates with age; 2) Patients in AF demonstrate similar changes in HRV on HUT to an age-matched cohort in SR; 3) The non-linear measures of HRV appear more discriminatory in both AF compared with the conventional linear methods (time and frequency domain).

The effect of age on cardiovascular responses to HUT in SR patients

Haemodynamics

The process of shifting from a supine to an upright position results in an immediate reduction in venous return and up to a 20% reduction in stroke volume. In response, there is an activation of various homeostatic pathways, one of which is the ANS, which functions to maintain cerebral blood flow and prevent syncope (Mourot et al., 2007). In our cohort, we demonstrated that our HUT protocol was effective in inducing an adequate haemodynamic stress. We observed a reduction in stroke volume index, which was associated with an increase in blood pressure, heart rate and total peripheral resistance index.

In the supine position, the oldest tertile had the lowest stroke volume and compared with the youngest tertile had higher blood pressures and vascular resistance. Upon HUT, the increase in heart rate was less pronounced in the older cohort as was the decrease in stroke index. Cumulatively, these responses seek to maintain cardiac output (heart rate x stroke volume indexed for body surface area). Laitinen and colleagues described the effect of ageing upon response to HUT in 63 individuals and found results different from ours (Laitinen, et al., 2004). Similar to our data they showed that elderly subjects had smaller increases in heart rate, however, in contrast to our findings they showed that this cohort also had a larger decrease in stroke volume and larger increase in total peripheral resistance upon HUT. The most likely explanation is that the two studies have examined different populations. Laitinen and colleagues studied a healthy population who were not on any medication compared with our cohort who all had previously experienced syncope and at least a fifth had other significant co-morbidities. Medications are known to influence cardiovascular responses but in our SR cohort there were no significant differences between the tertiles of age with respect to blood pressure lowering medications. Furthermore, there were differences between our studies with respect to when data were collected. In Laitinen's study all patients were rested supine for 3 hours before a 5 minute baseline recording of heart rate was obtained (our protocol mandated a 10 minute rest period) and were sampled at minutes 5-10 after HUT (our protocol mandated minutes 0-5).

Heart rate variability

Ageing affects HRV both at rest and under dynamic testing using HUT. Consistent with the wider literature our data confirm that HRV reduces with age, both at rest and in response to HUT (Sosnowski, et al., 2011). At rest, we did not find a change in either DFA-α1 or DFA-α2 with age. Others have reported similar results whilst some groups have shown a decrease in DFA-α1 and an increase in DFA-α2 with advancing age in health volunteers (Shiogai, Stefanovska, & McClintock, 2010; Voss, Schroeder, Heitmann, Peters, & Perz, 2015). The likely contributors to this discrepancy are: 1) our study population were not healthy volunteers; 2) approximately a fifth of our population in SR were on cardiovascular medications (V. D. Corino et al., 2013); and 3) our study numbers were modest and hence our investigation may be underpowered.

In the youngest tertile (0-30 years), seven of the nine HRV parameters changed significantly on HUT. Only SDRR and its correlate SD2 did not change.(Hoshi, Pastre, Vanderlei, & Godoy, 2013) In the 30-60 years of age cohort, RMSSD also failed to change significantly with HUT. Finally in the 60 years and older cohort, only two HRV parameters changed (at $p<0.005$), DFA-α2 and sample entropy, both of them non-linear parameters. Studies examining the effect of HUT upon HRV, using time and frequency domain, mirror our findings and have concluded that the response to HUT in younger individuals reflects parasympathetic withdrawal at the cardiac level, which diminishes with ageing and is associated with a concomitant increase in sympathetic tone to the periphery (Laitinen, et al., 2004).

Comparison of responses in patients with AF and SR

Frequency domain analyses of HRV in AF have failed to detect changes in response to manoeuvres known to affect HRV in SR and our data lends further support to this assertion (Hayano, et al., 1997; Leung et al., 2005). DFA-α2 and sample entropy are the only two HRV parameters that changed significantly ($p<0.005$) in patients in AF and/or age-matched SR upon HUT. Though the direction of change was identical between the two groups, the magnitude of difference is likely to be different (though we are statistically underpowered to demonstrate the latter). Furthermore there were three other HRV parameters that changed in patients with SR but did not in AF, when the type 1 error rate was reduced to 0.05: SDRR, SD2, LFnu (the former two have previously been shown to be well positively correlated).(Hoshi, et al., 2013) It is not unexpected to see differences in HRV effects, since our two populations are different and because of this we would always recommend analysing HRV in patients with AF separately from those in SR. Nonetheless, our findings suggest that though HRV data may be less interpretable in AF, certain parameters do have discriminatory values rather than just the “random chaos” of ventricular response.

However, a feature of note is how few HRV changes were actually observed on HUT, even in the SR cohort. This highlights the importance of ageing on HRV as discussed above. In both the oldest tertile in SR and the AF group, the non-linear measures were the only parameters that changed significantly.

One might ask why there is a differential effect depending on which measure of HRV is employed. There is no gold standard technique for measuring HRV and currently no one method can be described as superior to another; rather each provides complementary information (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Though many groups have attempted to attribute individual HRV parameters to a particular limb of the autonomic nervous system, to do so is an oversimplification of what is a complicated network. At best, HRV allows an insight into autonomic modulation; as tone increases, modulation increases but once tone remains elevated and saturation occurs, modulation decreases.

Non-linear measures of HRV

There is little doubt that heart rate and its variability are complex phenomena which arise from an intricate network of regulatory pathways. Heart rate is likely to be sensitive to initial conditions but this dependence is likely to diverge exponentially with time. In mathematics these types of systems are best described as non-linear, which are fundamentally different from linear systems (examples of which include time and frequency domain analyses).This description of the underlying principles of non-linear methods makes it immediately appealing as a technique for AF due to the apparent randomness of the latter. We examined three types of non-linear analysis: Poincaré plots (Hoshi, et al., 2013), DFA(Castiglioni et al., 2011) and entropy (Porta et al., 2007).

SD1 and SD2 represent the standard deviation in the minor and major axis of a fitted ellipse to a plot of RR interval against the subsequent RR interval. However, as derivation of these variables is based on simple statistics, groups have questioned whether analysis of Poincaré plots truly reflects a non-linear technique. Hoshi and colleagues performed linear correlation amongst time domain, frequency domain and non-linear HRV parameters in 65 healthy individuals and 114 patients with coronary artery disease. Their data showed that SD1 is highly correlated to RMSSD (r=0.99) and SD2 to SDRR (r=0.95) (Hoshi, et al., 2013). Tulppo and colleagues showed a strong correlation between SD1 and HF (r=0.94) as well as SD2 and SDRR (r=0.99) (Tulppo, Makikallio, Takala, Seppanen, & Huikuri, 1996). Our data similarly reproduced these correlations (Table 5).

DFA detects self-similarity. An α value of 0.5 suggests that the signal is truly random (white-noise) with larger values suggesting less noise (Brownian motion). When healthy volunteers were sequentially challenged with atropine, propranolol and clonidine, it was shown that DFA values rise with vagal blockade and decrease with sympathetic blockade (Millar, Cotie, St Amand, McCartney, & Ditor, 2010).

Sample entropy measures regularity or randomness of heart rate variations. Higher values indicate greater irregularity and are commonly a feature of health. During HUT, it is expected that sample entropy decreases and this has been shown to be proportional to the angle of HUT (Porta, et al., 2007).

Other data supporting the validity of using HRV in AF

Measuring HRV in AF is not implausible; however, there remains a marked under appreciation of it. The most basic search on PubMed reveals >18000 articles containing the words ‘heart rate variability’ but only 402 articles using the combination of ‘heart rate variability’ and ‘atrial fibrillation’. A selection of the key publications are summarised below, however, from a broader perspective, further work in this field is required especially using the less validated non-linear techniques.

Just as in SR there is a circadian rhythm of HR, a similar one is found in AF (Bollmann, et al., 2006). Following on from this, the prognostic significance of HRV in large populations of SR patients has been widely published and though there is a similar trend in patients with AF, the literature is sparse (Frey et al., 1995; Platonov & Holmqvist, 2011). Yamada and colleagues showed in 107 patients with AF and predominately a preserved left ventricular ejection fraction, that HRV (non-linear markers only) could predict mortality (Yamada et al., 2000). In the reduced ejection fraction population of MADIT-II, in a sub-group of patients with AF (n= 68), those with a pNN20 <87 had a higher mortality (V. D. Corino et al., 2015). Finally in a cohort of 155 patients with heart failure and AF who were enrolled into the Muerte Subita en Insufficiencia Cardiaca (MUSIC) study, only non-linear HRV parameters were found to be predictors of mortality, sudden cardiac death and heart failure progression.(Cygankiewicz, et al., 2015)

Our focus was on whether reactive changes in HRV could be identified in patient with AF after a dynamic challenge. Van den Berg and colleagues compared the role of vagal activity by using intravenous propanolol (SNS inhibitor) and methylatropine (PNS inhibitor) in 16 patients with chronic AF and 12 healthy men in SR (van den Berg et al., 1997). They demonstrated that though there were significant differences at baseline between the two groups in respect of HRV (SDRR, RMSDD, LF and HF), these parameters changed in patients with AF in a similar direction albeit visually different magnitudes to healthy individuals when subjected to pharmacological sequential autonomic blockade. In a subsequent blinded crossover trial in 60 patients with permanent AF, it was shown that both beta-blockers and rate limiting calcium channel blockers lower heart rate and time domain parameters (SDRR, RMSDD), whilst beta-blockers also increased irregularity (sample entropy)(V. D. Corino, et al., 2015). Nagayoshi and colleagues documented RR interval and SDRR in 23 patients (mean age 61 years) in response to tilt, Valsalva, hand grip and showed that the response in patients with AF was similar to those of a historic SR population (Nagayoshi, Janota, Hnatkova, Camm, & Malik, 1997).

Limitations

This is a retrospective study and is exposed to the inherent biases that are common with this design. We have tried to minimise selection bias by sampling consecutive patients. Observer bias was limited as the tilt-time around which the analysis was performed was based upon what was recorded at the time of the HUT. Ideally we would have wanted to study more patients with AF but we were surprised to find only 31 patients in total at two centres spanning in combination, 9 years of data in total. Our findings are applicable to patients with permanent AF who are above the age of 60 and likely to be on heart rate lowering or blood pressure lowering medications. Drugs, duration of AF (often difficult accurately to ascertain if the condition is asymptomatic) and other diagnoses (hypertension, heart failure, diabetes mellitus) are all known to induce autonomic remodelling and it is likely to account for the heterogeneity in response to HUT in our study. However, due to our limited sample size of patients with AF, we are unable confidently to perform further subgroup analyses to investigate the relative contributions of each of these explanatory variables on HRV. Comparisons of HRV and response to HUT between the population in AF and SR are confounded by the significantly increased use of cardiovascular medications and prevalence of hypertension in the AF group (Table 1). A larger and prospective study, with a broad spectrum of AF patients matched by an equally broad spectrum of SR patients, might overcome a number of these problems. Others have analysed blood pressure variability in patients with AF and found less 'white-noise' artefact when compared to spectral analysis of heart rate (V. D. A. Corino, Lombardi, & Mainardi, 2014). Future work may also study blood pressure variability and baroreceptor function as markers of the ANS in patients with AF.

We corrected for multiple statistical testing using a Bonferroni correction. However, an accepted weakness is that it often results in a reduction in power, i.e. a conclusion that there is no change, when one genuinely exists. To demonstrate the effects of this correction explicitly, we have also provided those results that achieved significance at the conventional critical p value of 0.05.

Conclusion

Our findings confirm the feasibility of using HRV in patients with AF. In particular, we were able to detect changes in HRV in response to HUT using non-linear methods (DFA-α2 and sample entropy) as compared to traditional linear methods in individuals with AF. However, this finding invites a larger multicentre validation study. These findings may also prove to be of value in assessing the effect of novel ANS-modulating treatments such as renal denervation for diseases that predispose to AF, such as heart failure or hypertension.

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Conflict of interests

None.

References:

Ardell, J. L., Cardinal, R., Beaumont, E., Vermeulen, M., Smith, F. M., & Andrew Armour, J. (2014). Chronic spinal cord stimulation modifies intrinsic cardiac synaptic efficacy in the suppression of atrial fibrillation. *Auton Neurosci, 186*, 38-44. doi: 10.1016/j.autneu.2014.09.017

Bilchick, K. C., Fetics, B., Djoukeng, R., Fisher, S. G., Fletcher, R. D., Singh, S. N., . . . Berger, R. D. (2002). Prognostic value of heart rate variability in chronic congestive heart failure (Veterans Affairs' Survival Trial of Antiarrhythmic Therapy in Congestive Heart Failure). *Am J Cardiol, 90*(1), 24-28.

Bollmann, A., Husser, D., Mainardi, L., Lombardi, F., Langley, P., Murray, A., . . . Sornmo, L. (2006). Analysis of surface electrocardiograms in atrial fibrillation: techniques, research, and clinical applications. *Europace, 8*(11), 911-926. doi: 10.1093/europace/eul113

Carrara, M., Carozzi, L., Moss, T. J., de Pasquale, M., Cerutti, S., Ferrario, M., . . . Moorman, J. R. (2015). Heart rate dynamics distinguish among atrial fibrillation, normal sinus rhythm and sinus rhythm with frequent ectopy. *Physiological Measurement, 36*(9), 1873-1888. doi: 10.1088/0967-3334/36/9/1873

Castiglioni, P., Parati, G., Di Rienzo, M., Carabalona, R., Cividjian, A., & Quintin, L. (2011). Scale exponents of blood pressure and heart rate during autonomic blockade as assessed by detrended fluctuation analysis. *J Physiol, 589*(Pt 2), 355-369. doi: 10.1113/jphysiol.2010.196428

Corino, V. D., Cygankiewicz, I., Mainardi, L. T., Stridh, M., Vasquez, R., Bayes de Luna, A., . . . Platonov, P. G. (2013). Association between atrial fibrillatory rate and heart rate variability in patients with atrial fibrillation and congestive heart failure. *Ann Noninvasive Electrocardiol, 18*(1), 41-50. doi: 10.1111/anec.12019

Corino, V. D., Ulimoen, S. R., Enger, S., Mainardi, L. T., Tveit, A., & Platonov, P. G. (2015). Rate-control drugs affect variability and irregularity measures of RR intervals in patients with permanent atrial fibrillation. *J Cardiovasc Electrophysiol, 26*(2), 137-141. doi: 10.1111/jce.12580

Corino, V. D. A., Lombardi, F., & Mainardi, L. T. (2014). Blood pressure variability in patients with atrial fibrillation. *Auton Neurosci, 185*(0), 129-133. doi: http://dx.doi.org/10.1016/j.autneu.2014.08.002

Cygankiewicz, I., Corino, V., Vazquez, R., Bayes-Genis, A., Mainardi, L., Zareba, W., . . . Platonov, P. G. (2015). Reduced Irregularity of Ventricular Response During Atrial Fibrillation and Long-term Outcome in Patients With Heart Failure. *Am J Cardiol, 116*(7), 1071-1075. doi: 10.1016/j.amjcard.2015.06.043

Frey, B., Heinz, G., Binder, T., Wutte, M., Schneider, B., Schmidinger, H., . . . Pacher, R. (1995). Diurnal variation of ventricular response to atrial fibrillation in patients with advanced heart failure. *Am Heart J, 129*(1), 58-65.

Hayano, J., Sakata, S., Okada, A., Mukai, S., & Fujinami, T. (1998). Circadian rhythms of atrioventricular conduction properties in chronic atrial fibrillation with and without heart failure. *J Am Coll Cardiol, 31*(1), 158-166.

Hayano, J., Yamasaki, F., Sakata, S., Okada, A., Mukai, S., & Fujinami, T. (1997). Spectral characteristics of ventricular response to atrial fibrillation. *Am J Physiol, 273*(6 Pt 2), H2811-2816.

Hillebrand, S., Gast, K. B., de Mutsert, R., Swenne, C. A., Jukema, J. W., Middeldorp, S., . . . Dekkers, O. M. (2013). Heart rate variability and first cardiovascular event in populations without known cardiovascular disease: meta-analysis and dose-response meta-regression. *Europace, 15*(5), 742-749. doi: 10.1093/europace/eus341

Hoshi, R. A., Pastre, C. M., Vanderlei, L. C., & Godoy, M. F. (2013). Poincare plot indexes of heart rate variability: relationships with other nonlinear variables. *Auton Neurosci, 177*(2), 271-274. doi: 10.1016/j.autneu.2013.05.004

Laitinen, T., Niskanen, L., Geelen, G., Lansimies, E., & Hartikainen, J. (2004). Age dependency of cardiovascular autonomic responses to head-up tilt in healthy subjects. *J Appl Physiol, 96*(6), 2333-2340. doi: 10.1152/japplphysiol.00444.2003

Leung, R. S., Bowman, M. E., Diep, T. M., Lorenzi-Filho, G., Floras, J. S., & Bradley, T. D. (2005). Influence of Cheyne-Stokes respiration on ventricular response to atrial fibrillation in heart failure. *J Appl Physiol, 99*(5), 1689-1696. doi: 10.1152/japplphysiol.00027.2005

Lim, P. B., Malcolme-Lawes, L. C., Stuber, T., Koa-Wing, M., Wright, I. J., Tillin, T., . . . Kanagaratnam, P. (2011). Feasibility of multiple short, 40-s, intra-procedural ECG recordings to detect immediate changes in heart rate variability during catheter ablation for arrhythmias. *Journal of Interventional Cardiac Electrophysiology, 32*(2), 163-171. doi: 10.1007/s10840-011-9580-2

Mehlsen, J., Kaijer, M. N., & Mehlsen, A. B. (2008). Autonomic and electrocardiographic changes in cardioinhibitory syncope. *Europace, 10*(1), 91-95. doi: 10.1093/europace/eum237

Millar, P. J., Cotie, L. M., St Amand, T., McCartney, N., & Ditor, D. S. (2010). Effects of autonomic blockade on nonlinear heart rate dynamics. *Clin Auton Res, 20*(4), 241-247. doi: 10.1007/s10286-010-0058-6

Mourot, L., Bouhaddi, M., Gandelin, E., Cappelle, S., Nguyen, N. U., Wolf, J.-P., . . . Regnard, J. (2007). Conditions of autonomic reciprocal interplay versus autonomic co-activation: Effects on non-linear heart rate dynamics. *Auton Neurosci, 137*(1–2), 27-36. doi: http://dx.doi.org/10.1016/j.autneu.2007.06.284

Nagayoshi, H., Janota, T., Hnatkova, K., Camm, A. J., & Malik, M. (1997). Autonomic modulation of ventricular rate in atrial fibrillation. *Am J Physiol, 272*(4 Pt 2), H1643-1649.

Patel, H. C., Rosen, S. D., Lindsay, A., Hayward, C., Lyon, A. R., & di Mario, C. (2013). Targeting the autonomic nervous system: Measuring autonomic function and novel devices for heart failure management. *Int J Cardiol, 170*(2), 107-117. doi: 10.1016/j.ijcard.2013.10.058

Petersen, M. E., Williams, T. R., Gordon, C., Chamberlain-Webber, R., & Sutton, R. (2000). The normal response to prolonged passive head up tilt testing. *Heart, 84*(5), 509-514.

Platonov, P. G., & Holmqvist, F. (2011). Atrial fibrillatory rate and irregularity of ventricular response as predictors of clinical outcome in patients with atrial fibrillation. *J Electrocardiol, 44*(6), 673-677. doi: 10.1016/j.jelectrocard.2011.07.024

Porta, A., Gnecchi-Ruscone, T., Tobaldini, E., Guzzetti, S., Furlan, R., & Montano, N. (2007). Progressive decrease of heart period variability entropy-based complexity during graded head-up tilt. *J Appl Physiol, 103*(4), 1143-1149. doi: 10.1152/japplphysiol.00293.2007

Rawles, J. M., & Rowland, E. (1986). Is the pulse in atrial fibrillation irregularly irregular? *Br Heart J, 56*(1), 4-11.

Shiogai, Y., Stefanovska, A., & McClintock, P. V. E. (2010). Nonlinear dynamics of cardiovascular ageing. *Physics Reports, 488*(2-3), 51-110. doi: 10.1016/j.physrep.2009.12.003

Sosnowski, M., Macfarlane, P. W., & Tendera, M. (2011). Determinants of a reduced heart rate variability in chronic atrial fibrillation. *Ann Noninvasive Electrocardiol, 16*(4), 321-326. doi: 10.1111/j.1542-474X.2011.00458.x

Tarvainen, M. P., Niskanen, J. P., Lipponen, J. A., Ranta-Aho, P. O., & Karjalainen, P. A. (2014). Kubios HRV--heart rate variability analysis software. *Comput Methods Programs Biomed, 113*(1), 210-220. doi: 10.1016/j.cmpb.2013.07.024

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. (1996). 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, 17*(3), 354-381.

Tulppo, M. P., Makikallio, T. H., Takala, T. E., Seppanen, T., & Huikuri, H. V. (1996). Quantitative beat-to-beat analysis of heart rate dynamics during exercise. *Am J Physiol, 271*(1 Pt 2), H244-252.

van den Berg, M. P., Haaksma, J., Brouwer, J., Tieleman, R. G., Mulder, G., & Crijns, H. J. (1997). Heart rate variability in patients with atrial fibrillation is related to vagal tone. *Circulation, 96*(4), 1209-1216.

Voss, A., Schroeder, R., Heitmann, A., Peters, A., & Perz, S. (2015). Short-Term Heart Rate Variability—Influence of Gender and Age in Healthy Subjects. *PLoS One, 10*(3), e0118308. doi: 10.1371/journal.pone.0118308

Yamada, A., Hayano, J., Sakata, S., Okada, A., Mukai, S., Ohte, N., & Kimura, G. (2000). Reduced ventricular response irregularity is associated with increased mortality in patients with chronic atrial fibrillation. *Circulation, 102*(3), 300-306.

	Atrial Fibrillation (n=31)	Sinus Rhythm (n=69)	p
Age	74.3 (68.9, 83.8)	70.3 (62.9, 77.7)	0.056
Male	20 (64.5%)	46 (66.7%)	1.000
Diabetes	5 (16.1%)	5 (7.2%)	0.277
Hypertension	23 (74.2%)	11 (15.9%)	<0.001
Heart Failure	2 (6.5%)	0 (0%)	0.094
Medications			
ACEi/ARB	15 (48.4%)	12 (17.4%)	0.002
Beta-blockers	10 (32.3%)	3 (4.3%)	<0.001
CCB	8 (25.8%)	3 (4.3%)	0.003
Digoxin	7 (22.6%)	0 (0%)	<0.001
Diuretics	7 (22.6%)	6 (8.7%)	0.103
Median	2 (1, 2)	0 (0, 0)	<0.001

Table 1: Demographics, past medical and medication history of the patients with atrial fibrillation and age matched sinus rhythm. Data are presented as median (quartile 1, quartile 3) or count (%). ACEi- angiotensin converting enzyme inhibitor; ARB- angiotensin receptor blocker; CCB- calcium channel blocker

		Baseline			Change from baseline after HUT		
	Cohort	AF (n=31)	SR (n=81)	P (AF vs SR)	AF	SR	P (AF vs SR)
Haemodynamics	SBP (mmHg)	126.5 (112.0, 139.1)	123.1 (112.7, 134.9)	0.469	+5.2 (-2.5, 12.1)	+6.6 (-1.7, 16.6)**	0.431
	DBP (mmHg)	79.2 (68.9, 83.5)	77.8 (70.6, 84.5)	0.871	+4.8 (-0.6, 13.1)*	+10.4 (1.5, 16.1)**	0.100
	HR (beats/min)	74.9 (67.6, 87.5)	70.7 (60.9, 78.2)	0.018	+3.8 (1.4, 7.1)**	+4.6 (1.7, 8.2)**	0.776
	Stroke index (ml/m^2)	36.8 (25.9, 42.2)	34.7 (29.9, 41.5)	0.729	-5.2 (-8.1, 1.0)*	-5.7 (-10.0, -1.1)**	0.417
	Cardiac index (L/[min.m^2])	2.41 (2.12, 2.94)	2.52 (2.17, 2.98)	0.663	-0.13 (-0.44, 0.26)	-0.26 (-0.54, 0.06)**	0.326
	TPR index (dyne*s*m^2/cm^5)	3464 (2666, 3792)	2930 (2585, 3488)	0.252	+612 (-168, 1092)*	+628 (106, 1028)**	0.515
Time	SDNN (ms)	102.8 (28.3, 160.4)	39.2 (25.3, 57.1)	0.003	+0.9 (-13.9, 18.4)	+3.4 (-6.7, 18.8)*	0.396
	RMSSD (ms)	139.2 (18.4, 208.1)	26.9 (15.9, 47.2)	0.001	-5.5 (-21.4, 4.2)	-2.2 (-22.1, 21.5)	0.291

	LF (ms²)	2075 (135, 5243)	254 (156, 612)	0.005	65.1 (-223, 320)	-21.6 (-151, 137)	0.364
	HF (ms²)	3921 (106, 10491)	209 (75, 539)	0.001	-92.5 (-2124, 119)*	-28.0 (-234, 36)*	0.149
	LF/HF	0.58 (0.46, 1.02)	1.39 (0.80, 2.25)	<0.001	+0.10 (-0.12, 0.35)*	+0.61 (-0.32, 2.33)**	0.216
	LFnu (%)	36.4 (31.4, 50.4)	58.0 (44.3, 69.2)	<0.001	+4.4 (-4.0, 9.9)	+7.1 (-4.3, 17.5)**	0.341
Frequency	HFnu (%)	63.1 (49.6, 68.2)	41.8 (30.7, 55.6)	<0.001	-4.2 (-9.8, 3.8)	-7.1 (-17.2, 4.3)**	0.331
	SD1 (ms)	98.5 (13.0, 147.4)	19.0 (11.3, 33.4)	0.001	-3.9 (-15.2, 3.0)	-1.4 (-10.9, 5.8)	0.393
	SD2 (ms)	110.6 (34.9, 163.9)	49.3 (33.6, 66.4)	0.006	+1.9 (-14.4, 29.3)	+4.7 (-6.4, 26.7)**	0.669
	DFA-α1	0.70 (0.61, 1.01)	1.06 (0.84, 1.22)	<0.001	+0.01 (-0.11, 0.09)	+0.07 (-0.14, 0.45)*	0.179
	DFA-α2	0.69 (0.54, 0.86)	0.94 (0.80, 1.11)	<0.001	+0.14 (-0.03, 0.32)**	+0.14 (-0.03, 0.36)**	0.806
Non linear	Sample Entropy	1.78 (1.26, 2.05)	1.26 (0.94, 1.55)	<0.001	-0.17 (-0.50, -0.01)**	-0.25 (-0.55, 0.04)**	0.887

Table 2: Baseline and change with HUT of haemodynamic and HRV data. *= p≤0.05 (within group delta from baseline, paired t-test) **=p≤0.005 (within group delta from baseline, paired t-test). DFA1, DFA2 and Sample Entropy are dimensionless.

SBP- systolic blood pressure; DBP- diastolic blood pressure; HR- heart rate; TPR- total peripheral resistance; SDNN- standard deviation of the RR interval; RMSSD- root of the mean squared differences of successive RR intervals; LFnu- low frequency power in normalized units; HFnu- high frequency power in normalized units; SD1- minor axis on Poincaré plots; SD2- major axis on Poincaré plots, DFA- detrended fluctuation analysis

Tertiles of age	0-30 (n=50)	30-60 (n=69)	60+ (n=57)	p
Age	22.0 (18.5, 24.9)	47.1 (38.4, 52.4)	72.7 (66.2, 79.4)	<0.001
Male	20 (40.0%)	34 (49.3%)	38 (66.7%)	0.018
Diabetes	9 (18.0%)	5 (7.2%)	4 (7.0%)	0.131
Hypertension	10 (20.0%)	14 (20.3%)	9 (15.8%)	0.807
Heart Failure	1 (2.0%)	0 (0%)	0 (0%)	0.284
Medications				
ACEi/ARB	6 (12.0%)	10 (14.5%)	10 (17.5%)	0.659
Beta-blockers	4 (8.0%)	6 (8.7%)	2 (3.5%)	0.522
CCB	5 (10.0%)	6 (8.7%)	3 (5.3%)	0.648
Diuretics	4 (8.0%)	3 (4.3%)	5 (8.8%)	0.562
Total	0.0 (0.0, 0.3)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.947

Table 3: Demographics, past medical and medication history of the patients in sinus rhythm across tertiles of age. Data are presented as median (quartile 1, quartile 3) or count (%). Abbreviations as per Table 1

		Baseline				Change from baseline after HUT			
	Age Tertiles (years)	0-30 (n=50)	30-60 (n=69)	60+ (n=57)	P (between groups)	0-30	30-60	60+	P (between groups)
Haemodynamics	SBP (mmHg)	114.5 (108.4, 122.6)	124.9 (112.8, 135.1)	123.3 (113.2, 135.0)	0.004	+9.3 (3.7, 11.0)**	+6.6 (0.3, 17.0)**	+6.7 (-1.4, 14.7) **	0.419
	DBP (mmHg)	73.8 (67.7. 81.5)	83.6 (76.5, 88.6)	75.9 (69.8, 81.6)	<0.001	+14.3 (6.2, 19.3) **	+10.6 (2.2, 10.6) **	+10.3 (2.5, 15.7) **	0.102
	HR (beats/min)	72.5 (64.8, 79.7)	72.2 (61.9, 80.6)	71.4 (61.9, 78.5)	0.800	+8.8 (5.6, 14.9) **	+6.1 (2.8, 11.2) **	+4.4 (1.1, 7.3) **	<0.001
	Stroke index (ml/m^2)	50.2 (42.1, 57.3)	38.2 (31.3, 44.6)	34.8 (29.2, 40.5)	<0.001	-9.8 (-15.6, -4.5) **	-7.8 (-13.0, -1.7) **	-5.1 (-9.0, -1.5) **	0.004
	Cardiac index ($L/[min.m^2]$)	3.39 (3.06, 4.08)	2.68 (2.16, 3.15)	2.52 (2.20, 3.05)	<0.001	-0.35 (-0.78, 0.06) **	-0.33 (-0.65, 0.02) **	-0.25 (-0.59, 0.09) **	0.582
	TPR index ($dyne*s*m^2/cm^5$)	1985 (1714, 2479)	2960 (2450, 3675)	2925 (2516, 3305)	<0.001	+532 (162, 750) **	+661 (328, 1224) **	+617 (7.7, 1030) **	0.218
Time-domain	SDRR (ms)	70.2 (48.9, 88.5)	43.7 (29.6, 62.4)	40.2 (26.3, 58.3)	<0.001	+0.2 (-9.5, 8.6) **	+7.0 (-7.0, 19.6)*	+1.8 (-7.8, 18.7)	0.144
	RMSSD (ms)	47.8 (37.0, 82.2)	30.2 (17.4, 55.20	27.9 (16.7, 52.7)	<0.001	-19.3 (-49.9, -4.2) **	-4.0 (-18.5, 14.5)	+0.6 (-26.8, 19.0)	0.001
Frequency-domain	LF (ms^2)	1092 (651, 2381)	492 (235, 806)	251 (142, 600)	<0.001	-14.8 (-350, -15) **	+24.2 (-150, 430)	-52.6 (-155, 126)	0.357

	HF (ms^2)	1085 (456, 2485)	310 (108, 926)	189 (76, 584)	<0.001	-592 (-1651, -592) **	-71.9 (-392, 21) **	-23.6 (-227, 62)	<0.001
	LF/HF	1.13 (0.68, 1.85)	1.69 (0.93, 2.37)	1.25 (0.68, 2.13)	0.037	+1.56 (0.82, 3.15)**	+1.23 (0.40, 3.57)**	+0.33 (-0.25, 2.33)**	0.005
	LFnu (%)	53.0 (40.3, 64.8)	62.7 (48.0, 70.3)	55.6 (40.4, 67.9)	0.037	+20.3 (12.5, 26.3) **	+12.2 (3.6, 23.5) **	+6.3 (-4.8, 19.4)*	<0.001
	HFnu (%)	46.9 (35.1, 59.6)	37.2 (29.6, 51.8)	44.4 (32.0, 59.1)	0.037	-20.2 (-26.2, -12.3)**	-12.1 (-23.4, -3.58)**	-6.3 (-19.1, 4.8)*	<0.001
	SD1 (ms)	33.9 (26.2, 58.2)	21.4 (12.3, 39.1)	19.8 (11.8, 37.3)	<0.001	-14.5 (-24.7, -2.8) **	-3.3 (-14.0, 1.9) **	-1.4 (-10.4, 7.9)	<0.001
	SD2 (ms)	84.9 (62.7, 108.8)	55.7 (38.8, 81.3)	49.3 (34.1, 66.4)	<0.001	+5.2 (-8.5, 14.3)	+9.4 (-6.2, 28.4) **	+3.5 (-7.3, 27.2)	0.406
	DFA-α1	1.00 (0.84, 1.26)	1.13 (0.92, 1.33)	1.04 (0.77, 1.19)	0.082	+0.28 (0.11, 0.48)**	+0.24 (-0.06, 0.42) **	+0.06 (-0.21, 0.49)	0.010
	DFA-α2	0.84 (0.78, 0.94)	0.87 (0.77, 1.05)	0.94 (0.80, 1.06)	0.142	+0.13 (-0.04, 0.28) **	+0.12 (-0.03, 0.25) **	+0.18 (-0.3, 0.37) **	0.260
Non linear	Sample Entropy	1.58 (1.36, 1.77)	1.42 (1.19, 1.63)	1.20 (0.86, 1.49)	<0.001	-0.45 (-0.66, -0.18) **	-0.30 (-0.60, -0.05) **	-0.20 (-0.55, 0.13) **	0.070

Table 4: Baseline and change with HUT of haemodynamic and HRV data across the tertiles of age in patients with sinus rhythm. Data are presented as median (quartile 1, quartile 3). *= p≤0.05 (within group delta from baseline) **=p≤0.005 (within group delta from baseline). Abbreviations as per Table 2.

AF / SR	SDNN (ms)	RMSSD (ms)	LF (ms²)	HF (ms²)	LF/HF	LFnu (%)	HFnu (%)	SD1 (ms)	SD2 (ms)	DFA-α1	DFA-α2	Sample Entropy
SDNN (ms)		0.992 P<0.001	0.926 P<0.001	0.943 P<0.001	-0.476 P=0.007	-0.614 P<0.001	0.613 P<0.001	0.992 P<0.001	0.996 P<0.001	-0.572 P=0.001	-0.737 P<0.001	0.703 P<0.001
RMSSD (ms)	0.892 P<0.001		0.908 P<0.001	0.935 P<0.001	-0.514 P=0.003	-0.664 P<0.001	0.663 P<0.001	1.000 P<0.001	0.976 P<0.001	-0.637 P<0.001	-0.762 P<0.001	0.735 P<0.001
LF (ms²)	0.871 P<0.001	0.740 P<0.001		0.970 P<0.001	-0.366 P=0.043	-0.474 P=0.007	0.474 P=0.007	0.908 P<0.001	0.930 P<0.001	-0.461 P=0.009	-0.676 P<0.001	0.663 P<0.001
HF (ms²)	0.841 P<0.001	0.878 P<0.001	0.765 P<0.001		-0.399 P=0.26	-0.530 P=0.002	0.529 P=0.002	0.935 P<0.001	0.939 P<0.001	-0.506 P=0.004	-0.659 P<0.001	0.696 P<0.001
LF/HF	-0.227 P=0.002	-0.444 P<0.001	-0.106 P=0.160	-0.327 P<0.001		0.904 P<0.001	-0.905 P<0.001	-0.514 P=0.003	-0.453 P=0.010	0.886 P<0.001	0.393 P=0.029	-0.448 P=0.012
LFnu (%)	-0.274 P<0.001	-0.553 P<0.001	-0.063 P=0.403	-0.444 P<0.001	0.823 P<0.001		-1.000 P<0.001	-0.664 P<0.001	-0.580 P=0.001	0.956 P<0.001	0.595 P<0.001	-0.555 P=0.001
HFnu (%)	0.275 P<0.001	0.553 P<0.001	0.065 P=0.389	0.446 P<0.001	-0.823 P<0.001	-1.000 P<0.001		0.663 P<0.001	0.579 P=0.001	-0.955 P<0.001	-0.595 P<0.001	0.555 P=0.001
SD1 (ms)	0.892 P<0.001	1.000 P<0.001	0.740 P<0.001	0.879 P<0.001	-0.444 P<0.001	-0.553 P<0.001	0.553 P<0.001		0.976 P<0.001	-0.637 P<0.001	-0.762 P<0.001	0.735 P<0.001
SD2 (ms)	0.986 P<0.001	0.807 P<0.001	0.880 P<0.001	0.785 P<0.001	-0.150 P=0.047	-0.163 P=0.031	0.164 P=0.029	0.807 P<0.001		-0.538 P=0.002	-0.713 P<0.001	0.678 P<0.001
DFA-α1	-0.242 P=0.001	-0.539 P<0.001	-0.066 P=0.381	-0.375 P<0.001	0.731 P<0.001	0.907 P<0.001	-0.904 P<0.001	-0.539 P<0.001	-0.123 P=0.103		0.551 P=0.001	-0.598 P<0.001
DFA-α2	-0.081 P=0.287	-0.227 P=0.002	-0.189 P=0.012	-0.100 P=0.188	0.187 P=0.013	0.156 P=0.039	-0.154 P=0.041	-0.227 P=0.002	-0.032 P=0.674	0.231 P=0.002		-0.698 P<0.001
Sample Entropy	-0.024 P=0.754	0.111 P=0.141	0.096 P=0.204	0.175 P=0.020	-0.279 P<0.001	-0.162 P=0.032	0.164 P=0.029	-0.111 P=0.141	-0.057 P=0.453	-0.178 P=0.018	-0.189 P=0.012	

Table 5: Correlations (r) between heart rate variability parameters in the sinus rhythm (SR- in white) population (N=176) and atrial fibrillation (AF- in grey) population (N=31). Abbreviations as per Table 2.