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Circadian variation of variability and irregularity of heart rate in patients with permanent atrial fibrillation: Relation to symptoms and rate-control drugs

Short title: 24-h variability and irregularity during AF

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Abstract

The aim of this study is to evaluate the diurnal variation of the variability and irregularity of the heart rate (HR) in patients with permanent atrial fibrillation (AF), with and without rate-control drugs. Thirty-eight patients with permanent AF were part of an investigator-blind cross-over study, comparing diltiazem, verapamil, metoprolol, and carvedilol. We analyzed five Holter recordings per patient: at baseline (no rate-control drug) and with each of the four drug regimens. HR, variability (standard deviation, pNN20, pNN50, pNN80, and rMSSD) and irregularity (approximate (APEn) and sample entropy) parameters were computed in 20-minute long non-overlapping segments. Circadian rhythmicity was evaluated using the cosinor analysis to each parameter series, that is characterized by the 24-h mean (MESOR) and the excursion over the mean (the amplitude). Arrhythmia-related symptoms were assessed by a questionnaire measuring symptoms severity (SS) and frequency (SF). HR and variability parameters showed a significant circadian variation in most patients, whereas only a small minority of the patients had circadian variation of irregularity parameters.

The patients with circadian ApEn at baseline had more severe symptoms (SS = 9±4 vs. 6±5, $p<0.05$; circadian vs. non-circadian variation). All drugs decreased the MESOR of HR and increased the MESOR of variability parameters. Only carvedilol and metoprolol decreased the normalized amplitude over the 24-h of all parameters and HR. In conclusion, HR and RR variability parameters present a circadian variation in patients with permanent AF, whereas few patients demonstrated circadian fluctuations in irregularity parameters, suggesting different physiological mechanisms.

Keywords: Circadianity; variability; irregularity; β-blockers; calcium-channel blockers

New & Noteworthy statement

Patients with permanent atrial fibrillation showed a circadian variation in heart rate and ventricular response variability parameters in most patients. In contrast, few patients showed circadian variation in irregularity parameters, and these were more symptomatic than other patients. Beta blockers and calcium channel blockers influenced irregularity parameters differently.

Introduction

Irregularity measures have been suggested as risk indicators in patients with atrial fibrillation (AF). Studies analyzing the variability and irregularity of the RR series have found that a reduced irregularity of RR intervals in permanent AF is associated with poor outcome (5, 14, 15, 22). Despite the accumulating data that suggest potential use of irregularity measures as risk indicators in patients with AF, it is not known to what extent they are affected by variation of autonomic tone over 24 hours and whether diurnal variation of these parameters, if exists, is affected by commonly used rate-control drugs.

Recently, the RATe control in Atrial Fibrillation (RATAF) study, compared the effects of four once-daily drug regimens (metoprolol, diltiazem, verapamil and carvedilol) on ventricular heart rate (HR) and arrhythmia related symptoms, in patients with permanent AF (21). In a recent study (3)we analyzed the RATAF data and found that calcium channel blockers and β-blockers influenced AV node conduction differently. Both calcium channel blockers and β-blockers reduced HR and increased time-domain measures of heart rate variability, but only β-blockers increased the irregularity measures.

However, 24-h variations of variability and irregularity measures in patients with AF have not been evaluated in controlled settings with and without rate-reducing drug administration.

Few studies investigated circadian variations in patients with AF. Hayano et al. (7, 8) examined the circadian variations in atrioventricular (AV) conduction properties during AF by a technique based on the Lorenz plot of successive ventricular response intervals. Their results suggested that AV node properties during AF may show a circadian rhythm that could contribute to the circadian variation of the ventricular response. Moreover, they found that the circadian rhythm was attenuated in patients with congestive heart failure (CHF) (8) and that the circadian rhythm of AV conduction was an independent risk for cardiac death in patients with chronic AF (7). Sandberg et al. explored the circadian variation in atrial

fibrillatory rate (19), showing that circadian variation was present in most patients with long-standing persistent AF though the short-term variation in the AF frequency was considerable and should be taken into account.

Irregularity of ventricular response obtained from short-time recordings during AF have demonstrated their value for prediction of outcome (5, 14, 15, 22), however limits of applicability of this methodology remain to be delineated including the optimal recording length, time of the day and potential impact of rate-control drugs, which are commonly used in patients with AF. The possible prognostic meaning of circadian variation of these same parameters is not known and we have recently shown that some of these parameters can be affected by drug treatment (3). The present study is the first to address these questions. Our objective was to evaluate the 24-h variation of the variability and irregularity of the RR-intervals in patients with permanent AF, at baseline and during metoprolol, carvedilol, diltiazem and verapamil administration.

2. Materials and Methods

2.1. Protocol

The RATAF study was a prospective, randomized, investigator-blind, cross over study designed to compare four drug regimens used to reduce the HR in patients with permanent AF. Patients without CHF or ischemic heart disease were recruited from the AF outpatient clinic at Bærum Hospital (Bærum, Norway) from May 2006 to June 2010. Detailed protocol of the study is described elsewhere (21). The study was approved by the regional ethics committee and the Norwegian medicines agency, and all patients signed informed consent. Clinical characteristics are shown in Table 1.

The participants received the following drug regimens in a randomized cross-over design: i) metoprolol slow-release tablets 100 mg/day, ii) diltiazem sustained-release capsules 360 mg/day, iii) verapamil modified-release tablets 240 mg/day, and iv) carvedilol immediate-

release tablets 25 mg/day. Each drug was given for at least three weeks to ensure steady-state plasma concentrations and an adequate period of wash out of the previous treatment.

Arrhythmia-related symptoms were assessed using a self-administered questionnaire (21): the Symptom Checklist-Frequency and Severity (SCL) in Norwegian translation. The frequency and severity of 16 symptoms potentially associated with arrhythmias, thereby generating frequency and severity scores, with higher scores representing worse symptoms. Total scores of symptoms frequency (SF) and severity (SS) were calculated on the basis of all 16 symptoms included in the checklist. The patients were given the questionnaires on each visit, i.e., at the end of wash-out and at the end of each treatment period, filled them out at home and returned them the next day.

2.2. Ventricular response analysis

We analyzed five Holter recordings per patient: at baseline (no rate-reducing drug) and with each of the four drug regimens. Variability and irregularity parameters were computed in 20-minute long non-overlapping segments, therefore for each parameter, a series of N values is obtained (where N = 24*3 = 72 if the recording lasts exactly 24 hours). Variability and irregularity are not synonyms: variability is related to the dispersion of data, whereas irregularity is related to the degree of unpredictability of the data fluctuations, therefore they offer complementary information. A visual explanation of the difference between variability and irregularity is shown in Figure 1.

2.2.1 RR variability

Time domain analysis includes the HR, the standard deviation (SD) of all normal RR intervals, the root of the mean squared differences of successive RR intervals (rMSSD) and the percentage of interval differences of successive RR intervals greater than 20ms (pNN20), 50ms (pNN50) and 80ms (pNN80)(6).

2.2.2 RR irregularity

Irregularity of RR intervals was assessed by the approximate (ApEn) and sample (SampEn) entropy.

The approximate entropy (ApEn) is a regularity statistic quantifying the unpredictability of fluctuations in a time series such as an instantaneous heart rate time series. The presence of repetitive patterns of fluctuation in a time series makes it more predictable than a time series in which such patterns are absent. ApEn reflects the likelihood that similar patterns of observations will not be followed by additional similar observations. A time series containing many repetitive patterns, i.e., a regular and predictable series, has a relatively small ApEn; a less predictable, i.e., more complex, process has a higher ApEn (13). The ApEn algorithm counts each sequence as matching itself, and this makes the ApEn biased. Therefore, the sample entropy (SampEn), not counting self-matches, has been introduced (18).

2.3. Circadian Analysis

To evaluate the circadian rhythmicity of the variations, the cosinor analysis is applied, i.e., a single-component cosinor with a 24 h period is fitted to the parameter series to determine if there is a circadian variation. Briefly, the following variables that characterize circadian rhythmicity are estimated (see Figure 2): the MESOR (Midline Statistic Of Rhythm, a rhythm-adjusted mean); A, the amplitude (a measure of half the extent of predictable variation within a cycle); the acrophase (a measure of the time of overall high values recurring in each cycle). The period (duration of one cycle) is supposed to be known and equal to 24 hours. The normalized amplitude A_{norm} was also computed as A/MESOR, for a better comparison among patients and phases, i.e., A_{norm} is the percentage of variation during the day over the average value (MESOR). The three parameters MESOR, A, and acrophase are determined using a nonlinear least squares method (1).

2.4. Statistical Analysis

To determine whether a circadian variation was present, the zero-amplitude test was used. Briefly, the sum of squared differences between the estimated values based on the fitted model and the arithmetic mean (MSS) and the sum of squared differences between the data and the estimated values from the fitted model (RSS) are computed. The model is statistically significant when the model sum of squares (MSS) is large relative to the residual sum of squares (RSS), as determined by the F test

$F = (MSS/2)/(RSS/(N-3)$

where 2 and N-3 are the numbers of degrees of freedom attributed to the model (k = 3 parameters – 1) and to the error term (N-k). The null hypothesis (H_0) that there is no rhythm (the amplitude is zero) is rejected when $F > F1-\alpha(2, N-3)$, where α relates to the chosen probability level for testing H_0 and was chosen equal to 0.001(4).

One-way repeated measures ANOVA test was performed to compare the computed parameters during baseline and drug regimens; if the p-value of the ANOVA test was significant, a paired t-test or Wilcoxon test with Holm's correction was applied.

The Wilcoxon rank sum test was used to test symptom scores between patients who presented circadian variation and those who did not.

A p-value <0.05 was considered statistically significant. All analyses and statistical tests were performed using MATLAB® R2012b (The MathWorks, USA).

3. Results

3.1. Patient characteristics and data availability

In total, 60 patients (age 71±9 years, 42 men) with permanent AF were included in the RATAF study. For the current analyses, we included the 38 patients that had five ECG recordings lasting ≥ 20 hours (minimum duration for circadian analysis). Clinical characteristics are presented in Table 1.

3.2. 24-h variation at baseline

Figure 3 (left column) shows an example of 24-h trends for HR, a variability (rMSSD) and an irregularity parameter (SampEn) for one patient. It can be noted that both HR and rMSSD show a circadian variation, whereas SampEn does not. These results are confirmed on the whole database and for all parameters, as shown in Table 2, first column, which reports the number of patients whose parameters were found to present a circadian variation, established by the zero-amplitude test. At baseline, variability parameters show a circadian variation in 87% of the patients (range 82-95%), whereas one third of the patients have a circadian rhythm in irregularity parameters.

3.3. Rate-control drugs effect: 24-h variation

Heart rate

Figure 3 (top row) shows an example of 24-h trends for HR for one patient. It can be noted that a significant circadian variation is present in HR trends during all drug administration, however the MESOR and amplitude are lower when compared to baseline. These results are confirmed on the whole database as shown in Tables 3 and 4.

Figure 4 shows an average cosinor of HR for all patients, during the five phases of analysis, after normalizing by the average MESOR and considering the same acrophase for all, in order to emphasize the difference in the normalized amplitude. It can be observed that all drugs decreased the normalized amplitude, diltiazem being the drug maintaining the maximal excursion in HR. This result was confirmed in almost all parameters as shown in Table 4. Only verapamil significantly decreased the normalized amplitude of HR compared to the baseline value.

Variability parameters

Figure 3 (middle row) shows an example of 24-h trends for a variability (rMSSD) parameter for one patient. rMSSD shows a circadian variation during drug administration and larger MESOR comparing to baseline. Tables 3 shows that the MESOR of variability parameters is

higher during drug administration compared to baseline. Diltiazem was the drug which increased the MESOR of variability parameters the most. The normalized amplitude using β-blockers carvedilol and metoprolol is lower than during baseline.

Irregularity parameters

Figure 3 (bottom row) shows an example of 24-h trends an irregularity (SampEn) parameter for one patient. SampEn did not show any significant circadian variation during baseline or drug administration. On the whole database (Tables 3) the MESOR of irregularity parameters is higher during β-blockers administration compared to baseline, whereas there is no significant difference when calcium channel blockers are used.

The normalized amplitude for the irregularity parameters using β-blockers carvedilol and metoprolol is lower than at baseline.

3.4. Circadianity and symptoms

We investigated the relation between symptoms and the presence of circadian rhythm in variability and irregularity parameters. A trend between symptoms and circadian variation in both variability and irregularity parameters can be observed: patients with circadian variation in variability parameters have less frequent and less severe symptoms. On the contrary patients with circadian variation in irregularity parameters have more frequent and more severe symptoms. At baseline, significant differences in symptoms severity is found in rMSSD and ApEn (rMSSD = 6±5 vs. 11±5, ApEn = 9±4 vs. 6±5, circadian vs. non-circadian variation). Patients with circadian variation in irregularity parameters tend to have lower actual irregularity (as an example during baseline ApEn: 1.79±0.10 vs. 1.91±0.07 $p < 0.0001$, circadian vs. non circadian variation).

4. Discussion

In normal subjects, a lower HR during sleep is well established, though few studies have undertaken a detailed analysis of the circadian properties of the curve (11),(12), as both HR

and HR variability depend on the autonomic nervous system. In addition, previous studies suggest that in normal subjects also the complexity of short-term RR series depends on the state of the autonomic nervous system: it is usually reduced during experimental conditions inducing an increase of the sympathetic modulation (16, 17). A reduction of RR series complexity during tilt was also found in a small group of patients with AF (2). However, to the best of our knowledge, this is the first time the effect of rate-control drugs on heart rate variability and irregularity over 24 hours is assessed in patients with AF in the setting of randomized prospective cross-over designed study.

The main findings of this study are: i) the existence of a circadian variation in HR and variability parameters in almost all patients at baseline, ii) the lack of circadianity in irregularity parameters in most of the patients; iii) both β-blockers and calcium-channel blockers decreased the 24-h mean (MESOR) of HR and increased the MESOR of variability parameters; iv) β-blockers decreased the normalized amplitude over the 24-h, i.e., the excursion of variation of all parameters and HR.

The existence of a circadian variation in HR and variability parameters in patients with AF can be considered a sign that the autonomic nervous system still works properly in these patients. The effect of the autonomic nervous system during AF has been highlighted in recent studies on arterial blood pressure, where fluctuations in the low frequency band have been related to the influence of the sympathetic fibers acting on the cardiovascular system (9). Moreover, a previous study suggested that the blunted circadian rhythms of AV conduction properties may reflect blunted circadian rhythm of autonomic cardiac modulation, which may be in accordance with the fact that reduced circadian variation in heart rate variability, an index of cardiac autonomic activity, is associated with an increased risk of mortality in patients after myocardial infarction (10).

We have recently shown that calcium channel blockers and β-blockers influence AV node conduction differently. Both calcium channel blockers and β-blockers reduced HR and increased time-domain measures of heart rate variability, but only β-blockers increased the irregularity measures (3) and the present results on MESOR are in agreement with the previous ones. β-blockers decreased the normalized amplitude over the 24-h, i.e., the excursion of variation of all parameters and HR. Previous studies showed a marked attenuation in the circadian variation of the low-frequency component after β blockade (20).

Even if irregularity parameters have been shown to be good risk indicators in patients with AF (5, 14, 15, 22), interpretation of the prognostic impact of RR irregularity is rather complex. The few studies analyzing variability and irregularity of the RR series showed that a reduced irregularity of RR intervals in permanent AF was associated with poor outcome. Reduced variability and irregularity of RR intervals during AF were found to be an independent predictor of all cause mortality in patients with left ventricular dysfunction following myocardial infarction (14) and in patients with mild to moderate heart failure (5), respectively. In this study, irregularity measures show circadian behavior in the minority of patients (about one third). The patients who have circadian variation in irregularity parameters tend to have worse and more frequent symptoms, these same patients have lower irregularity, which has been shown to be a poor prognostic sign (5, 14, 15, 22).

In conclusion, in the majority of patients, HR and RR variability parameters present a circadian variation as in normal subjects, showing that the autonomic nervous system works quite properly even in patients with permanent AF. In contrast, irregularity parameters have a circadian variation only in few patients. The circadianity parameter MESOR is influenced by calcium channel blockers and β-blockers, whereas the normalized amplitude is attenuated only by β-blockers, i.e., HR and variability and irregularity parameters are forced to have a smaller range of variation. Finally, irregularity parameters do not generally demonstrate

circadian fluctuations, which may suggest that they may prove to be more robust as risk predictors in patients with AF.

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346 **Table 1**: Demographic characteristics and cardiovascular history in study population

Variable	Value
Age (yrs)	71 ± 8
Gender (male/female)	30 / 8
AF duration (months)	23 (2-92)
BMI (kg/m^2)	27 ±4
Stroke or transitory ischemic attack	5 (13%)
Diabetes mellitus	3 (8%)
Hypertension	17 (45%)
Left atrial diameter (long-axis view) (mm)	50 ± 6
Left ventricular ejection fraction (%)	61 ± 7
Warfarin	35 (92%)
Aspirin	3 (8%)
Angiotensin receptor blocker or angiotensin-converting enzyme inhibitor	15 (40%)
Diuretics	7 (18%)
Statins	10 (26%)

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Table 2: Number of patients whose parameters were found to have a circadian variation at baseline and during drug administration (of the total 38 patients).

	Baseline	Carvedilol	Metoprolol	Diltiazem	Verapamil
HR	36	35	34	34	28
SD	33	31	34	37	30
pNN20	32	24	29	32	21
pNN50	31	29	31	33	24
pNN80	32	29	31	34	25
rMSSD	33	33	32	35	30
ApEn	9	7	5	9	12
SampEn	14	7	9	8	17

Table 3: Mean ± SD for the MESOR for all parameters during baseline and during drug administration.

	Baseline	Carvedilol	Metoprolol	Diltiazem	Verapamil
HR (bpm)	96±12	85±11 *	83±12 *†	77±10 *†‡	81±12 *†§
SDNN (ms)	154±32	171±37 *	182±43 *†	194±46 *†‡	177±40 *§
pNN20 (%)	89±3	91±2 *	91±2 *	91±3 *	90±2 *
pNN50 (%)	73±6	78±4 *	79±5 *	79±6 *	77±5 *§
pNN80 (%)	60±7	66±6 *	67±6 *	68±8 *	65±6 *§
rMSSD (ms)	206±43	234±50 *	248±58 *†	266±69 *†‡	238±57 *§
ApEn (a.u.)	1.88±0.10	1.92±0.07*	1.93±0.06 *	1.90±0.07	1.90±0.07 †‡
SampEn (a.u.)	1.72±0.14	1.78±0.11 *	1.78±0.10 *	1.76±0.11	1.75±0.10

* $p<0.05$ comparison with baseline

† $p<0.05$ comparison with carvedilol

‡ $p<0.05$ comparison with metoprolol

§ $p<0.05$ comparison with diltiazem

Table 4: Percentage of the variation over the average (MESOR) during the day (A_{norm}) for all parameters during baseline and drug administration.

	Baseline	Carvedilol	Metoprolol	Diltiazem	Verapamil
HR	17±6	10±4 **	13±6 ** †	16±7 † ‡	13±6 **
SDNN	20±1	13±6 **	15±7 *	21±10 † ‡	17±9
pNN20	3±2	1±1 **	2±1 **	3±4	3±3
pNN50	8±6	4±3 **	5±3 *	7±8	6±6
pNN80	13±8	7±4 **	7±5 *	11±10	10±8
rMSSD	22±11	14±7 **	17±8 *	23±11 † ‡	18±10
ApEn	2±2	1±2 *	1±1 *	2±3	3±3 † ‡
SampEn	5±5	3±3 *	2±2 *	3±4	5±5

* $p<0.05$, ** $p < 0.001$ comparison with baseline

† $p<0.05$ comparison with carvedilol

‡ $p<0.05$ comparison with metoprolol

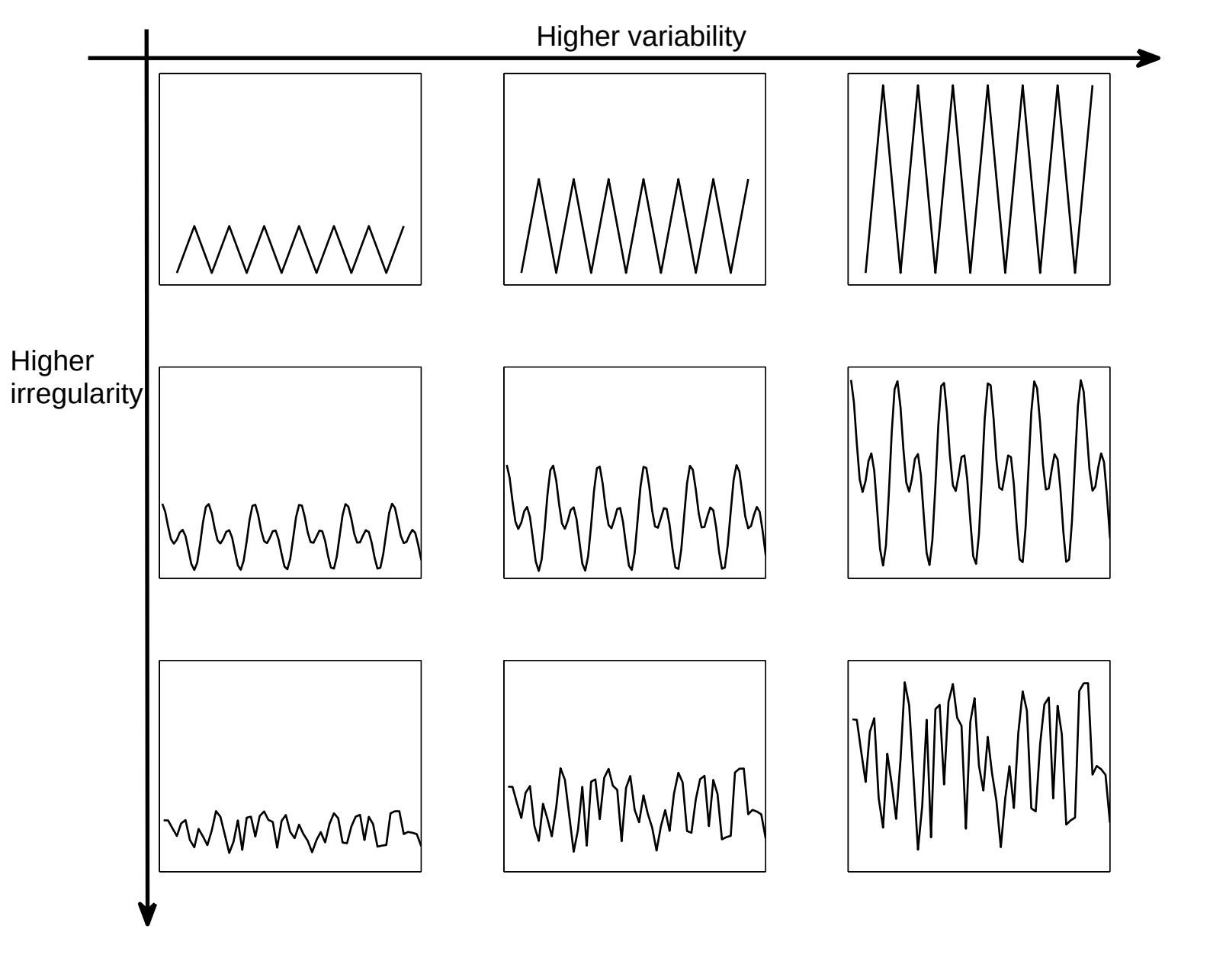
Figures caption:

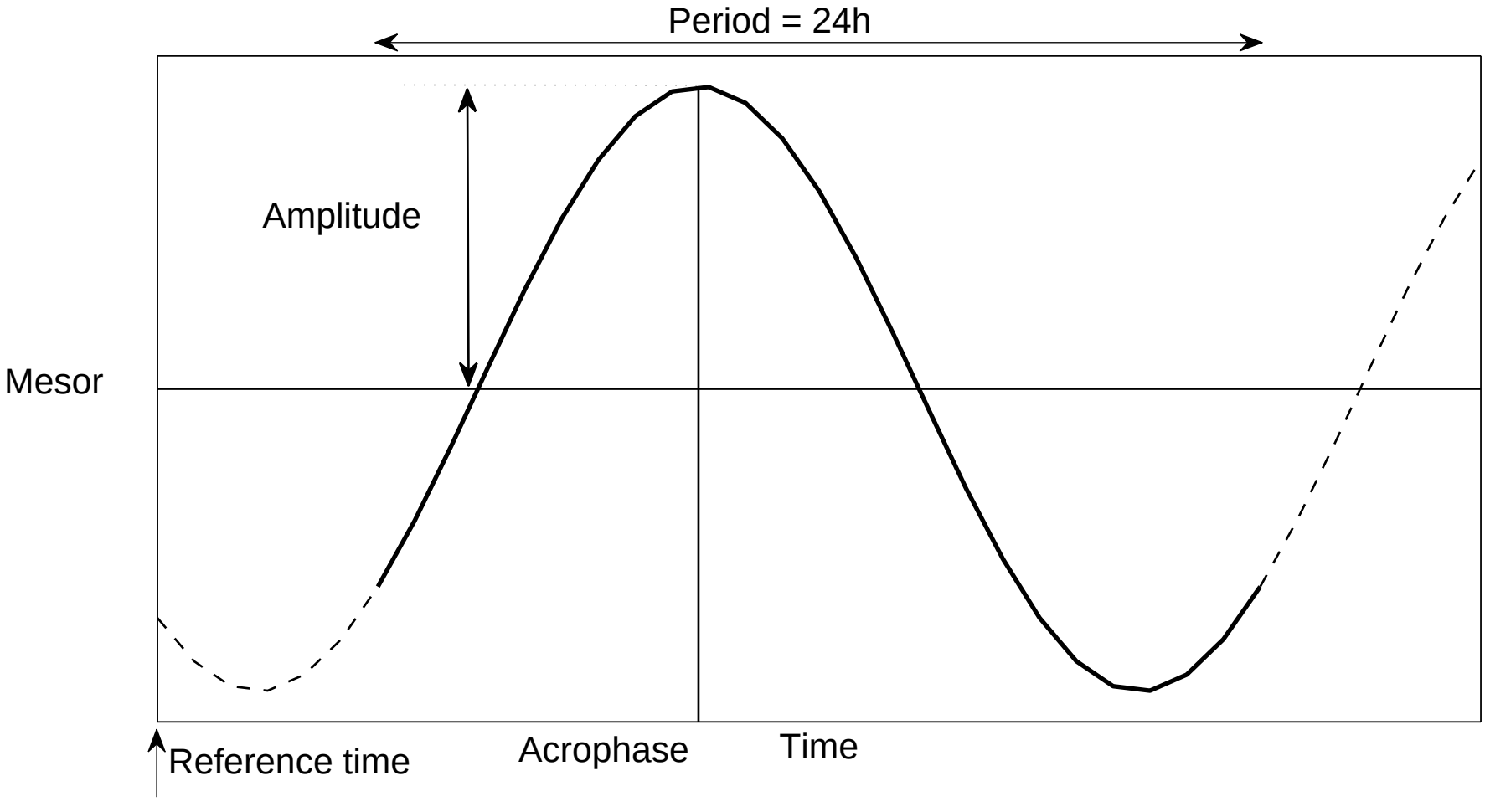
Figure 1: Figure explaining the difference between variability and irregularity in time series. Each row shows series with the same irregularity but increasing variability going from the left to the right, whereas each column shows series with the same variability but different increasing irregularity moving from the top to the bottom.

Figure 2: Schematic representation of the variables that characterize a circadian rhythm. The MESOR is a rhythm-adjusted mean; the amplitude (A) is a measure of half the extent of predictable change within a cycle; the acrophase is a measure of the timing of overall high values recurring in each cycle, and the period is the duration of one cycle.

Figure 3: Example of 24-h trends for HR, a variability (rMSSD) and an irregularity (SampEn) measure for a patient. The parameter values (circles) are fitted by the cosinor (dashed line), and the MESOR line is superimposed (dash-dotted line). A circadian variation is present in HR and rMSSD trends during baseline and drug administration; the MESOR and amplitude during drugs are lower (higher for rMSSD) when compared to baseline. SE shows no significant circadian variation during baseline or drug administration.

Figure 4: Average normalized cosinors of HR for all patients, during baseline and drug administration. The cosinor is normalized by the average MESOR, and the same acrophase is considered. All drugs decreased the normalized amplitude, being diltiazem the drug maintaining the maximal excursion in HR.


Higher variability
Higher irregularity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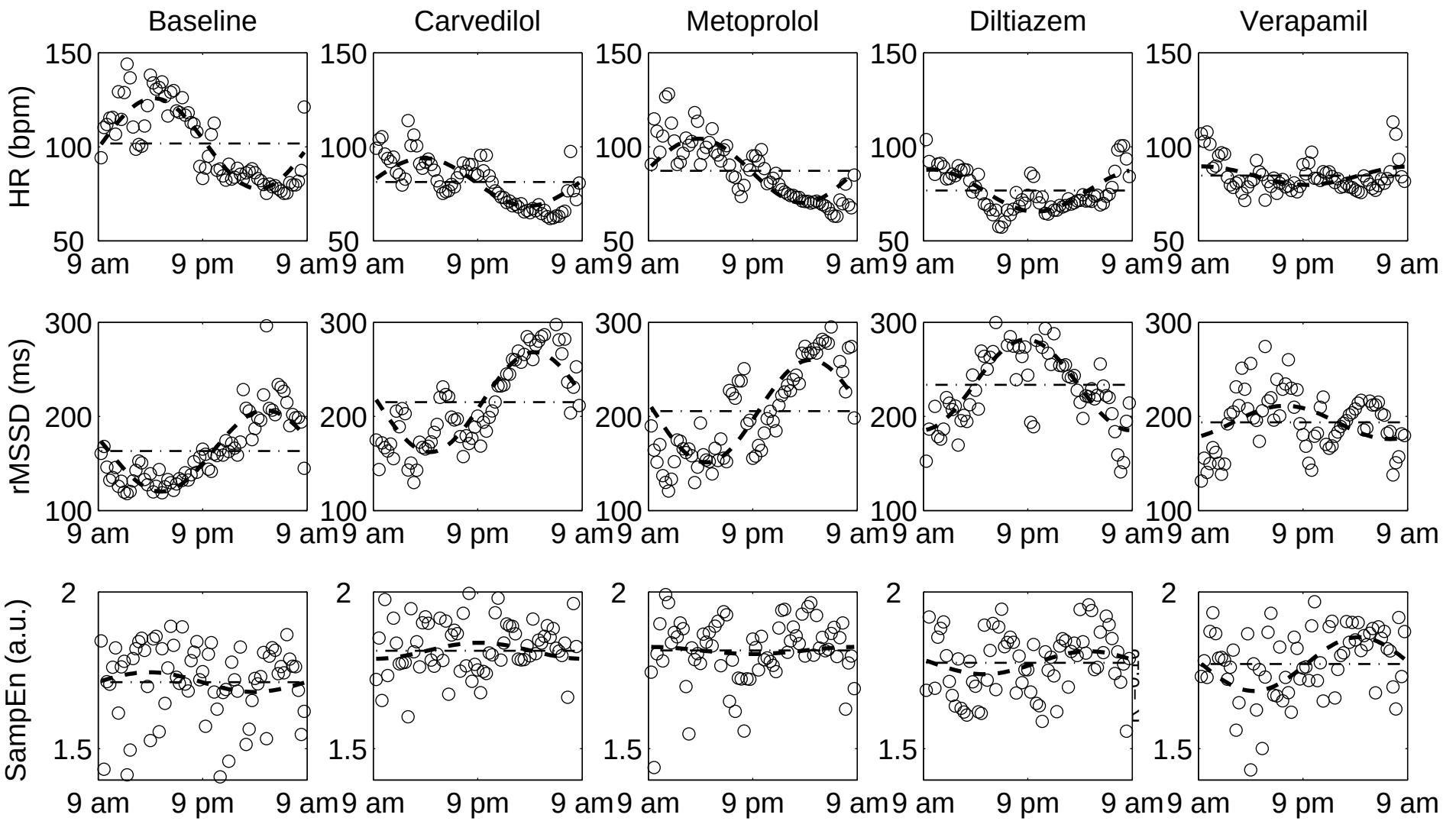
Baseline
Carvedilol
Metoprolol
Diltiazem
Verapamil
HR (bpm)
rMSSD (ms)
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150
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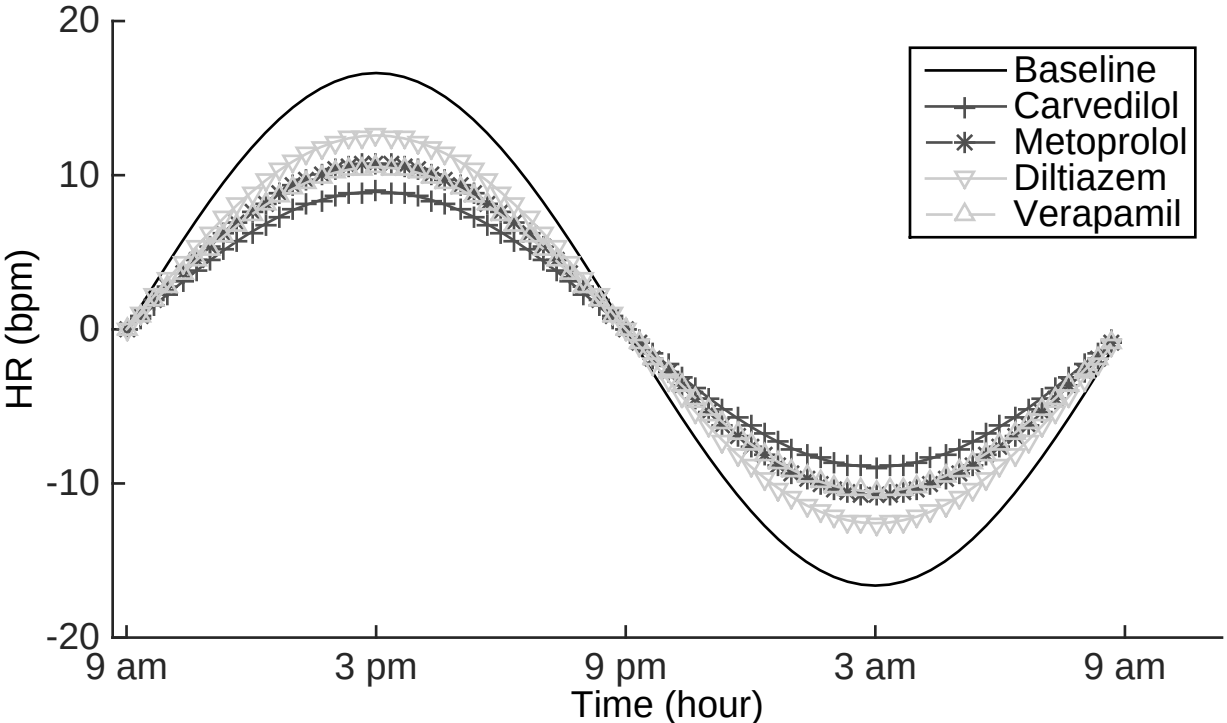
9 pm
9 am


20
10
0
-10
-20
HR (bpm)
9 am
3 pm
9 pm
3 am
9 am
Time (hour)
Baseline
Carvedilol
Metoprolol
Diltiazem
Verapamil