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**Beta-blockade and A1-adenosine receptor agonist effects on atrial fibrillatory rate and atrio-ventricular conduction in patients with atrial fibrillation.**

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## **Abstract**

**Aim:** Reduced irregularity of RR intervals in permanent atrial fibrillation (AF) has been associated with poor outcome. It is not fully understood, however, whether modification of AV conduction using rate control drugs affects RR variability and irregularity measures. We aimed at assessing whether atrial fibrillatory rate (AFR) and variability and irregularity of the ventricular rate are modified by a selective A1 adenosine receptor agonist tecadenoson, beta-blocker esmolol and their combination.

**Methods:** Twenty-one patients (age  $58 \pm 7$  years, 13 men) with AF were randomly assigned to either 75, 150 or 300  $\mu$ g i.v. tecadenoson. Tecadenoson was administered alone (Dose Period 1) and in combination (Dose Period 2) with esmolol (100  $\mu$ g/kg/min for 10 minutes then 50  $\mu$ g/kg/min for 50 minutes). Heart rate, AFR were estimated for every 10-min long recording segment. Similarly for every 10-min segment, variability of RR intervals was assessed, as SD, pNN20, pNN50, pNN80 and RMSSD, and irregularity was assessed by nonlinear measures such as regularity index (R) and approximate entropy (ApEn).

**Results:** A marked decrease in HR was observed after both tecadenoson injections, whereas almost no changes could be seen in AFR. The variability parameters were increased after the first tecadenoson bolus injection. In contrast, the irregularity parameters did not change after tecadenoson. When esmolol was infused, all the variability parameters further increased.

**Conclusion:** Modification of AV node conduction can increase RR variability but does not affect regularity of RR-intervals, or AFR.

**Keywords:** variability, irregularity, tecadenoson, RR series, atrial fibrillation frequency

## **Condensed abstract**

Aim of this study was to assess whether atrial fibrillatory rate (AFR) and variability and irregularity of the ventricular rate are modified by a selective A1 adenosine receptor agonist tecadenoson, beta-blocker esmolol and their combination.

Tecadenoson significantly decreased HR and increased variability of RR intervals, AFR and irregularity were unchanged.

**What's new? Max 150 words**

1. The selective A1-receptor agonist tecadenoson aiming at reduction of ventricular rate during atrial fibrillation, does not affect atrial fibrillatory rate. This suggests that selective A1-receptor agonist action does not shorten atrial refractoriness and lacks the atrial fibrillation provoking effects associated with non-selective adenosine receptor agonist.
2. Modification of AV node conduction using beta-blocker and A1-receptor agonist can increase RR variability but does not affect irregularity of RR-intervals. Relative stability of RR-irregularity measures during atrial fibrillation supports the use of non-linear indices of RR behaviour for outcome prediction in large-scale trials.

## **1. Introduction**

The irregular and usually high rate ventricular activity during AF is largely determined by atrioventricular (AV) node properties, being some atrial electrical impulses delayed, and other blocked by the AV node. However, the exact relationships between the atrial and ventricular rate during AF are not fully understood at this point. Electrophysiological factors such as intrinsic refractoriness of the AV node and concealed conduction are known to influence the ventricular response<sup>1</sup>. Due to the AV node intrinsic refractoriness, many of the impulses are blocked when reaching the AV node<sup>2</sup>. Concealed conduction of a single atrial impulse, occurring when the impulse traverses part of the AV node but is not conducted to the ventricles, influences the conduction of subsequent beat or beats<sup>3,4</sup>.

Although ventricular response during AF is highly irregular, it is not completely random on short<sup>5</sup> or long term analysis<sup>6</sup>, thus assessment of variability and irregularity of the RR series could provide useful insights into the arrhythmia. 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<sup>7,8,9,10</sup>. The very first study by Yamada<sup>7</sup> showed that a reduced RR irregularity in a 24-hour ambulatory ECG had an independent prognostic value for cardiac mortality during long-term follow-up in patients with chronic AF. More recently, in a post hoc analysis, reduced variability of RR intervals during AF, likely caused by autonomic dysfunction, was found to be an independent predictor of all cause mortality in patients with left ventricular dysfunction following myocardial infarction<sup>8</sup>. Reduced irregularity was an independent predictor of all cause mortality, as well as sudden death and heart failure progression in patients with mild to moderate heart failure<sup>9</sup>. Nevertheless, the effect of rate-control drugs on irregularity of ventricular response has not been studied in controlled settings.

Higher AF rate (AFR) has recently been associated to increased irregularity of the RR series<sup>7</sup> in a large population of patients with AF, taking various antiarrhythmic drugs. However, this study also indicated variable effects of AV blocking agents on ventricular response, being the RR irregularity measures stronger associated with AFR in patients not taking antiarrhythmic drugs while this correlation is much weaker in the treated patients, which likely results from the unequal effects of antiarrhythmic drugs on atrial and AV nodal electrophysiology<sup>11</sup>. Whether irregularity of ventricular response depends exclusively on the AV node properties or it is also affected by AFR is not fully elucidated at this point. It is not known to what extent AFR and irregularity of ventricular response are stable characteristics of fibrillatory process that can be considered intrinsic features of AF substrate or whether they are affected by drugs. The aim of the present study was to assess whether the atrial fibrillatory rate (AFR) and the variability and irregularity of the ventricular rate are modified by a selective A1 adenosine receptor agonist tecadenoson and beta-blocker esmolol.

## **2. Methods**

### **2.1 Tecadenoson**

Tecadenoson (CVT-510) is a selective A1-adenosine receptor agonist with an immediate onset of action (less than one minute) and a half-life of approximately 30 minutes<sup>12</sup> (but with no documented effect on ventricular conduction or refractory period) developed specifically to exploit the A1-adenosine receptor-mediated effect of slowing conduction through the AV node<sup>12,13</sup> while avoiding effects mediated by the A2 and A3 receptors (eg, vasodilation and bronchospasm as seen with adenosine)<sup>12,14</sup>.

### **2.2. Protocol**

The analysis is based on the data collected in a phase II, open-label, sequential-group, dose-escalation trial of tecadenoson administered i.v. alone and in combination with esmolol.

Detailed study protocol is as accessible via <http://www.clinicaltrials.gov/ct2/show/study/NCT00713401>. The study was aimed at assessment of tolerability and safety of a range of i.v. bolus doses of tecadenoson administered alone to patients with AF. By study protocol, twenty-one patients with AF in need of treatment for rate control but otherwise clinically stable were randomly assigned to receive either 75 (group A), 150 (group B) or 300 (group C) µg i.v. tecadenoson.

Tecadenoson was administered alone (Dose Period 1) and in combination (Dose Period 2) with esmolol (100 µg/kg/min for 10 minutes then 50 µg/kg/min for 50 minutes), a short-acting beta-blocker with a distribution half-life of two minutes and an elimination half-life after i.v. infusion of approximately nine minutes. The ECG recording started within 15 minutes prior to Dose Period 1. The start of esmolol infusion was to commence at least 75 minutes but no more than 150 minutes after the administration of the tecadenoson bolus injection in Dose Period 1. Following the tecadenoson bolus injection in Dose Period 2, ECG was recorded for 20 minutes. See Figure 1 for the protocol phases.

Any concomitant antiarrhythmic therapy (including AV nodal blocking agents) must have been temporarily discontinued from no later than 8:00 pm on the day prior to study drug dosing until completion of the last dose period assessment. Blood samples for plasma levels of antiarrhythmics and AV nodal blocking agents were collected prior to tecadenoson bolus in both Periods 1 and 2.

The study complied with the Declaration of Helsinki, the research protocol was approved by ethics committee, and the informed consent was obtained from all the subjects.

### **2.2.1. Phase definition**



The Holter recording was divided in non-overlapping 10-minute segments, thus the following segments were considered:

- baseline1: the first 10-minute segment, defined so that it ended at the time of the first tecadenoson bolus
- six post-dose1 segments, among whom the first one is named Tec (all patients had at least 60 minutes after the first tecadenoson bolus)
- baseline2: one 10-minute segment defined so that it ended at the time of the esmolol injection
- three 10-minute segments of esmolol maintenance, among whom the first one is named Esmo
- two post-dose2 segments after the second tecadenoson bolus, with esmolol still maintained, among whom the first one is named Tec+Esmo.

### **2.3. RR variability**

Time domain analysis includes the heart rate (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)<sup>15</sup>.

### **2.4. RR irregularity**

Irregularity of RR intervals was assessed by non-linear measures such as regularity index (R) and approximate entropy (ApEn). For a visual explanation of the difference between variability and irregularity of RR series see Figure 2.

#### **2.4.1. Approximate 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. Intuitively, 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<sup>16</sup>.

#### **2.4.2. Regularity**

Conditional entropy may be used to estimate a regularity index,  $R$ , defined as the degree of recurrence of a pattern in a signal. The conditional entropy represents the amount of information carried by the most recent sample of a normalized realization of the series when its past  $L-1$  samples are known.  $R$  tends to zero if the series is an unpredictable process and tends to one if the series is a periodic signal and it assumes intermediate values for those processes that can be partially predicted by the knowledge of the past samples<sup>17</sup>.

#### **2.5. Atrial fibrillatory rate**

The AFR was computed in one-minute segment using spatiotemporal QRST cancellation and time frequency analysis<sup>18</sup> and the resulting fibrillatory signal was downsampled to 50 Hz and subjected to spectral analysis. The time-frequency distribution of the atrial signal (obtained by short-term Fourier transform) was decomposed such that each spectrum can be modeled as a frequency-shifted and amplitude-scaled version of the spectral profile. This procedure is based on a spectral profile, dynamically updated from previous spectra, which is matched to each new spectrum using weighted least squares estimation<sup>19</sup>. The frequency shift needed to achieve optimal matching then yields a measure of instantaneous fibrillatory rate of a 2.5-s

ECG segment (overlapping with one segment each second) and was trended as a function of time. Frequencies were converted to fibrillatory rates with its unit fibrillations per minute (fpm, i.e., rate = frequency x 60). Mean fibrillatory rate (in fpm) was defined as the average of the instantaneous fibrillatory rates over the ten-minute ECG segment.

## **2.6. Statistical Analysis**

all the computed parameters were estimated for every 10-minute segment. A paired t-test or Wilcoxon-Mann-Whitney was applied for comparison between the different phases of the protocol for each dose regimen.

A p-value <0.05 was considered statistically significant. All analyses and statistical tests were performed using MATLAB® R2008a (The MathWorks, USA).

## **3. Results**

### **3.1. Patient characteristics and data availability**

In total, 21 patients (age  $58 \pm 7$  years, 13 men) were included in the study, seven in each dosing group A, B and C. Twelve patients had longstanding persistent AF (defined as AF duration > 12 months). Clinical characteristics are presented in Table 1. All patients dosed with tecadenoson were included in statistical summaries by dose regimen. One patient in group B was excluded from the study because of a lengthy gap in the ECG recording, beginning just before the second tecadenoson bolus dose administration and ending approximately one hour later.

For rate control purposes, three patients were treated with verapamil and six with bisoprolol. Nine patients received amiodarone prior to inclusion in the study. Blood samples were collected for antiarrhythmic agent plasma concentration prior to tecadenoson infusion start and appeared below the therapeutic concentrations in all but one patient (group A) who was

treated with amiodarone (102% of the lower limit).

Non-antiarrhythmic medications included vitamin K antagonists (76% took warfarin), ACE inhibitors (19% took captopril, 19% took enalapril, and 19% took perindopril), platelet aggregation inhibitors (29% took acetylsalicylic acid). There was no difference in drug use between the groups.

### **3.2. Dosage effect**

Figure 3 shows the trend of normalized HR and AFR at different dosages. In all groups, a marked decrease in HR can be observed after both tecadenoson injections, whereas almost no changes can be seen in AFR.

The first tecadenoson injection produced a decrease in HR of about 6% and in all patients but three (one of group A and two in group B). In particular, a decrease of  $5 \pm 5$  bpm ( $p < 0.05$ ),  $1 \pm 2$  bpm (ns) and  $8 \pm 6$  bpm ( $p < 0.01$ ) was found in group A, B and C, respectively, after the first bolus. Similar results were found after second tecadenoson injection ( $2 \pm 2$  bpm ( $p < 0.05$ ),  $3 \pm 5$  bpm (ns) and  $7 \pm 3$  bpm ( $p < 0.01$ ) in group A, B and C, respectively). Esmolol further decreased HR in most patients.

On the other hand, the AFR was unaffected immediately after the first tecadenoson injection, however, in groups B and C, esmolol decreased the AFR (Table 2). The second tecadenoson bolus on the top of ongoing esmolol infusion further decreased the AFR. In all dosage groups the combination of esmolol and tecadenoson resulted in lower AFR than tecadenoson alone (significant difference between Tec vs. Tec+Esmo).

Tables 2, 3, 4 present the variability and irregularity parameters values for the most relevant phases of the protocol.

In patients of group A, all the variability parameters were significantly increased after the first tecadenoson bolus injection: both in the ten-minute segment immediately after injection and after 30 minutes, i.e., half-life of tecadenoson (except for rMSSD). On the contrary, irregularity parameters did not change after tecadenoson. When esmolol was infused, all the variability parameters further increased (both compared to tecadenoson only and to baseline2). When assessing variability during the combination of tecadenoson and esmolol with tecadenoson alone they all were significantly higher.

In patients of group B, the variability parameters were not increased after the first tecadenoson bolus injection. During esmolol infusion and during its combination with tecadenoson, the parameters were significantly higher than during tecadenoson alone. In these patients, ApEn was significantly lower after esmolol and during its combination with tecadenoson when compared to tecadenoson alone.

In patients of group C, only the SD of RR series and rMSSD were significantly increased after the first tecadenoson bolus injection: both in the ten-minute segment immediately after injection and after 30 minutes. Irregularity parameters did not change after tecadenoson. When esmolol was infused, all the variability parameters further increased but not significantly. The combination of tecadenoson and esmolol significantly increased almost all the variability parameters in comparison to tecadenoson or esmolol alone.

Figure 4 shows the trend of a variability (rMSSD) and an irregularity (R) measure in the three groups of patients. It can be noted that the variability measure is affected by tecadenoson whereas the irregularity measure is not.

## **Discussion**

The main findings of this study suggest that the selective A1-receptor agonist tecadenoson

reduces HR, increases time-domain measures of heart rate variability without effect on irregularity parameters and has neutral effect on AFR. Beta-blockade with intravenous esmolol further increased all the variability parameters, decreased HR and AFR.

To the best of our knowledge, any long-term clinical benefit of modulation of variability and regularity of AV conduction during AF, apart from the effect of ventricular rate reduction, has not been demonstrated. Therefore, our findings should be interpreted as an attempt to clarify, in a controlled manner, the effect rate-control drugs have on AV conduction characteristics in order to assess reliability of RR variability and irregularity indices that appear to be linked to prognosis in patients with AF<sup>7,8,9</sup>. Reduced irregularity of the RR intervals in a 24-hour ambulatory ECG appeared to be an independent predictor of cardiac mortality during long-term follow-up in patients with chronic AF<sup>7</sup>. More recently, a reduced variability of RR intervals during AF during long-term follow-up in patients with chronic AF, likely caused by autonomic dysfunction, was found to be an independent predictor of all cause mortality in patients with left ventricular dysfunction following myocardial infarction<sup>10</sup> and in patients with mild to moderate heart failure<sup>9</sup>. Interpretation of the prognostic impact of RR-irregularity measures is, however, rather complex since majority of patients with permanent AF take rate-control medication. In our earlier study<sup>20</sup>, we did not observe any difference in RR-irregularity parameters during AF in patients with congestive heart failure regardless of the antiarrhythmic drug use. The current study, in which antiarrhythmics were administered in a controlled manner, demonstrate that RR-irregularity measures, which were significantly associated with the long-term outcome in earlier studies, seem to be unaffected by rate control using beta-blocker therapy and tecadenoson. Thus, the use of (at least) beta-blockers is not a concern that one should adjust the model for when assessing the hazard ratio of

reduced regularity in AF population. Both RR irregularity and AFR seem to be stable parameters not affected by rate-control drug tecadenoson or beta-blocker.

In regard to the AFR response to antiarrhythmic drug use, earlier studies have shown antiarrhythmic class I and class III drugs propensity to prolong atrial fibrillatory cycle length and thus reduce AFR. Procainamide<sup>21,22,23,24</sup>, propafenone<sup>21</sup>, disopyramide<sup>25</sup>, cibenzoline<sup>26</sup>, sotalol<sup>27</sup> and ibutilide<sup>24,28</sup> have all been shown to reduce the average frequency of fibrillatory activity. The magnitude of slowing appears to correlate with the drug effect. Boahene et al.<sup>21</sup> noted that procainamide- and propafenone-induced slowing of atrial cycle length was greater in patients who were successfully converted from AF to sinus rhythms.

In addition, some rate control drugs such as verapamil have also been reported to reduce AFR but not to the extent sufficient for restoration of sinus rhythm<sup>29</sup>. In regard to the beta-blockade, their effect on the AFR slowing has been uncertain even though beta-blockers possess moderate antiarrhythmic effect against AF recurrence<sup>30</sup> and esmolol administration was associated with higher rate of sinus rhythm restoration in patients with AF after coronary artery bypass surgery<sup>31</sup>. In one study by Sticherling et al.<sup>32</sup>, the effect of esmolol on atrial fibrillatory cycle length was assessed in patients with pacing-induced AF and appeared to be neutral. Our study included patients with permanent AF and showed that beta-blocker therapy alone, at least when given intravenously, can result in AFR reduction in a similar manner, even though in a lesser extent, as earlier reported for class I and class III antiarrhythmics.

Finally, the lack of tecadenoson effect on AFR suggests that despite potent effect of A1-adenosine receptor agonist on AV conduction resulting in significant slowing of ventricular response, electrophysiological properties of atrial myocytes are minimally affected. This in

contrast to the effect of non-selective A-receptor agonist adenosine that is used in acute treatment of supraventricular tachycardias but its use can provoke AF and is associated with shortening of atrial refractoriness<sup>33</sup>. In the atrial-paced isolated guinea-pig heart, tecadenoson has shown its potential to shorten the atrial, but not ventricular, monophasic action potential<sup>13</sup>, which could however not be translated in modification of AFR in our clinical study. Apart from the difference between species that might explain the lack of tecadenoson effect on AFR, the patients who received tecadenoson in our study had significant cardiovascular comorbidities that may have had impact on the atrial substrate and associated with atrial structural remodelling that would affect responsiveness of atrial myocytes to A1-receptor agonist.

In conclusion, modification of AV node conduction using beta-blockade and A1-receptor agonist can increase RR variability but does not affect irregularity of RR-intervals. Relative stability of RR-irregularity measures during AF supports the use of non-linear indices of RR behavior, such as ApEn, for prediction of clinical outcome in patients with AF in large-scale trials. Esmolol possesses modest effect on AFR slowing in patients with clinical AF while tecadenoson did not show AF provoking effect associated with non-selective adenosine receptor agonist.

### **Study Limitations**

Even though antiarrhythmic drugs were discontinued at least 24 hours prior to start of the study drug infusion, as per study protocol, this is certainly less than five half-lives commonly used in electrophysiological studies. We can therefore not completely rule out the residual effect of concomitant medications on AV conduction. However, plasma concentrations of anti-



arrhythmic drugs were checked prior to infusion start and were found to be below the therapeutic range in all but one subject who was treated with amiodarone.

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**Conflict of interest:** none declared.

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Table 1. Demographic characteristics and cardiovascular history in study population

| Variable             | Group A (7) | Group B (6) | Group C (7)  |
|----------------------|-------------|-------------|--------------|
| Age (yrs)            | 57 ± 9      | 58 ± 7      | 58 ± 8       |
| Gender (male/female) | 6/1         | 3/3         | 4/3          |
| AF duration (months) | 14 (1-168)  | 20.5 (1-60) | 60 (0.5-122) |
| BMI                  | 27.4 ± 2.8  | 29.0 ± 2.5  | 26.9 ± 3.5   |
| Heart failure        | 3           | 3           | 4            |
| Diabetes             | 0           | 0           | 0            |
| Hypertension         | 6           | 5           | 4            |
| Dyslipidemia         | 3           | 3           | 4            |
| Previous MI          | 0           | 0           | 0            |

Group A (patients taking tecadenoson dose 75), Group B (patients taking tecadenoson dose 150), Group C (patients taking tecadenoson dose 300). AF duration is reported as median and range (minimum-maximum).

**Table 2:** Mean and SD of computed parameters for the most significant phases of the protocol for group A (patients taking tecadenoson dose 75).

|                    | <b>Baseline1</b> | <b>Tec</b>  | <b>Baseline 2</b> | <b>Esmo</b> | <b>Tec+Esmo</b> |
|--------------------|------------------|-------------|-------------------|-------------|-----------------|
| <b>HR (bpm)</b>    | 94 ± 16          | 91 ± 14*    | 88 ± 13           | 85 ± 10°+   | 83 ± 11 °# §    |
| <b>AFR (fpm)</b>   | 437 ± 30         | 436 ± 30    | 432 ± 30          | 428 ± 22+   | 427 ± 28§       |
| <b>SD (ms)</b>     | 142 ± 31         | 154 ± 32 *  | 161 ± 35          | 171 ± 32 °+ | 177 ± 40 °§     |
| <b>pNN20 (%)</b>   | 90 ± 3           | 92 ± 2 *    | 92 ± 3            | 92 ± 2      | 92 ± 1          |
| <b>pNN50 (%)</b>   | 74 ± 5           | 78 ± 4 *    | 79 ± 5            | 80 ± 4+     | 80 ± 3 §        |
| <b>pNN80 (%)</b>   | 64 ± 7           | 67 ± 5*     | 69 ± 6            | 71 ± 5 °+   | 70 ± 4 §        |
| <b>rMSSD (ms)</b>  | 196 ± 45         | 217 ± 44 *  | 227 ± 49          | 241 ± 43°+  | 250 ± 55°§      |
| <b>ApEn (a.u.)</b> | 1.63 ± 0.08      | 1.62 ± 0.06 | 1.61 ± 0.07       | 1.59 ± 0.06 | 1.61 ± 0.08     |
| <b>R (a.u.)</b>    | 0.04 ± 0.02      | 0.04 ± 0.02 | 0.04 ± 0.01       | 0.05 ± 0.01 | 0.04 ± 0.02     |

\* p<0.05 comparison with Baseline1, ° p<0.05 comparison with Baseline2 , # p <0.05 Esmo vs Tec+esmo, § p<0.05 Tec vs Tec+Esmo

**Table 3:** Mean and SD of computed parameters for the most significant phases of the protocol for group B (patients taking tecadenoson dose 150).

|                    | <b>Baseline1</b> | <b>Tec</b>  | <b>Baseline2</b> | <b>Esmo</b>  | <b>Tec+Esmo</b> |
|--------------------|------------------|-------------|------------------|--------------|-----------------|
| <b>HR (bpm)</b>    | 87 ± 9           | 86 ± 8      | 83 ± 8           | 81 ± 7+      | 79 ± 9 ° §      |
| <b>AFR (fpm)</b>   | 426 ± 90         | 427 ± 90    | 422 ± 91         | 418 ± 93 °+  | 415 ± 97°§      |
| <b>SD (ms)</b>     | 163 ± 20         | 166 ± 14    | 175 ± 13         | 181 ± 23+    | 194 ± 28 #§     |
| <b>pNN20 (%)</b>   | 92 ± 2           | 91 ± 1      | 93 ± 2           | 93 ± 1+      | 93 ± 2          |
| <b>pNN50 (%)</b>   | 80 ± 2           | 79 ± 3      | 80 ± 4           | 81 ± 2+      | 81 ± 3 §        |
| <b>pNN80 (%)</b>   | 70 ± 4           | 69 ± 4      | 71 ± 4           | 72 ± 4+      | 72 ± 5 §        |
| <b>rMSSD (ms)</b>  | 229 ± 26         | 233 ± 16    | 243 ± 29         | 252 ± 33     | 269 ± 37§#      |
| <b>ApEn (a.u.)</b> | 1.61 ± 0.04      | 1.62 ± 0.05 | 1.60 ± 0.03      | 1.58 ± 0.02+ | 1.55 ± 0.04§#   |
| <b>R (a.u.)</b>    | 0.05 ± 0.01      | 0.04 ± 0.02 | 0.06 ± 0.03      | 0.06 ± 0.02  | 0.04 ± 0.02     |

° p<0.05 comparison with Baseline2, + p<0.05 Esmo vs. Tec, # p <0.05 Esmo vs Tec+Esmo, § p<0.05 Tec vs Tec+Esmo



**Table 4:** Mean and SD of computed parameters for the most significant phases of the protocol for group C (patients taking tecadenoson dose 300).

|                    | <b>Baseline1</b> | <b>Tec</b>  | <b>Baseline2</b> | <b>Esmo</b> | <b>Tec+Esmo</b> |
|--------------------|------------------|-------------|------------------|-------------|-----------------|
| <b>HR (bpm)</b>    | 96 ± 17          | 87 ± 11*    | 86 ± 8           | 85 ± 10     | 76 ± 9 °§#      |
| <b>AFR (fpm)</b>   | 407 ± 49         | 409 ± 47    | 422 ± 46         | 397 ± 46°+  | 393 ± 50°§      |
| <b>SD (ms)</b>     | 123 ± 21         | 136 ± 27 *  | 139 ± 26         | 142 ± 19    | 174 ± 30°#§     |
| <b>pNN20 (%)</b>   | 88 ± 3           | 89 ± 2      | 91 ± 1           | 90 ± 2      | 92 ± 1 §        |
| <b>pNN50 (%)</b>   | 71 ± 7           | 73 ± 5      | 75 ± 2           | 76 ± 3      | 78 ± 3 °§#      |
| <b>pNN80 (%)</b>   | 59 ± 10          | 61 ± 6      | 63 ± 5           | 65 ± 5+     | 68 ± 4 °§#      |
| <b>rMSSD (ms)</b>  | 161 ± 38         | 182 ± 39 *  | 191 ± 37         | 196 ± 28    | 235 ± 37°§#     |
| <b>ApEn (a.u.)</b> | 1.61 ± 0.06      | 1.59 ± 0.07 | 1.59 ± 0.05      | 1.60 ± 0.07 | 1.54 ± 0.07§#   |
| <b>R (a.u.)</b>    | 0.08 ± 0.03      | 0.06 ± 0.02 | 0.04 ± 0.01      | 0.05 ± 0.03 | 0.06 ± 0.03     |

\* p<0.05 comparison with Baseline1, ° p<0.05 comparison with Baseline2, + p<0.05 Esmo vs. Tec, # p <0.05 Esmo vs Tec+Esmo, § p<0.05 Tec vs Tec+Esmo

### Figures caption:

**Figure 1:** Protocol phases and drugs timing. Tecadenoson was administered alone (Dose Period 1) and in combination with esmolol (Dose Period 2). The ECG recording started within 15 minutes prior to Dose Period 1 and was continuously recorded throughout the whole protocol. The start of esmolol infusion was to commence at least 75 minutes but no more than 150 minutes after the administration of the tecadenoson bolus injection in Dose Period 1. ECG recording continued until 20 min after the tecadenoson bolus in Dose Period 2.

**Figure 2:** 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 3:** Trend of normalized heart rate (black circles) and normalized atrial fibrillatory rate (empty circles) plus standard deviation, for the three groups of patients taking (a) 75, (b) 150 and (c) 300 mcg. Each dot represents the value of a 10-minute segment, the timing of tecadenoson injections is shown by the dashed line, while the grey area represents esmolol maintenance. A significant decrease of HR can be noted after both tecadenoson injections, whereas AFR remains unchanged. The first seven segments are normalized to baseline1 (1<sup>st</sup> point) whereas the others are normalized to baseline2 (9<sup>th</sup> point).

**Figure 4:** Trend of rMSSD (a variability parameter) and R (an irregularity parameter) plus standard deviation for the three groups of patients taking (a) 75, (b) 150 and (c) 300 mcg. Each dot represents the value of a 10-minute segment, the timing of tecadenoson injections is shown by the dashed line, while the grey area represents esmolol maintenance. A significant increase of rMSSD can be noted after both tecadenoson injections, whereas R remains unchanged.

**Figure 1**

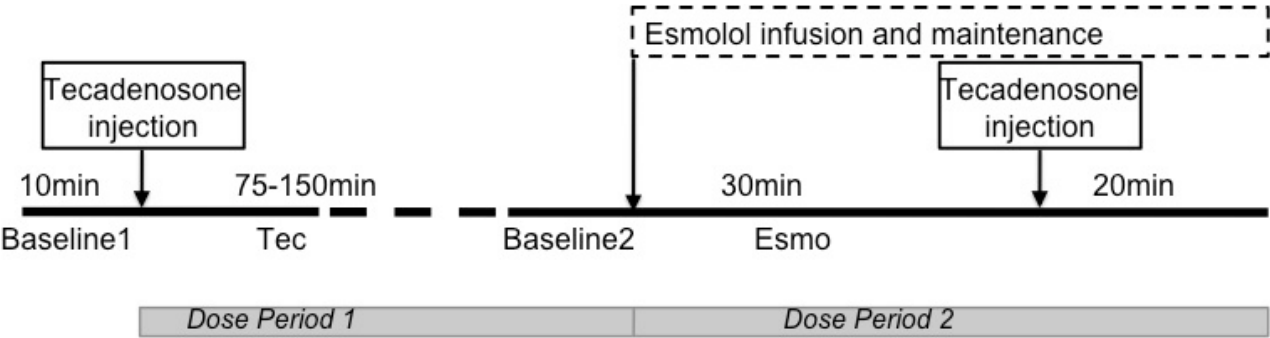


Figure 2

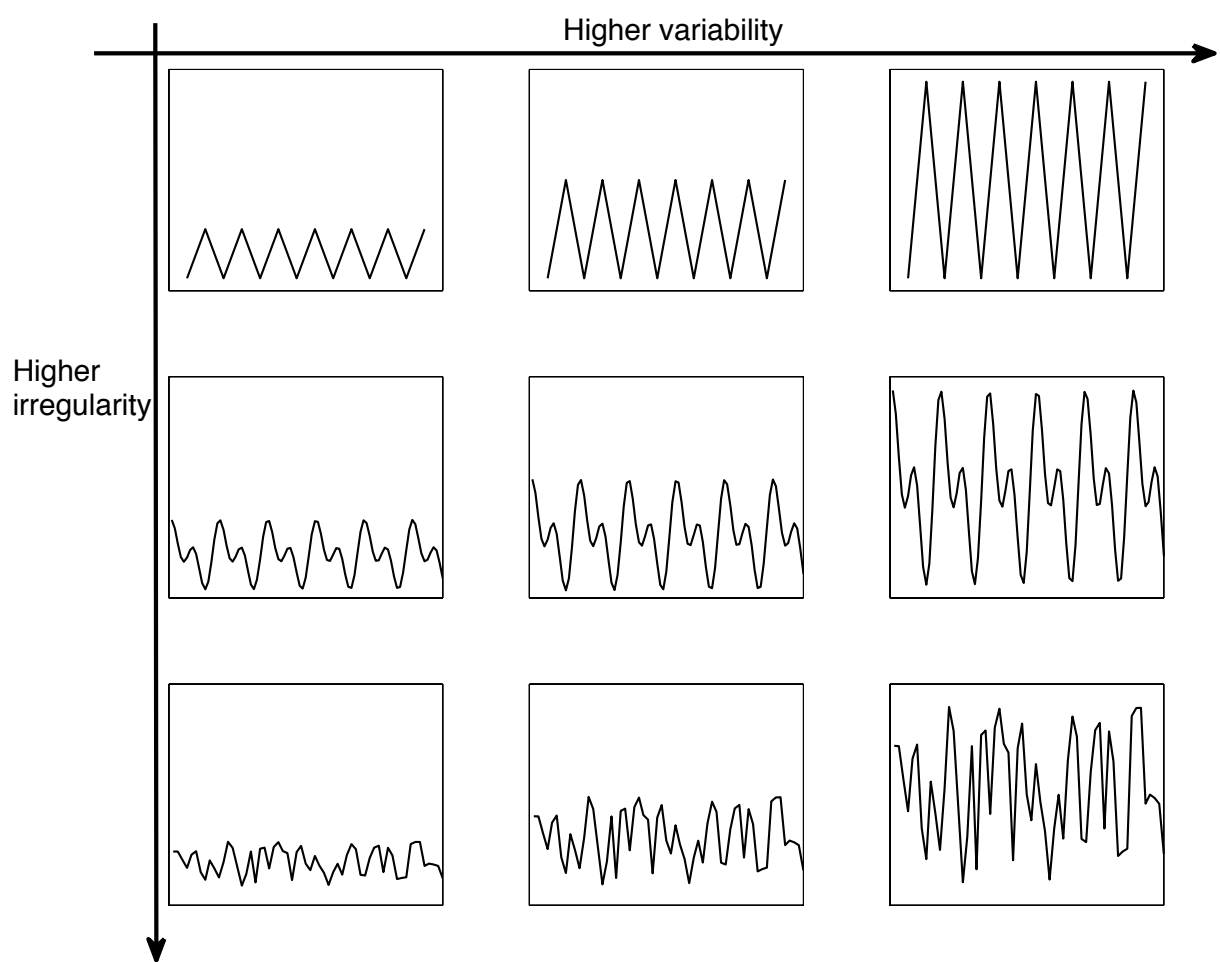
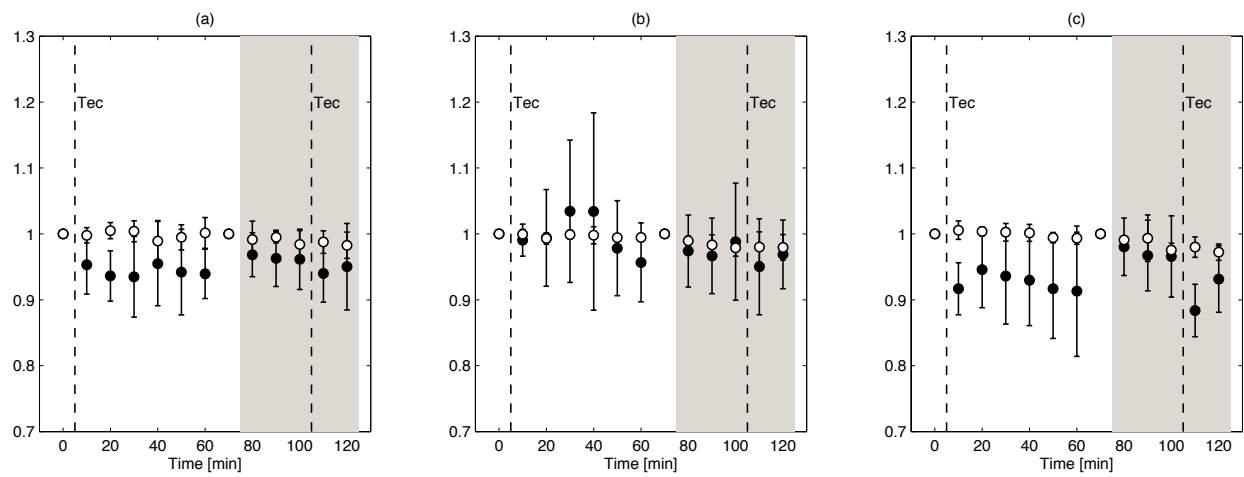


Figure 3



**Figure 4:**

