# In-vivo and Ex-vivo HRV Discrimination by Complex Correlation Measure

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## Abstract

*Heart rate variability (HRV) in in-vivo and isolated hearts was evaluated by complex correlation measure (CCM) and Poincaré descriptors SD1 and SD2. CCM quantifies temporal aspect of Poincaré plot; SD1 and SD2 quantify final shape of Poincaré diagram only.*

*Five in-vivo and five isolated New Zealand rabbit hearts were used in the study.*

*When used for distinguishing between in-vivo and ex-vivo condition of the heart, CCM has been proved as a more sensitive quantifier of Poincaré plot as compared to SD1 and SD2. Decrement of CCM, caused by heart excision, was statistically significant in contrast to decrements of SD1 and SD2, respectively.*

## 1.    Introduction

Poincaré plot is a valuable technique for heart rate variability (HRV) assessment due to its ability to visualise nonlinear aspect of time series. Poincaré plot can be evaluated by visual perception, where categorization of diagram shape provides information about heart condition. Another approach for Poincaré plot evaluation consists in quantification of resulting diagram by descriptors SD1 and SD2 [1].

However, descriptors SD1 and SD2 can quantify final diagram only. In contrast, recently published descriptor Complex Correlation Measure (CCM) can quantify the temporal aspect of the Poincaré plot [2]. In the depressed patients, CCM has been proved as a more sensitive measure of HRV, compared to SD1 and SD2 of the Poincaré plot distribution [3].

The aim of this study was to compare sensitivity of CCM with that of SD1 and SD2 in in-vivo and ex-vivo conditions.

## 2.    Methods

### 2.1.    In-vivo hearts

ECG signals of five New Zealand rabbits were included in the study. The signals were recorded using a SEIVA recording system. Body surface wire electrodes were attached to the skin with miniature clips. Location of electrodes did not restrict free posturing of the animal in sitting position. In order to get stable signals in awaked animals, rabbits were placed in a plastic box. The box was sufficiently high for preventing of rabbit's looking out, which makes the animal restless.

### 2.2.    Isolated hearts

All experiments followed the guidelines for animal treatment approved by local authorities and conformed to the EU law. Five New Zealand rabbits were included in the study. Their isolated hearts were perfused according to Langendorff in the mode of constant perfusion pressure (85mmHg). In deep anaesthesia with xylasin and ketamin, the hearts were excised and fixed on perfusion apparatus filled with Krebs-Henseleit (K-H) solution ($1.25mMCa^{2+}$, 37°C) and placed in a thermostatically-controlled bath. The hearts were stabilized for 30 minutes.

ECG signal was recorded by touch-less method [4, 5]. Briefly, three orthogonal Ag-AgCl disc electrodes were placed in the walls of the bath which is a part of the perfusion system. ECG signals were recorded by data acquisition multifunction card PCI-6111E (National Instruments, USA) with sampling frequency of 2000Hz. ECG signals were acquired by software designed in LabView 7.1 (Texas Instrument, 2008). The 12-bit analogue to digital conversion was used. The digital signal was stored on a hard disk for off-line processing.

## 2.3. Data processing and analysis

ECG signals degraded by noise were excluded from further processing. R-peaks were detected automatically by own R-wave detector designed in Matlab R2006a (MathWorks, 2006). The results of automatic analysis were reviewed and errors in detection were corrected manually.

Parameters SD1, SD2, and CCM from five-minute long segments of ECG signal were computed in custom made software in Matlab R2006a (MathWorks, 2006).

SD1 was computed from Poincaré diagram as standard deviation of the points perpendicular to the line-of-identity ($RR_j = RR_{j+1}$). SD2 was computed in the same manner as standard deviation of the points along the line-of-identity.

CCM was computed in windowed manner, which embeds the temporal information of the signal [3]. Moving window with a length of three consecutive points has been one-sample shifted over *N-2* points of Poincaré plot, where *N* represents total number of Poincaré plot points. CCM composed of all overlapping windows was calculated as [3]:

$$CCM = \frac{1}{Cn(N-2)} \sum_{i=1}^{N-2} \|A(i)\|$$

where $Cn$ represents the normalizing constant, which is equal to $\pi \cdot SD1 \cdot SD2$, N represents total number of points in Poincaré plot sequence, *A* represents the area of the triangle formed by three points, and *i* is the number of windows.

Ability to distinguish between in-vivo and isolated heart was compared in CCM and standard Poincaré plot parameters SD1 and SD2. Two-sample t-test has been used for evaluating statistically significant ($\alpha = 0.05$) differences between in-vivo and ex-vivo conditions.

## 3. Results

Spontaneously beating in-vivo heart beats with high variability. Points of Poincaré plot are therefore widely scattered around line-of-identity. A typical representative of in-vivo Poincaré plot with its specific shape is shown in Figure 1.

Excision of the heart from the body significantly decreases heart rate variability. Ex-vivo Poincaré plot has compact shape with points situated closely one to other. Ex-vivo Poincaré plot is depicted in Figure 2. Both figures are drawn with the same scale.

Besides low variability, the extraction of the heart from the animal causes prolongation of RR interval.

Quantifiers of Poincaré diagram, reflecting extraction-based decrement of heart rate variability, decrease in
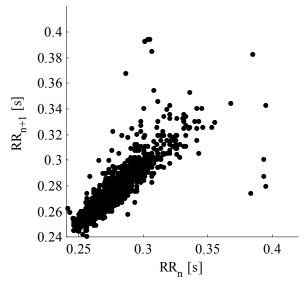


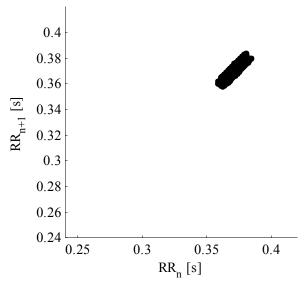Figure 1. A typical representative of in-vivo Poincaré plot.



Figure 2. A typical representative of ex-vivo Poincaré plot. Scale is the same as in Figure 1.

all three studied parameters: SD1, SD2, and CCM. SD1 decreases from 5.6±2.3ms in in-vivo hearts to 4.5±6.7ms in ex-vivo hearts, respectively. Difference between in-vivo and ex-vivo HRV, represented by Poincaré descriptor SD1, is shown in Figure 3.

SD2 decreased from 19.5±6.6ms in-vivo hearts to 11.6±8.4ms in ex-vivo hearts, respectively. It can be seen in Figure 4.

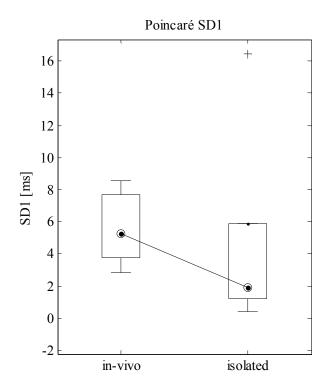It is worth mentioning, that both decrements are insignificant.

Figure 3. Difference between in-vivo and ex-vivo HRV, represented by Poincaré descriptor SD1.
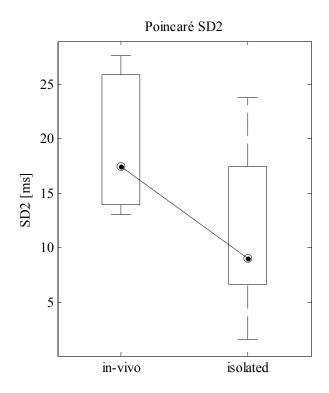


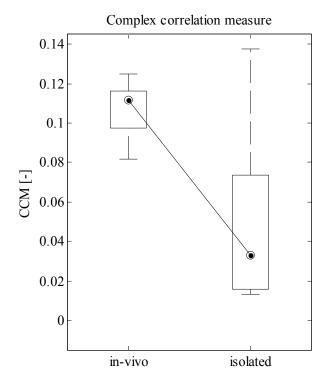Figure 4. Difference between in-vivo and ex-vivo HRV, represented by Poincaré descriptor SD2.



Figure 5. Difference between in-vivo and ex-vivo HRV, represented by parameter CCM.

Unitless parameter CCM decreases from 0.1±0.01 in in-vivo hearts to 0.03±0.05 in the ex-vivo hearts, respectively. CCM decrement is statistically significant.

All changes of Poincaré plot are summarized in Table 1. It can be clearly seen that only CCM has ability to distinguish significantly between in-vivo and ex-vivo Poincaré plot.

Table 1. Comparison of standard Poincaré descriptors and CCM parameter in in-vivo and ex-vivo hearts.

|          | in-vivo  | isolated  | statistically significant difference |
|----------|----------|-----------|--------------------------------------|
| SD1 [ms] | 5.6±2.3  | 4.5±6.7   | -                                    |
| SD2 [ms] | 19.5±6.6 | 11.6±8.4  | -                                    |
| CCM      | 0.1±0.01 | 0.03±0.05 | $\alpha < 0.05$                      |

# 4. Conclusions

The complex correlation method, a measure of non-linear HRV that evaluates the beat-to-beat dynamics, has been investigated in this study. Sensitivity of CCM in in-vivo and ex-vivo conditions has been compared with SD1 and SD2, standard non-linear quantifiers obtained from the Poincaré plot distribution. CCM has been proved as a more sensitive quantifier of Poincaré plot as compared to SD1 and SD2, when used for distinguishing between in-vivo and ex-vivo conditions of the heart.

Isolation of the heart increases almost two-times RR interval duration. The same effect has been observed in isolated rat hearts [6]. Despite prolongation of RR interval, HRV is noticeably decreased by heart isolation. It may be caused by elimination of sympathetic and parasympathetic nervous system influence on the heart. HRV persists even in isolated heart, indicating that heart has its own controlling mechanism(s) for heart rate modulation. Further studies are needed for clarification of the effects of heart isolation on HRV.

## References

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