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Poincaré plot indexes of heart rate variability capture dynamic adaptations after haemodialysis in chronic renal failure patients

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Summary

The hypothesis that the Poincaré plot indexes of heart rate variability (HRV) detect dynamic changes after haemodialysis (HD) over the HRV in haemodynamically stable chronic renal failure (CRF) patients was examined. Minor axis (SD1), major axis (SD2) and the SD1/SD2 ratio were compared against standard HRV indexes in time and frequency domain, in a group of healthy subjects and in a group of CRF patients before and after HD. These indexes were estimated from Poincaré plots reconstructed with lags of one, two and four heartbeats. The surrogate data analysis technique was applied in order to discern if only random components or linear features of HRV contribute to its dynamics. None of the standard linear HRV indexes changed after HD. The Poincaré plot indexes measured from CRF patients were smaller than the ones measured from healthy subjects. In CRF patients the SD1/SD2 ratio decreased after HD, when a lag of four heartbeats was used ($0{\cdot}68 \pm 0{\cdot}19$ before HD versus $0{\cdot}55 \pm 0{\cdot}12$ after HD, $P<0{\cdot}05$). The presence of deterministic components in HRV were confirmed for all measures of the SD1/SD2 ratio. Moreover, a loss of non-linear components after HD was detected by the surrogate analysis over the SD1/SD2 ratio with a lag of four heartbeats. In conclusion, the SD1/SD2 ratio measured at lag of 4 heartbeats capture dynamic changes after HD upon the HRV of CRF patients that are not solely related to linear autocorrelations of HRV. This suggests that the SD1/SD2 ratio reflects non-linear information of HRV.

Introduction

The Poincaré plot analysis is a geometrical and non-linear method to assess the dynamics of heart rate variability (HRV) (Tulppo *et al.*, 1996, 1998; Toichi *et al.*, 1997; Hayano *et al.*, 1999). The Poincaré plot is a representation of a time series into a cartesian plane (or phase space), where the values of each pair of successive elements of the time series define a point in the plot. The theoretical background that supports the use of a phase space is the Takens theorem (Takens, 1981). According to Takens, it is possible to reconstruct the attractor of a dynamical system by mapping a scalar measurement into a phase space using a given time delay and embedding dimension. The Poincaré plot is a very simplified phase space with dimension two and delay or lag of one beat (i.e. each R-R interval is plotted as a function of the previous R-R interval). The 'true' attractor of HRV is certainly not displayed by the Poincaré plot as the HRV has a higher estimated dimension than two (Bogaert *et al.*, 2001). However, the Poincaré plot gives a useful visual contact to the R-R data by representing both short- and long-term variations included in the recording (Tulppo *et al.*, 1996).

Analysis of Poincaré plots can be performed by a simple visual inspection of the shape of the attractor (like torpedo or butterfly shape), which has been used to classify the signal (Hayano *et al.*, 1999). In chronic renal failure (CRF) patients this approach has proved to be useful to evaluate the survival prognosis in the presence of coronary disease (Hayano *et al.*, 1999). However, the assessment and standardization of these qualitative classifications are difficult because they are highly subjective. A quantitative analysis of the HRV attractor displayed by the Poincaré plot can be made by adjusting it to an ellipse (Tulppo *et al.*, 1996). Using this technique, three indexes can be obtained: the standard deviation (SD) of the instantaneous beat-to-beat R-R interval variability (minor axis of the ellipse or SD1), the SD of the long term R-R interval variability (major axis of the ellipse or SD2) and the axes ratio (SD1/SD2). It has been shown that SD1 and SD2 can be expressed as second-order statistical moments or as autocorrelation functions, as long as

the stationary condition is met (Brennan *et al.*, 2001). However, as it is known that HRV is highly non-stationary (Braun *et al.*, 1998), we expect that some features of the HRV (probably of non-linear nature) could be detected by the Poincaré plot indexes. In fact, it was found that statistical and spectral indexes are highly correlated with both axes of the ellipse but not with the axes ratio at rest (Tulppo *et al.*, 1996). In this work we used the surrogate analysis (Theiler *et al.*, 1992) in order to prove two different null hypothesis about the behaviour of the HRV time series: (a) HRV is a random process of independent random variables and (b) HRV is a linear autocorrelated Gaussian process. If the first hypothesis is rejected, then the original HRV time series may be generated by deterministic mechanisms and their corresponding SD1/SD2 ratio would contain information about these mechanisms. If the second hypothesis is rejected, then the original HRV time series could contain non-linear information that is captured by the estimated SD1/SD2 ratio.

The original proposal of Poincaré plot indexes included a lag of one beat only, with the aim to explore the HRV dynamics in terms of 'beat-to-beat' basis (Tulppo *et al.*, 1996). Then the lag was naturally adjusted to the R-R mean (i.e. the greater the R-R mean the greater is the time lag). In our study, besides the lag of one beat, we tested the influence of longer lags (two and four beats) over the Poincaré plot indexes. It is expected that greater lags would increase the size of SD1 and would decrease SD2, thereby increasing the SD1/SD2 ratio.

The standard methods for analysing HRV are statistical, geometrical and power spectral analysis (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Power spectral analysis revealed the potential to measure autonomic nervous system activity in a non-invasive way, causing great expectation about its application in physiology and medicine (Akselrod *et al.*, 1981; Eckberg, 1997). For example, it has been observed that there is a significant loss of variance in the HRV of CRF patients, which is reflected in the reduction of total power in the power spectra (Cloarec-Blanchard *et al.*, 1992). More importantly, it has been shown that there is a dominant activity of the sympathetic nervous system in these patients (Cloarec-Blanchard *et al.*, 1992). This has clinical relevance since it is known that augmented sympathetic activity is related to an increased electrical instability of the myocardium and the increased occurrence of cardiac dysrhythmias in CRF patients (Thomson *et al.*, 1991; Deligiannis *et al.*, 1999). The spectral analysis technique is able to discriminate between hypotension-resistant and hypotension-prone patients during haemodialysis (HD) sessions where blood pressure and heart rate were not different between groups (Cavalcanti *et al.*, 1998). However, time and frequency domain methods have some technical limitations such as the stationary requirement and the linear assumptions in which these techniques are based. Moreover, the standard linear indexes of HRV did not change during uncomplicated HD and hypotension-prone patients only exhibit a reduced low frequency to high frequency ratio (LF/HF) during HD (Ligtenberg *et al.*, 1996; Cavalcanti *et al.*, 1998). Therefore, the potential effect of HD over the cardiac dynamics in hypotension-resistant patients is not evident by these linear analysis techniques.

The objectives of this work were: (i) to compare the Poincaré plots indexes of HRV of CRF patients against the ones of healthy subjects, (ii) to evaluate if the Poincaré plot indexes of HRV change after the HD in haemodynamically stable patients and (iii) to discern if there is non-linear information hidden in the SD1/SD2 ratio as measured from Poincaré plots of HRV. At the end of the paper we discuss the physiological implications of the present results.

Subjects and methods

Subjects

We studied a group of 10 clinically healthy subjects and another group of 10 CRF patients. Each group had five women and five men paired by sex and age between groups. To select a participant, each candidate was clinically examined by a physician and underwent a conventional 12-lead electrocardiogram (ECG). All subjects gave their informed consent to participate in this study. This work was approved by the Ethics Committee of the hospital (Instituto Nacional de Cardiología 'Ignacio Chávez', México).

The volunteers selected for the healthy group fulfilled the following inclusion criteria: normal clinical diagnosis from the physical examination, normal 12-lead ECG, sex and age matched to one of the CRF patients previously selected, and no known history of diabetes mellitus, cardiovascular disease or any other kind of chronic or acute disease.

The selected stable CRF patients had HD performed three times weekly for more than 6 months and fulfilled the following inclusion criteria: creatinine extraction less than 5 ml per min, left ventricular ejection fraction more than 40%, no history of primary cardiovascular disease or diabetes mellitus, and none were receiving drugs known to affect the autonomic nervous system, anti-hypertensive drugs or erythropoietin. None of the selected patients had had experience of atrial fibrillation or conduction disorders in ECG. The selected patients did not suffer hypotension episodes during the previous six HD treatments. The hypotension episode was defined as a fall of systolic blood pressure below 95 mmHg with either a drop in blood pressure of at least 20 mmHg or with the presence of symptoms related to hypotension.

Equipment characteristics

We recorded lead II ECG using an equipment with the following characteristics: an amplifier with a common mode rejection ratio of 80 dB at 60 Hz and gain of 25, an antialiasing low-pass filter with cut-off frequency of 45 Hz and a high-pass filter with cut-off frequency of 0·5 Hz; total gain was 500. An analog-to-digital converter with a resolution of 10 bits, conversion time of 30 μs, a full scale range of ±5 V and sampling frequency of 250 Hz was used (Infante *et al.*, 1988). ECG were recorded and

monitored in real time by means of a computer program developed with Turbo Pascal 7·0., running in a Pentium PC.

Study protocol

We obtained two ECG records for each participant; the first began at 9 AM and the second at 4 PM. In renal failure patients, HD treatment was applied between the first record (in the morning) and the second one (in the afternoon). During the recording, each participant was in the supine position and at the end of each recording, the blood pressure was measured by conventional sphygmomanometry. All CRF patients were haemodynamically stable during HD.

Haemodialysis prescription

Haemodialysis was performed with volumetrically controlled ultrafiltration (2008, Fresenius, Bad Homburg, Germany). Each treatment session lasted 3–5 h, with blood flow rates between 300 and 500 ml min^{-1}, and bicarbonate dialysate flow rates of 500 ml min^{-1}. All patients received biocompatible synthetic high-flux membranes (polysulphone, Fresenius).

R-wave detection process

The R-wave was detected by a second-derivative algorithm which has been tested previously with synthetic ECG signals of heart rate fixed and different signal-to-noise ratios, and with ECG from normal volunteers at rest and supine position (Infante *et al.*, 1992; Lerma *et al.*, 2000). The R-wave detection has a maximum error of identification of four samples before and after the fiducial point where sampling frequency is 250 Hz. This gives a maximum possible error of eight samples (0·032 s). This can originate aliased frequencies around 31·25 Hz, which is far away from 0·4 Hz, the maximum frequency of interest of HRV (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Detection and elimination of artefacts and ectopic beats and analysis of HRV time series were performed according to standards (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996) in a system developed with LabVIEW 5·0 (Lerma *et al.*, 2000). Automatic detection of the R-wave was manually inspected to guarantee that no more than 5% beats were missed in the detection process.

HRV analysis

The HRV analysis was performed by measuring time domain, frequency domain and Poincaré plot indexes over the first 5000 heartbeats recorded. The *time domain indexes* were: mean, SD of all normal R-R intervals and SD of the differences between successive R-R intervals. The *spectral analysis indexes* calculated were: total power, low frequency (LF) band power, high frequency (HF) band power and the LF/HF ratio. The power spectrum was calculated using a fast Fourier transform algorithm. To obtain the indexes, the area under different bands of the power spectrum was measured. Total power was computed considering the frequency range from 0·003 to 0·4 Hz the mean power of the LF band was obtained from 0·04 to 0·15 Hz and the mean power of the HF band was obtained from 0·15 to 0·4 Hz. Measurements of LF and HF power components were made in absolute values of power (ms^2) and normalized units (n.u.) which represent the relative value of each power component in proportion to the total power minus the power below 0·04 Hz. It is generally considered that the LF power reflects sympathetic and vagal tone, the HF reflects purely vagal tone, and the LF/HF ratio reflects the sympatho–vagal balance, although there is still some debate about the physiological mechanisms that are actually measured on the different frequency bands of the power spectrum (Akselrod *et al.*, 1981; Eckberg, 1997). However, we used power spectral analysis as a standard comparison methodology because its theoretical basis has been thoroughly examined (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). We measured the spectral indexes of non-overlapped epochs of 5 min for the entire 5000 heartbeats recording. The spectral indexes reported for the entire HRV time series were the averages of all 5-min epochs. The LF and HF indexes are reported in total and normalized units.

The *Poincaré plot* is a diagram in which each R-R interval of a tachogram or HRV time series is plotted as a function of the previous R-R interval. We used pairs of R-R intervals lagged by one, two, and four beats. The *quantitative analysis of the Poincaré plot* is based on the notion that each R-R interval is influenced by previous vagal and sympathetic modulations over the heart rate and therefore the pairs of successive R-R intervals form an attractor in the Poincaré plot. An ellipse was adjusted to the Poincaré plot attractor after calculation of the minor and the major axes as follows: the Poincaré plot is first turned 45° counterclockwise and the SD of the data along the horizontal axis (SD1 or minor axis) is then computed. SD1 shows the SD of the instantaneous beat-to-beat variability of the data. The SD of the continuous long-term R-R intervals (SD2 or major axis) is measured along the horizontal axis, after the plot is turned 45° clockwise. The point where both axes intersect corresponds to the total mean of the R-R intervals (Tulppo *et al.*, 1996).

The surrogate data tests

The surrogate data analysis technique (Theiler *et al.*, 1992) was used to prove two different null hypothesis about the content of the HRV time series:

HRV is a random process of independent random variables

The original HRV time series was shuffled, changing all R-R data into an arbitrary place within the time series. The statistical moments (mean, variance, etc.) were preserved but all

autocorrelations were destroyed, so we obtained a non-deterministic surrogate time series. If the SD1/SD2 ratio measured from the original HRV time series turned out to be different from the ones measured from the surrogate time series, then we rejected the null hypothesis that the SD1/SD2 ratio estimated from the original time series comes from an uncorrelated time series (e.g. the original HRV time series may be generated by deterministic mechanisms and their corresponding SD1/SD2 ratio would contain information about these mechanisms).

HRV is a linear autocorrelated Gaussian process

In order to obtain a surrogate time series where non-linearities are destroyed we applied the following procedure: the original HRV time series was transformed by a discrete Fourier transform, the phases were randomized and the inverse discrete Fourier transform was calculated. When phases are randomized, the non-linearities that result from the interactions among phases in the original time series disappear and the new surrogate time series is a sum of only linear autocorrelations. If the SD1/SD2 ratio measured from the original HRV time series turned out to be different from the ones measured from the surrogate time series, then we rejected the null hypothesis that the SD1/SD2 estimated from the original time series comes from linearly autocorrelated time series (e.g. the original HRV time series could contain non-linear information that is captured by the estimated SD1/SD2 ratio).

We used the computer program Chaos Data Analyzer (Sprott & Rowlands, 1995) to transform each HRV time series into surrogate time series, under the two null hypothesis described previously. The SD1/SD2 index calculated from each surrogated time series was compared against its corresponding SD1/SD2 index obtained from the original HRV time series.

Statistical analysis

The normality of the data distribution was verified by the Kolmogorov–Smirnov test. Then a one-way ANOVA followed by a Tukey honest significant difference test was applied (STATISTICA for Windows 5·5., 2000; StatSoft, Inc., Tulsa, OK, USA). Results are reported as mean ± SD. In order to compare each group of SD1/SD2 indexes measured from original HRV time series against its corresponding group of SD1/SD2 indexes measured from the surrogate HRV time series, a paired Student's t-test was used.

Results

Clinical data

In the healthy group the age, height, weight and body mass index (BMI) were 24·1 ± 3·9 years, 1·6 ± 0·5 m, 60·3 ± 6·0 kg and 23·5 ± 2·3, respectively. In the morning, systolic and diastolic blood pressures were 105 ± 10·1 mmHg and 76 ± 9·3 mmHg, respectively. In the afternoon, systolic and diastolic blood pressures were 107 ± 10·5 and 79 ± 9·1 mmHg, respectively. There was no significant difference in blood pressure between the morning and the afternoon measurement in the healthy group.

In the CRF group the average age, height, weight, BMI and creatinine extraction were 22 ± 3·5 years, 1·6 ± 0·9 m, 52·7 ± 15·3 kg, 21·4 ± 4·5 and 0·73 ± 0·7 ml min^{-1}, respectively. The CRF patients had 1·2 ± 0·7 years under HD treatment, and haemoglobin of 8·8 ± 3·0 mg dl^{-1}. Before HD treatment, the CRF group had systolic and diastolic blood pressures of 129 ± 11·4 and 78 ± 11·0 mmHg, respectively. After HD, systolic and diastolic blood pressures were 130 ± 9·0 and 73 ± 8·3 mmHg, respectively. HD treatments took 3·6 ± 0·7 h and the total extracted volume was 2·4 ± 1·1 l. There was no significant difference in blood pressure between the pre-HD and the post-HD values. Before HD the blood urea nitrogen, potassium, phosphate and calcium levels were 66·5 ± 1·7 mg dl^{-1}, 5·2 ± 0·8 mEq l^{-1}, 4·19 ± 1·2 mg dl^{-1} and 8·94 ± 0·9 mg dl^{-1}, respectively. After HD the blood urea nitrogen, potassium, phosphate and calcium levels were 25·56·2 mg dl^{-1}, 3·8 ± 0·7 mEq l^{-1}, 3·44 ± 0·6 mg dl^{-1} and 9·18 ± 1·0 mg dl^{-1}, respectively. All electrolytes changed significantly after HD ($P<0·5$) except for calcium. Anthropometric measures were not significantly different between the healthy and the CRF groups, although the mean weight and BMI was slightly greater in the healthy group. Blood pressure measures were not statistically different between the healthy and the CRF group, except for the systolic blood pressure that was smaller in the healthy than in the CRF group ($P<0·001$), for both the morning versus pre-HD measures and the afternoon versus post-HD measures.

Statistical and power spectra analysis

In Fig. 1 panel A, typical examples of HRV time series of a healthy subject (left) and a CRF patient before HD (right) are shown. Table 1 shows global results from the statistical and power spectral analysis in both groups. Note that the R-R mean, the SD of all normal R-R intervals and the SD of successive differences are larger in the healthy subject than in the CRF patient, and that these differences between groups were even greater after HD treatment (Fig. 1, panel A; Table 1). The power spectrum in the healthy subject (Fig. 1, panel B) exhibits visible peaks in the low frequency band (LF: 0·04–0·15 Hz) and in the high frequency band (HF: 0·15–0·4 Hz), whereas in the CRF patient, the power spectral peaks almost vanished at all frequency bands.

The power expressed in total units was larger in both the LF and HF bands in the healthy group as compared with the CRF group (Table 1, $P\leq0·01$). However, when the LF and HF bands are compared in normalized units, the power of the LF band was smaller and the power of the HF band was larger in healthy subjects than in CRF patients. This is reflected in the LF/HF index, which is almost three times greater in the CRF patients.

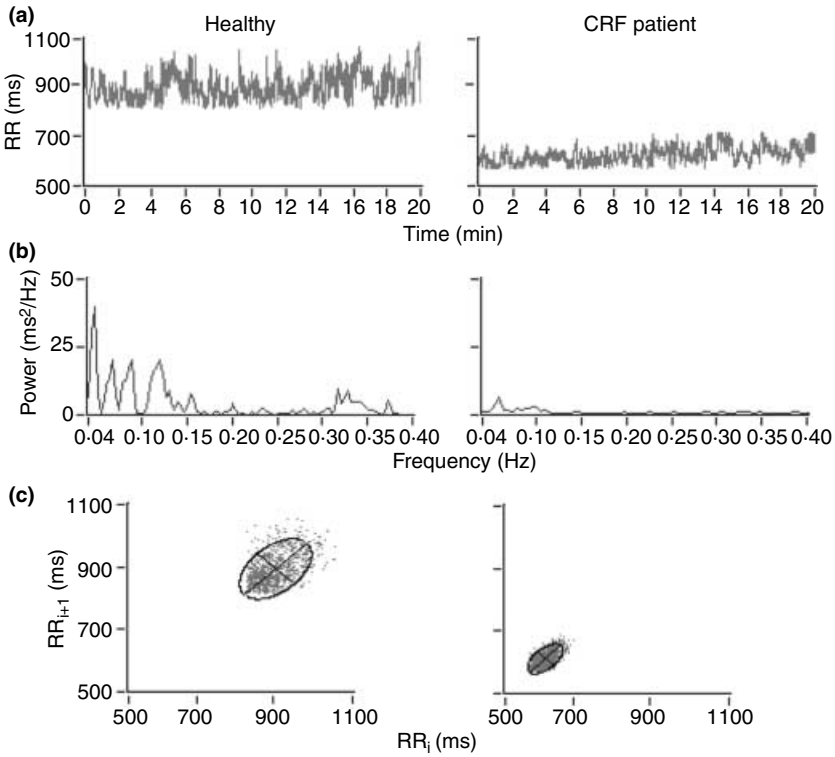

Figure 1 Heart rate variability time series (a), power spectra (b) and Poincaré plots (c) from a healthy subject at morning and from a chronic renal failure patient at morning, before haemodialysis.

Table 1 Results of the time and frequency domain analysis of HRV time series.

	Healthy (n = 10) Morning	**CRF (n = 10) Pre-HD**
Mean (ms)	1024 ± 130*·**	679 ± 58
SD of all normal R-R (ms)	60 ± 14**	26 ± 10
SD of differences (ms)	51 ± 19**	14 ± 6
Total power (ms^2)	1·12 ± 0·33**	0·28 ± 0·24
Low frequency or LF (ms^2)	0·28 ± 0·12**	0·12 ± 0·13
(n.u.)	48 ± 10**	72 ± 11
High frequency or HF(ms^2)	0·31 ± 0·15**	0·04 ± 0·03
(n.u.)	52 ± 10**	28 ± 11
LF/HF	1·13 ± 0·51**	3·61 ± 1·52
	Afternoon	**Post-HD**
Mean (ms)	954 ± 64**	633 ± 75
SD of all normal R-R (ms)	63 ± 24**	25 ± 12
SD of differences (ms)	55 ± 25**	13 ± 7
Total power (ms^2)	1·16 ± 0·60**	0·22 ± 0·17
Low frequency or LF (ms^2)	0·30 ± 0·12**	0·08 ± 0·06
(n.u.)	48 ± 11**	67 ± 14
High frecuency or HF(ms^2)	0·27 ± 0·21**	0·04 ± 0·05
(n.u.)	52 ± 11**	33 ± 14
LF/HF	1·11 ± 0·46**	2·93 ± 1·55

Indexes are expressed as mean ± SD; n.u. means normalized units, which represent the relative value of each power component in proportion to the total power minus the power below 0·04 Hz.
*Significant differences ($P \leq 0{\cdot}05$, Tukey HSD test) of each index between morning and afternoon measurements in the healthy group and pre-HD versus post-HD in the CRF group.
**Significance ($P \leq 0{\cdot}01$) of a Tukey HSD test when comparing each index between the healthy and the CRF group.

In the healthy group the only index that changed during the afternoon compared with the morning record was the R-R mean. In the CRF group, none of the linear indexes changed significantly after HD.

Poincaré plot analysis

Typical Poincaré plots reconstructed with a lag of one heartbeat, corresponding to the HRV time series illustrated in panel A of Fig. 1, are shown in panel C of Fig. 1. The Poincaré plot of the healthy subject exhibits greater dispersion of points than that of the CRF patient. The adjusted ellipses have larger SD1 and SD2 in the healthy subject than in the CRF patient. The centre of the ellipse of the CRF patient is shifted down and to the left with respect to the centre of the ellipse of the healthy subject. Table 2 shows the Poincaré plot indexes measured from Poincaré plots reconstructed with a lag of *one* beat. Recall that both axes were greater in the healthy group than in the CRF group. The SD1/SD2 ratio was greater in the healthy group than in the CRF group. Note that no differences were detected by the Poincaré indexes in the healthy subjects between the morning and the afternoon record and that any change was detected after the HD treatment in the CRF patients with these indexes.

The Poincaré plot indexes measured over Poincaré plots reconstructed with a lag of *two* heartbeats are shown in Table 3. Both axes were larger in the healthy group than in the CRF group. During the morning and before HD, the SD1/SD2 ratio tended to be larger in the healthy group than in the CRF group,

Table 2 Poincaré plot indexes of the HRV measured from the Poincaré plots reconstructed with lag of *one* heartbeat.

	Healthy ($n = 10$) Morning	CRF ($n = 10$) Pre-HD
SD1 (ms)	47·9 ± 12·2*	15·7 ± 8·0
SD2 (ms)	75·1 ± 18·2*	31·6 ± 12·7
SD1/SD2	0·68 ± 0·17*	0·48 ± 0·13
	Afternoon	**Post-HD**
SD1 (ms)	52·5 ± 21·5*	13·4 ± 6·8
SD2 (ms)	76·1 ± 28·7*	30·7 ± 15·1
SD1/SD2	0·69 ± 0·14*	0·44 ± 0·08

Data are mean ± SD. There was no significant difference of indexes between morning and afternoon measurements in the healthy group and pre-HD versus post-HD in the CRF group.
*Significance ($P \leq 0{\cdot}01$) of a Tukey HSD test when comparing each index between the healthy and the CRF group.

Table 3 Poincaré plot indexes of the HRV measured from the Poincaré plots reconstructed with lag of two heartbeats.

	healthy ($n = 10$) Morning	CRF ($n = 10$) Pre-HD
SD1 (ms)	49·0 ± 11·3*	18·2 ± 10·7
SD2 (ms)	80·3 ± 19·0*	32·9 ± 14·7
SD1/SD2	0·62 ± 0·13	0·51 ± 0·15
	Afternoon	**Post-HD**
SD1 (ms)	50·2 ± 15·3*	14·7 ± 7·8
SD2 (ms)	82·5 ± 27·2*	31·1 ± 15·9
SD1/SD2	0·61 ± 0·09*	0·47 ± 0·07

Data are mean ± SD. There was not significant difference of indexes between morning and afternoon measurements in the healthy group and pre-HD versus post-HD in the CRF group.
*Significance ($P \leq 0{\cdot}01$) of a Tukey HSD test when comparing each index between the healthy and the CRF group.

although without any statistical significance. During the afternoon and after-HD, the SD1/SD2 ratio was larger in the healthy group than in the CRF group. With these indexes measured from Poincaré plots reconstructed with a lag of two heartbeats, neither afternoon nor after HD changes were detected.

Table 4 shows the Poincaré plot indexes estimated from Poincaré plots reconstructed with a lag of *four* heartbeats. Both axes were greater in the healthy group than in the CRF group. During the morning, the SD1/SD2 ratio of the healthy group was almost the same as the one observed from the CRF patients before HD. During the afternoon, the SD1/SD2 ratio in the healthy group was greater than in the CRF group observed after HD. In the healthy group the SD1/SD2 ratio measured in the morning and in the afternoon did not show significant difference, whereas in the CRF group the SD1/SD2 ratio was larger before HD than after HD.

Table 4 Poincaré plot indexes of the HRV measured from the Poincaré plots reconstructed with lag of *four* heartbeats.

	healthy ($n = 10$) Morning	CRF ($n = 10$) Pre-HD
SD1 (ms)	53·4 ± 11·4**	22·8 ± 12·3
SD2 (ms)	79·3 ± 19·2**	32·1 ± 12·3
SD1/SD2	0·69 ± 0·13	0·68 ± 0·19*
	Afternoon	**Post-HD**
SD1 (ms)	58·7 ± 22·6**	17·8 ± 8·8
SD2 (ms)	80·4 ± 27·1**	33·2 ± 17·1
SD1/SD2	0·73 ± 0·10**	0·55 ± 0·12

Data are mean ± SD.
*Significant differences ($P \leq 0{\cdot}05$, Tukey HSD test) of each index between morning and afternoon measurements in the healthy group and pre-HD versus post-HD in the CRF group.
**Significance ($P \leq 0{\cdot}01$) of a Tukey HSD test when comparing each index between the healthy and the CRF group.

The surrogate tests of the SD1/SD2 ratio

Fig. 2 shows the SD1/SD2 ratio measured from Poincaré plots reconstructed with lags of *one, two and four* heartbeats. Panel A shows the indexes measured during the morning in the healthy group (left) and before HD in the CRF group (right). Note that at lag of *one* heartbeat the estimates of the SD1/SD2 ratio from the original time series (○) for both groups was significantly greater than the ones obtained from the uncorrelated surrogate time series (▲) (a statistical significant value of $P<0{\cdot}05$ obtained from a Student's *t*-test between the original HRV and the uncorrelated time series is denoted by the symbol §). The SD1/SD2 ratios measured from the linearly correlated surrogate time series (X) were not significantly different from the ones obtained with the original time series. Panel B shows the SD1/SD2 ratios measured during the afternoon in the healthy group and after HD in the CRF group. Note that the SD1/SD2 ratio measured at lag of *one* heartbeat from both the uncorrelated surrogate time series (▲) and from the linearly correlated surrogate time series (X) are different from the original HRV time series (○), for both groups (a statistical significant value of $P<0{\cdot}05$ obtained from a Student's *t*-test between the original HRV and the linearly autocorrelated time series is denoted by the asterisk *).

For both groups the SD1/SD2 ratio measured with a lag of two heartbeats from the uncorrelated surrogate time series (▲) was greater than the one measured from the original HRV time series (○) ($P<0{\cdot}05$ is denoted by the symbol §). The SD1/SD2 ratio measured from the linearly correlated surrogate time series (X) with a lag of *two* heartbeats was different only for the healthy group, at afternoon ($P<0{\cdot}05$ is denoted by the asterisk *).

Note that for all cases in which a lag of *four* heartbeats was used, the SD1/SD2 ratios measured from the original HRV time series (○) were significantly smaller than the ones measured from their corresponding uncorrelated surrogate time series (▲) ($P<0{\cdot}05$ is denoted by the symbol §). The SD1/SD2 ratio

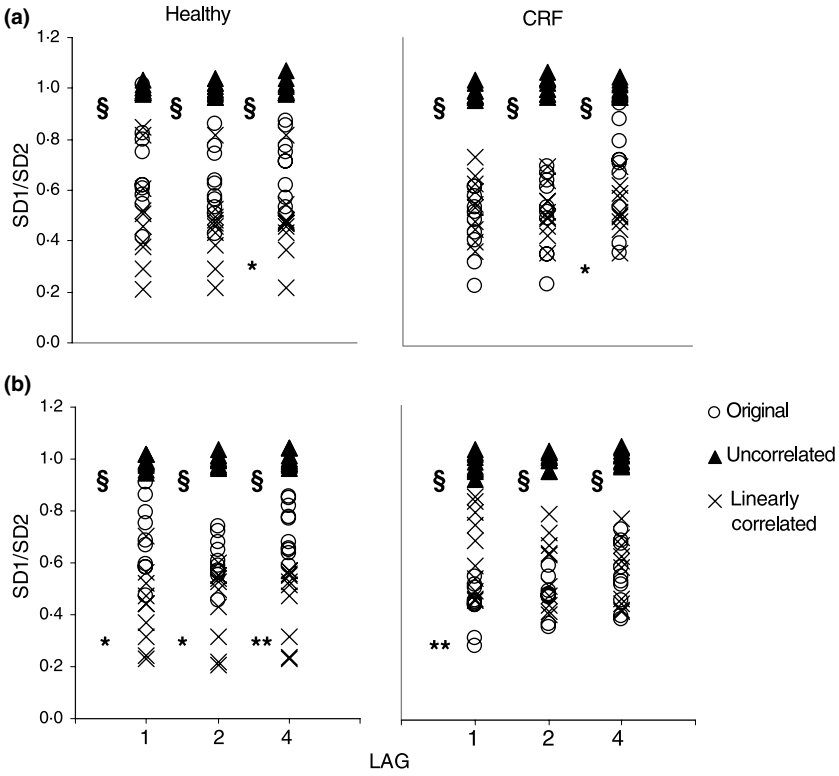

Figure 2 The axes ratio (SD1/SD2) measured from HRV Poincaré plots reconstructed with lags of *one, two and four* heartbeats for all healthy volunteers at morning and CRF patients at morning before HD (a), and for all healthy volunteers at afternoon and all CRF patients at afternoon after HD (b). The symbol ○ corresponds to the original HRV time series; the symbol ▲ corresponds to the uncorrelated surrogate time series; and the symbol X corresponds to the only linearly correlated surrogate time series. The symbol § denotes a $P<0{\cdot}05$ of a Student's t-test between the SD1/SD2 ratio measured from the original and from the uncorrelated time series. The asterisks denote a $P<0{\cdot}05$ (*) or $P<0{\cdot}01$ (**) of a Student's t-test between the SD1/SD2 ratio measured from the original and from the linearly correlated time series.

Table 5 The P-value from a Student's t-test between the SD1/SD2 ratio measured from each original HRV time series and the SD1/SD2 ratio measured from its corresponding linearly correlated surrogate time series*.

Lag	Healthy (n = 10) Morning	CRF (n = 10) Pre-HD
One beat	0·10	0·25
Two beats	0·06	0·87
Four beats	0·03	0·05
	Afternoon	**Post-HD**
One beat	0·00	0·01
Two beats	0·03	0·08
Four beats	0·00	0·73

*Only when $P\leq 0{\cdot}05$ the null hypothesis that the original HRV time series contain only linear correlations is rejected. In that case the original HRV time series contains non-linear components that were detected by the measured SD1/SD2 ratio from the corresponding Poincaré plot.

measured with a lag of *four* heartbeats from the linearly correlated surrogate time series (X) was significantly smaller than the one measured from their corresponding original HRV time series, for both groups during the morning. During the afternoon, the difference between the SD1/SD2 ratios measured from the linearly correlated surrogate time series and their corresponding original HRV time series of the healthy group was accentuated. However, in the CRF group, after HD, the use of a lag of four heartbeats, did not allow us to reject the null hypothesis that only linear autocorrelations are present in that HRV time series.

Table 5 shows a summary of the P-values obtained from a Student's t-test between the SD1/SD2 ratio measured from the linearly correlated surrogate time series and the SD1/SD2 ratio measured from their corresponding original HRV time series. In both groups, during the morning and pre-HD, the P-values did not show significant differences on the SD1/SD2 ratio measured from Poincaré plots reconstructed with lags of *one* and *two* beats. However, in both groups, the P-values showed significant differences on the SD1/SD2 ratio measured from Poincaré plots reconstructed with a lag of *four* heartbeats.

At afternoon and after HD, both groups showed different estimates of the SD1/SD2 ratio when a lag of *one* beat was used to reconstruct the Poincaré plots. The estimates of the SD1/SD2 ratio measured from Poincaré plots reconstructed with lags of *two* and *four* beats were different in the healthy group but were not significantly different in the CRF group.

Discussion

In this work we show that haemodynamically stable CRF patients have larger Poincaré plot indexes than their age and sex paired controls. The standard linear HRV indexes did not change after HD, while the SD1/SD2 ratio measured from Poincaré plots reconstructed with a lag of *four* beats decreased significantly after HD. Moreover, a loss of non-linear components after HD was detected by the surrogate analysis over the SD1/SD2 ratio with a lag of four heartbeats.

It is known that HRV in healthy subjects has non-linear components (Braun *et al.*, 1998), which is not surprising given the complex control mechanisms of the autonomic nervous system and the existence of feedback loops with time delays (Lefebvre & Goodings, 1993; Persson, 1996). The Poincaré plot is a geometrical representation that permits to identify the presence of non-linear structures in a time series that cannot be detected by other methods. However, it has been shown that the standard Poincaré plot indexes (SD1 and SD2) can be described as functions of statistical moments: the SD of all normal beats and SD of the differences between successive beats (Brennan *et al.*, 2001). These authors claim that both axes measure only linear features of HRV. In this study we present evidence that non-linear features of HRV can be detected by the SD1/SD2 ratio. The surrogate data tests, applied between each original HRV time series and its respective linearly correlated surrogate time series, showed evidence that the SD1/SD2 ratio is a discriminant index that is able to detect non-linear information contained in the original HRV time series.

The physiological meaning of Poincaré plot indexes has been explored in different experimental settings (Tulppo *et al.*, 1996, 1998; Toichi *et al.*, 1997). It is known that the sympatho–vagal interaction during exercise is reflected by the Poincaré plot indexes and that the SD1 axis reflects vagal activity in certain specific conditions (Kamen *et al.*, 1996). However, as far as we know, the Poincaré plot indexes had not been previously evaluated in the CRF disease and only a subjective classification of the Poincaré plot attractors has been explored in this pathology (Hayano *et al.*, 1999). We found that there is indeed a change in HRV dynamics after the HD treatment that was evident when a lag of four heartbeats was used to reconstruct the Poincaré plot (Fig. 2; Table 5). It is known that the CRF patients have reduced baroreflex sensitivity (Tomiyama *et al.*, 1980), which augments the delay in the heart rate response. Therefore, it is possible that the change after HD was not detected at lags of one and two heartbeats because the CRF patients have a larger delay in the heart rate control. Some theoretical studies support the notion that a change in the baroreflex delay may alter the HRV dynamics (Cavalcanti & Belardinelli, 1996; Ottesen, 1997). For the reconstruction procedure of the Poincaré plot it is necessary to choose the lag which indicates the pairs of beats that would define the coordinates of each point. This is important because the adequate unfolding depends on the lag we choose according to the Takens theorem (Takens, 1981). We proved lags of three, five, six, seven and eight heartbeats and we did not find significant differences between the indexes measured from lags of two and three. We also did not find a significant difference between the lags of four and five. With lags of six, seven and eight heartbeats the ellipse reached a 90° rotation and the indexes measured were the same than the ones obtained with smaller lags (not shown).

The statistical and spectral features of HRV in CRF patients are well established, either for hypotension-prone or for hypotension-resistant patients (Axelrod *et al.*, 1987; Cloarec-Blanchard *et al.*, 1992; Ligtenberg *et al.*, 1996; Severi *et al.*, 1997; Cavalcanti *et al.*, 1988; Barnas *et al.*, 1999; Hayano *et al.*, 1999; Pelosi *et al.*, 1999;). The loss of spectral power of HRV in CRF patients could be a sign of dominant activity of the sympathetic nervous system, although the accentuation of such a pattern after HD could also be influenced by anaemia and volume extraction (Cloarec-Blanchard *et al.*, 1992). This drives the heart to tachycardia and increased electrical instability, which augments the risk of suffering cardiac dysrhythmias (Thomson *et al.*, 1991; Deligiannis *et al.*, 1999). Therefore, the assessment of changes in HRV that may occur after HD could be important to identify patients at risk and hence the clinical surveillance at post-HD hours must be improved. The statistical and spectral indexes are related to the *magnitude* of variations in HRV but they do not reflect the inherent dynamical features of HRV, particularly the information of the phases whose interactions lead to the non-linear nature of HRV. We were able to determine if the HRV is formed by pure linear autocorrelations or if it has other components, probably of non-linear nature. For example, in healthy subjects, the SD1/SD2 ratio measured during the morning was the same as the one measured from linearly autocorrelated time series, while during the afternoon the HRV was different from the one measured from linearly autocorrelated time series. This observation suggests a circadian variation in HRV dynamics in healthy subjects. In CRF patients the SD1/SD2 ratio measured during the morning at lag of four heartbeats was also different from the one measured from their corresponding linear autocorrelation time series, while during the afternoon, after HD, such difference was not detected. This could be ascribed to the effect of HD, as it is known that circadian variations of mean heart rate are altered or lost in CRF patients when compared with healthy populations (Baumgart *et al.*, 1991; Nakamura *et al.*, 1995; Pikkujämsä *et al.*, 1999). In order to use the Poincaré plot indexes for clinical purposes, a larger and heterogeneous population should be evaluated. An extension of the present study design and a 24-h ambulatory recording are needed in order to discern the presence or absence of circadian variations of the SD1/SD2 ratio, both in healthy and CRF patients.

This work provides insight into the fact that non-linear dynamic changes may indeed occur in CRF hypotension-resistant patients concomitantly with HD treatment. These non-linear changes can be considered to be adaptive as they may avoid hypotension episodes given the high sympathetic activity. However, the loss of non-linearity of HRV after HD could mean a reduced capacity of response of the heart over different demands, which is an important clinical factor to consider during post-HD hours.

In conclusion, the SD1/SD2 ratio is a Poincaré plot index of HRV that capture non-linear dynamic changes in the hypotension-resistant CRF patients after HD, when a lag of four heartbeats is used to reconstruct the Poincaré plot.

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